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Guidelines for the clinical management and follow-up of infants with inconclusive cystic fibrosis diagnosis through newborn screening

Recommandations pour la prise en charge et le suivi des nourrissons pour lesquels un diagnostic de mucoviscidose n'a pu être conclu après dépistage néonatal

I. Sermet-Gaudelus[a,b,*], J. Brouard[c], M.-P. Audrézet[d], L. Couderc Kohen[e], L. Weiss[f], N. Wizla[g], S. Vrielynck[h], K. LLerena[i], M. Le Bourgeois[a], E. Deneuville[j], N. Remus[k], T. Nguyen-Khoa[a], C. Raynal[l], M. Roussey[m], E. Girodon[b,n]

[a] *Cystic fibrosis center, Necker-Enfants-Malades hospital, 75015 Paris, France*
[b] *Inserm U1151, 75993 Paris, France*
[c] *Cystic fibrosis reference center, hôpital de la Côte-de-Nacre, 14033 Caen, France*
[d] *Molecular genetic laboratory, CHRU de Brest, 29609 Brest, France*
[e] *Cystic fibrosis reference center, Charles-Nicolle hospital, 76000 Rouen, France*
[f] *Cystic fibrosis reference center, Hautepierre hospital, 67200 Strasbourg, France*
[g] *Cystic fibrosis reference center, Jeanne-de-Flandres hospital, 59000 Lille, France*
[h] *Cystic fibrosis reference center, child and mother hospital, 69677 Lyon, France*
[i] *Cystic fibrosis center, university hospital, 38700 Grenoble, France*
[j] *Cystic fibrosis center, CHU de Rennes, 35000 Rennes, France*
[k] *Cystic fibrosis center, Créteil intercommunal hospital, 94000 Créteil, France*
[l] *UMR 5535, molecular genetic institute, 34293 Montpellier, France*
[m] *Association française pour le dépistage et la prévention des handicaps de l'Enfant, 75015 Paris, France*
[n] *Molecular genetics laboratory, Cochin hospital, 75014 Paris, France*

Summary

Neonatal screening for cystic fibrosis (CF) can detect infants with elevated immunoreactive trypsinogen (IRT) levels and inconclusive sweat tests and/or *CFTR* DNA results. These cases of uncertain diagnosis are defined by (1) either the presence of at most one CF-associated *cystic fibrosis transmembrane conductance regulator* (*CFTR*) mutation with sweat chloride values between 30 and 59 mmol/L or (2) two *CFTR* mutations with at least one of unknown pathogenic potential and a sweat chloride concentration below 60 mmol/L. This encompasses various clinical situations whose progression cannot be predicted. In these cases, a sweat chloride test has to be repeated at 12 months, and if possible at 6 and 24 months of life along with extended *CFTR* sequencing to detect

Résumé

Les cas d'hypertrypsinémie au dépistage néonatal pour lesquels le diagnostic reste non conclu sont définis soit (1) par l'association d'une mutation au plus du gène *CFTR* associée à la mucoviscidose avec une concentration de chlorure sudoral intermédiaire entre 30 et 59 mmol/L, soit (2) par l'association de 2 mutations de *CFTR*, dont au moins une est de pathogénicité indéterminée avec une concentration de chlorure sudoral inférieure à 60 mmol/L. Ces situations regroupent des formes cliniques différentes dont il est impossible de prévoir l'évolution. Ceci impose de refaire un test de la sueur à 12 mois et si possible à 6 et 24 mois et de rechercher les mutations rares du gène *CFTR*. En l'absence de conclusion, des explorations fonctionnelles visant à établir une dysfonction de CFTR peuvent être proposées. Les

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* Corresponding author at: Cystic fibrosis center, Necker–Enfants-Malades hospital, 75015 Paris, France.
e-mail: isabelle.sermet@aphp.fr (I. Sermet-Gaudelus).

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rare mutations. When the diagnosis is not definite, CFTR functional explorations may provide a better understanding of CFTR dysfunction. The initial evaluation of these infants must be conducted in dedicated CF reference centers and should include bacteriological sputum analysis, chest radiology, and fecal elastase assay. The primary care physicians in charge of these patients should be familiar with the current management of CF and should work in collaboration with CF centers. A follow-up should be performed in a CF reference center at 3, 6, and 12 months of life and every year thereafter. Any symptom indicative of CF requires immediate reevaluation of the diagnosis. These guidelines were established by the "neonatal screening and difficult diagnoses" working group of the French CF society. Their objective is to standardize the management of infants with unclear diagnosis.

nourrissons concernés doivent avoir une évaluation initiale au sein d'un centre de ressources et de compétences pour la mucoviscidose (CRCM) comprenant une étude bactériologique des sécrétions bronchiques, une radiologie de thorax et un dosage de l'élastase fécale. Le praticien libéral référent doit être informé des particularités de la prise en charge et travailler en collaboration avec le CRCM. L'enfant doit être revu à 3, 6 et 12 mois, puis tous les ans au CRCM. L'apparition de symptômes évocateurs de mucoviscidose justifie une réévaluation. Ces recommandations établies par le groupe « Dépistage et formes de diagnostic difficile » de la Société française de la mucoviscidose visent à uniformiser les pratiques dans les CRCM pédiatriques français pour un suivi rationnel adapté et éthique.

1. Introduction

A key objective of newborn screening for cystic fibrosis (CF) is early diagnosis and prompt initiation of specialized care from a pediatric team of CF specialists to delay nutrition impairment and pulmonary complications and improve long-term outcomes. Current screening methods include immunoreactive trypsinogen (IRT) in dried blood spots and common *CFTR* (*cystic fibrosis transmembrane conductance regulator*) gene mutation analysis in infants with IRT levels $\geq$ 65 μg/L [1]. In newborns with presumptive CF, a sweat chloride testing is carried out to rule out or confirm a diagnosis. A diagnosis for CF is confirmed when the sweat chloride concentration is $\geq$ 60 mmol/L and the presence of one CF-causing mutation inherited from both parents has been established. These abnormalities result in loss or altered function of the CFTR protein and are associated with a high probability of developing clinical manifestations of CF later in life [2–5]. However, diagnosis for CF can be challenging in some subjects with intermediate sweat chloride results between 30 and 59 mmol/L and only one to no CF-causing mutation [3,4,6–10]. In those individuals with an atypical presentation, reliable predictions for disease progression are difficult. A subset of these infants will develop classic CF [3,4,6–11], sometimes later in life [12], while some might never exhibit any symptoms [13]. In others, clinical features associated with CFTR dysfunction might not develop until adolescence or adulthood, which is associated with better prognosis than classic CF. These forms may affect multiple organ systems and are usually characterized by mild pulmonary disease and the absence of exocrine pancreatic insufficiency. They may also involve a single organ with evidence of CFTR-related pathology such as enlarged airways (bronchiectasis), recurrent acute or chronic pancreatitis, or obstructive azoospermia with congenital bilateral absence of vas deferens in boys [14]. The prevalence of such cases is highly variable across populations, ranging from 1 to 6% in Australia and Canada [3,9], up to 10% in the US [6,15], and even 21% in California [16]. In France, the proportion of patients with uncertain diagnosis for CF is 1 in every 6.3 confirmed diagnoses, accounting for 184 children between 2002 and 2009 [17,18]. These patients are usually clinically asymptomatic (9% versus 63% of the children with established diagnosis according to the French CF newborn screening report) [18]. In some cases, these patients can present symptoms consistent with CF, but this is at a later stage than in individuals with confirmed diagnosis due to the mild clinical course of the disease (26% before 35 days of age and 70% before 56 days of age in patients with an inconclusive diagnosis versus 53% and 88% in patients with confirmed CF) [18]. This delay illustrates the challenge to ensure proper diagnosis management of these patients. Moreover, a wide variability of management approaches for these patients with inconclusive diagnosis across CF centers in France adds to the diagnostic dilemma. A survey conducted by the authors of this consensus has shown that 12 French CF care teams provided support and reassurance to parents, two others recommended routine follow-up visits similar to classic CF, while 11 teams scheduled spaced-out follow-ups and 13 did not have any defined strategy.

Parents of CF children face a great deal of anxiety about their infant's prognosis and uncertain future. Moreover, the desire for future pregnancies raises the indication of prenatal DNA diagnosis with the possible option of terminating the pregnancy if a genetic test proves positive for CF mutations in the fetus. It is therefore fundamental to clarify this situation by establishing adapted and homogenous diagnostic management and follow-up throughout France. The "Screening and uncertain diagnosis of cystic fibrosis" working group of the French cystic fibrosis society consists of physicians, biochemists, geneticists, nurses, and psychologists at CF centers. Their objective is to provide a standard of care for CF patients harmonized with international guidelines [19]. This group previously published recommendations for management and follow-up of newborns diagnosed with typical forms of CF [20,21]. The goal of the present recommendations is to

Table 1
Guidelines for the clinical management of infants with inconclusive cystic fibrosis diagnosis following newborn screening.

Definitions

1 Inconclusive diagnosis of CF is established when a newborn presents hypertrypsinemia, a sweat chloride test between 30 and 59 mmol/L and at most 1 *CFTR* mutation causing CF
2 Inconclusive diagnosis of CF is established when a newborn presents hypertrypsinemia, a sweat chloride test $<$ 60 mmol/L and 2 *CFTR* mutations with at least 1 of unknown pathogenic potential[a]

Additional diagnostic tests

3 Any intermediate sweat chloride test between 30 and 59 mmol/L should be confirmed with a second test within 3 months of the initial test
4 Any newborn presenting hypertrypsinemia, a sweat chloride test between 30 and 59 mmol/L and only 1 mutation found with the Elucigene CF kit must undergo a genetic analysis for rare *CFTR* mutations
5 Any newborn presenting persistent hypertrypsinemia, a sweat chloride test between 30 and 59 mmol/L and no mutation found with the Elucigene CF kit must undergo a genetic analysis for rare *CFTR* mutations.
6 Any newborn presenting hypertrypsinemia, a sweat chloride test $<$ 30 mmol/L and only 1 mutation found with the Elucigene CF kit should not undergo any additional diagnostic test for CF except c.3718-2477C>T (3849+10kb C>T)[b] carriers
7 Polymorphism c.1210-12T (polyT) will be investigated in cases of c.350G>A (R117H) mutation. R117H in *cis* with the T5 splicing variant is considered as a CF mutation. R117H in *cis* with the T7 normal variant is not considered as a CF mutation
8 Newborns with an inconclusive diagnosis for CF must have a sweat chloride test at 12 months. A test at 6 months and 2 years of age is recommended
9 A diagnosis for CF should be overturned when a sweat chloride test normalizes (returns below 30 mmol/L), in newborns carrying only 1 mutation causing CF following extended genetic *CFTR* analysis
10 A diagnosis for CF is confirmed when a sweat chloride test becomes $\geq$ 60 mmol/L
11 In cases presenting a sweat chloride test $<$ 60 mmol/L and 2 mutations detected following extended genetic *CFTR* analysis, including at least one of unknown pathogenic potential, a functional exploration of the CFTR protein using dynamic transepithelial potential tests (nasal transepithelial potential difference, and/or short-circuit current measurements on rectal biopsies) may be proposed according to the local CF center's procedures
12 In cases presenting a sweat chloride test between 30 and 59 mmol/L and only 1 mutation with unknown pathogenic potential found following extended genetic *CFTR* analysis, a functional exploration of the CFTR protein using transepithelial potential tests (nasal transepithelial potential differences, and/or short-circuit current measurements on rectal biopsies) may be proposed according to the local CF center's procedures
13 Parents will be informed about the uncertainty of the diagnosis and the need of a specialized care
14 Compound heterozygotes for R117H;T7 and a CF-causing mutation may in rare cases be symptomatic and require a clinical follow-up

Initial assessment

15 Newborns with an inconclusive diagnosis for CF must receive an evaluation in a CF center
16 Pulmonary evaluation includes a bacteriological analysis of bronchial secretions and a thoracic radiography
17 Nutritional evaluation includes anthropometric analysis and fecal elastase assessment
18 Genetic counseling will be offered to parents after identification of a CF mutation

Initial management

19 Newborns must receive proper immunization following annual recommendations
20 Influenza vaccination is recommended for newborns with an inconclusive diagnosis of CF from 6 months of age as well as for their family members
21 Systematic physiotherapy is not necessary unless the patient presents signs of lung disease
22 Sodium supplementation might be given at high ambient temperatures in cases where sweat chloride test is $>$ 30 mmol/L to limit risks for dehydration
23 A newborn with an inconclusive diagnosis for CF does not automatically qualify for long-term illness application. Each application will be assessed on a case-by-case basis

Follow-up

24 The primary care physician in charge will be informed of the specificities of the clinical management and will work in collaboration with the CF center
25 Newborns with an inconclusive diagnosis for CF will be managed in CF centers. Follow-up visits will be scheduled at 3, 6 and 12 months and yearly thereafter. The development of signs indicative of CF requires a reevaluation of the diagnosis
26 General hygiene practices identical to the ones for patients with CF will be followed
27 Systematic bacteriological bronchial secretion analysis and growth monitoring will be performed during each visit at the CF center
28 A visit at the CF center will be conducted upon recurrent pulmonary obstructive disease for reevaluation
29 A fecal elastase assessment will be performed in patients with growth retardation or signs of exocrine pancreatic insufficiency

CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; IRT: immunoreactive trypsinogen.
[a] Mutation with undetermined pathogenic potential or unknown clinical relevance. These are mainly rare or unknown missense mutations or mutations which might alter splicing and for which pathogenic potential and severity cannot be predicted. These are different from mutations with known and documented moderate pathogenic potential or associated with a wide phenotypic spectrum and for which the sweat chloride test may be intermediate or negative (see "b").
[b] CF patients compound heterozygous for the c.3718-2477C>T (3849+10kbC>T) mutation and a CF-causing mutation like c.1521_1523del (F508del) might have a negative or intermediate sweat chloride test.

standardize clinical management of infants with an inconclusive diagnosis following newborn screening.

2. Methods

Guideline statements, developed by a core committee of CF experts, were reviewed by participating pediatric CF centers using the Delphi methodology [22]. It was determined that 90% agreement among pediatricians would constitute an adequate consensus on a statement. If they disagreed or were unable to conclude, statements were rewritten and amended according to reviewer's suggestions and resubmitted to the core panel of experts for validation. Guidelines were divided into four sections:

- definitions;
- additional diagnostic testing;
- initial evaluation;
- follow-up (Table 1).

Twenty-five pediatric CF centers contributed to the Delphi process. Seven of the 29 statements had to be amended and consensus was achieved on all 29 statements after the second round of reviews.

3. Defining situations where the diagnosis of CF is inconclusive following newborn screening

Cases of infants with elevated IRT levels at newborn screening for CF for whom diagnosis remains uncertain are defined by (Table 2):

- the presence of at most one *CFTR* mutation causing CF associated with "intermediate" sweat chloride values between 30 and 59 mmol/L (Recommendation #1);
- or the presence of two *CFTR* mutations including at least one of unknown pathogenic potential associated with a sweat chloride level $<$ 60 mmol/L (Recommendation #2).

"Mutations that cause CF", also called "CF-causing mutations" or "Group A mutations" according to Castellani et al. [23] when they are found in *trans* with another CF-causing mutation, are defined by their association with a typical form of the disease and sweat chloride values $\geq$ 60 mmol/L or in vitro evidence of their pathogenic effect. This pathogenic effect is based on clinical observations, epidemiological data, electrophysiological testing, as well as data from bioinformatics analysis or various functional tests [23].

"Mutations that result in a CFTR-related disorder", called "CFTR-RD" or "Group B mutations", are mostly encountered in adult patients with an atypical form or a single-organ impairment [23]. They include mutations with allegedly moderate or minor deleterious effects that have not been found in patients with CF in isolation, i.e., with no other mutation in *cis*.

"Mutations associated with a wide phenotypic spectrum", or "CF/CFTR-RD" or "Group A/B mutations" may be observed in patients with CF, variable pancreatic impairment, and moderate pulmonary disease as well as in adult patients with monosymptomatic presentations [23].

"Mutations of unproven or uncertain pathogenicity" or "Group D mutations" are mutations for which observational, bibliographic, and functional data do not allow any conclusion on their susceptibility to inducing symptoms indicative of CF [23]. These mutations are rare, with a pathogenicity that is challenging to establish due to a lack of available data.

Finally, "mutations with no known pathogenic effect" or "Group C mutations" may be observed in *trans* with a CF mutation in asymptomatic individuals [23].

A nonexhaustive list of mutations detected through newborn screening and classified according to the four groups defined above is displayed in Table 3.

Mutation c.3718-2477C $>$ T (3849 + 10kbC $>$ T) is frequently associated with sweat chloride values $<$ 30 mmol/L. Most of the CFTR-RD mutations and some mutations with a wide phenotypic spectrum such as c.617T $>$ G (L206 W), c.1040G $>$ A (R347H), and c.3454G $>$ C (D1152H) can occasionally be associated with an intermediate or negative sweat chloride test [24–27].

Table 2
Definition of inconclusive diagnosis after nonatal screening.

Sweat chloride test	First allele	Second allele
30–59 mmol/L	No mutation found	CF mutation
		Wide spectrum
		CFTR-RD
		VUS
		No mutation found
$<$ 60 mmol/L	VUS	CF mutation
		Wide spectrum
		CFTR-RD
		VUS

CF mutation: mutation causing a typical form of CF when found in trans with another CF-causing mutation; VUS: variant of unknown significance; mutation CFTR-RD: mutation resulting in a CFTR-related disorder when in trans with a CF-causing mutation.

4. Diagnostic genetic testing (Recommendations 4–7 and 14)

Hypertrypsinemia is considered a sensitive marker of CFTR dysfunction. However, due to a lack of specificity in newborns [9], screening algorithms typically search for the 29 most common mutations in the French population. The diagnosis algorithm is shown in Fig. 1.

If only one mutation is detected with the mutation test panel, a sweat chloride test is performed. If the sweat chloride value is $<$ 30 mmol/L, the infant is classified as a healthy carrier, except when c.3718-2477C $>$ T (3849 + 10kbC $>$ T) is detected. In such cases, an extended genetic analysis must be perfor-

Table 3
Phenotypic classification of common *CFTR* mutations.

Groups of mutations and synonyms	Most common CF mutations			
Mutations that cause CF disease	**c.254G>A (G85E)**	**c.1523_1523del (F508del)**	c.1766+1G>A (1898+1G>A)	c.3302T>A (M1101K)
	c.262_263del (394delTT)	**c.1519_1521del (I507del)**	c.2012del (2143delT)	**c.3484C>T (R1162X)**
CF mutations (group A)	**c.178G>T (E60X)**	c.1545_1546del (1677delTA)	**c.2051_2052delinsG (2183AA>G)**	**c.3528del (3659delC)**
	c.366T>A (Y122X)	**c.1585-1G>A (1717-1G>A)**	c.2052dup (2184insA)	c.3472C>T (R1158X)
	c.489+1G>T (621+1G>T)	**c.1624G>T (G542X)**	c.2128A>T (K710X)	c.3731G>A (G1244E)
	c.579+1G>T (711+1G>T)	c.1646G>A (S549N)	c.2215del (2347delG)	**c.3752G>A (S1251N)**
	c.1000C>T (R334W)	c.1647T>G (S549R)	**c.2538G>A (W846X)**	c.3773dup (3905insT)
	c.1040G>C (R347P)	**c.1652G>A (G551D)**	c.2668C>T (Q890X)	**c.3846G>A (W1282X)**
	c.948del (1078delT)	c.1654C>T (Q552X)	**c.2988+1G>A (3120+1G>A)**	**c.3909C>G (N1303K)**
	c.1364C>A (A455E)	**c.1657C> (R553X)**	c.3196C>T (R1066C)	c.54-5940_273+10250del21080 (CFTRdele2,3)
	c.1477_1478del (1609delCA)	c.1679G>C (R560T)	**c.3276C>A (Y1092X)**	
		c.1680-886A>G (1811+1.6kbA>G)		
Mutations associated with a wide phenotypic spectrum CF/CFTR-RD (group A/B)	c.328G>C (D110H)[a]	c.1210-34TG[13]T[5] (TG13T5)[a]	**c.3140-26A>G (3272-26A>G)**	c.3454G>C (D1152H)[a]
	c.617T>G (L206W)[a]	**c.2657+5G>A (2789+5G>A)**	c.3208C>T (R1070W)[a]	**c.3718-2477C>T (3849+10kbC>T)**[a]
	c.1040G>A (R347H)[a]			
Mutations that result in a CFTR-related disorder CFTR-RD mutations (group B)	c.91C>T (R31C)	c.349C>T (R117C)	(R74W;D1270N)c.2991G>C (L997F)	
	c.350G>A (R117H;T7)	c.[220C>T;3808G>A] (R74W;D1270N)	(R74W;D1270N)c.1865G>A (G622D)	c.3154T>G (F1052V)
	c.1210-34TG[12]T[5]		(R74W;D1270N)c.[1327G>T; 1727G>C;2002C>T] (D443Y;G576A;R668C)	c.4056G>C (Q1352H)
Mutations of unproven or uncertain clinical relevance Group D	c.579+3A>G (711+3A>G)	c.1069G>A (A357T)	c.1666A>G (I556V)	c.2855T>C (M952T)
	c.695T>A (V232D)	c.1367T>C (V456A)	c.1801A>T (I601F)	c.869+5G>A (1001+5G>A)
	c.1055G>A (R352Q)			
Mutations with no known clinical consequence Group C	c.-8C>G (125G>C)	c.1210-34TG[11]T[5] (TG11T5)	c.1584G>A (E528E)	c.3469-20T>C (3601-20T>C)
	c.224G>A (R75Q)	c.1408G>A (M470V)	c.2562T>G (T854T)	c.3870A>G (P1290P)
	c.743+40A>G (875+40A>G)	c.1523T>G (F508C)	c.2620-15C>G (2752-15C>G)	

Mutations detected by neonatal screening using Elucigene CF30 kit (Gen Probe) are indicated in bold. Mutation c.350G>A (R117H) is no longer in the kit mutation panel.
[a] *CF-causing mutations or mutations associated with a wide phenotypic spectrum that are found in newborns with negative or intermediate sweat chloride tests when they are in trans with a CF mutation or a CFTR-RD mutation.*

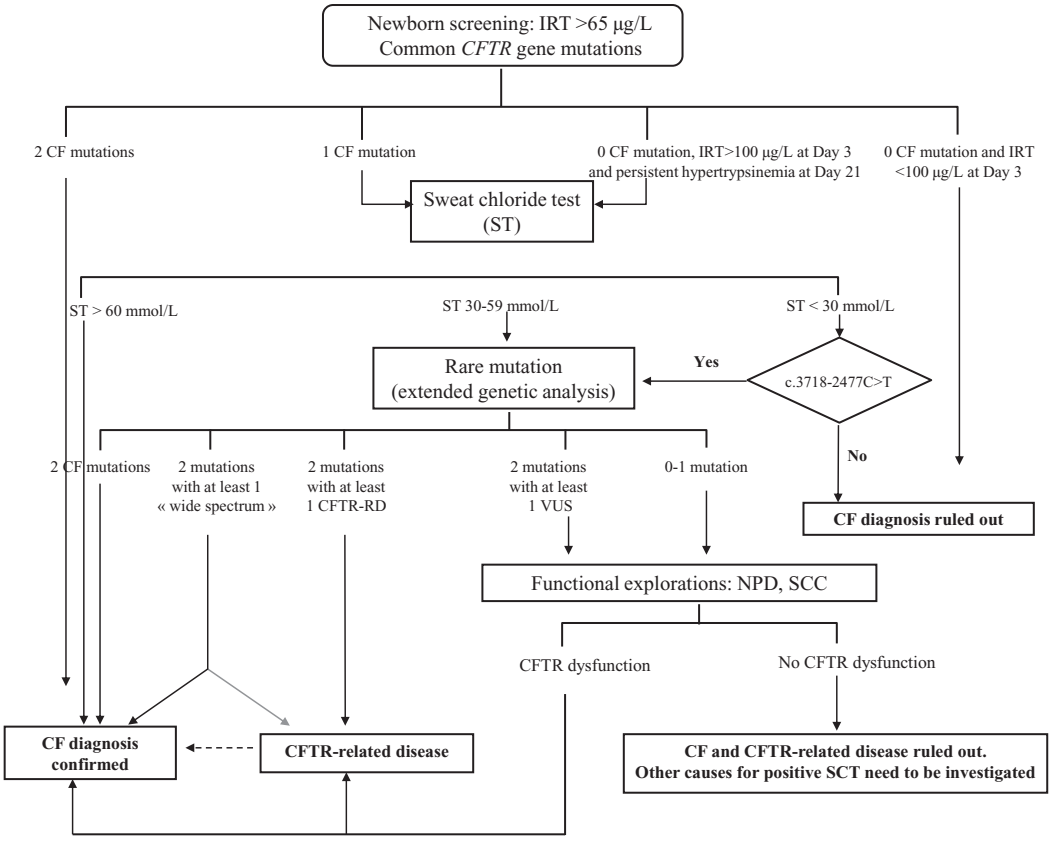

Figure 1. Diagnostic algorithm in infants with inconclusive diagnosis for cystic fibrosis at newborn screening. SCC: short circuit current measurement on rectal biopsy; NPD: transepithelial nasal potential difference; IRT: immunoreactive trypsinogen; ST: sweat chloride test; CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; Day 3: 3rd day of life; Day 21: 21st day of life. CF mutations: mutations known or presumed associated with a typical form of the disease, sweat chloride values ≥ 60 mmol/L, or evidence of their clinical pathogenic relevance. VUS (variants of unknown significance): mutations of unproven or uncertain clinical relevance for which their susceptibility to induce symptoms that may be attributed to CF cannot be concluded due to the scarcity of observational, bibliographic, and functional evidence. CFTR-RD mutations: mutations with documented pathogenicity yet associated with a single-organ disease or an atypical presentation suggestive of CF in adults. Identification of a CFTR-RD genotype with an intermediate SCT suggesting a CFTR-related disorder does not exclude, however, a possible clinical progression towards late CF.

med because this mutation might be associated with a negative sweat chloride test (Recommendation #6) [24–27]. When the sweat chloride test is intermediate between 30 and 59 mmol/L, a genetic analysis searching for rare *CFTR* variants must be conducted (Recommendation #4). An extended genetic analysis must also be performed if no targeted mutation has been found with the test panel, but hypertrypsinemia is > 100 µg/L at Day 3 after birth and persists at Day 21, and the sweat chloride test is ≥ 30 mmol/L (Recommendation #5). An exhaustive *CFTR* sequence analysis must be conducted in a specialized genetics laboratory and must include analysis of the full coding sequence and screening for major rearrangements such as deletions, duplications, or insertions. In this situation, the possible outcomes are listed below:

- identification of two CF mutations, one on each parental allele: CF diagnosis is confirmed;
- identification of a CF mutation associated with a wide phenotypic spectrum mutation in an infant presenting a sweat chloride value < 60 mmol/L. This indicates the risk of progression to CF and requires regular personalized follow-up visits;
- identification of at least one CFTR-RD mutation combined with a sweat chloride test < 60 mmol/L. The diagnosis for CF cannot be established but a CFTR-related disorder has been identified. In such cases, the patient might develop single-organ involvement (CFTR-related disorder), which has the potential to progress to multiorgan disease (for example, evidence of lung involvement in a patient presenting an absence of vas deferens). Such clinical manifestations might also be observed in patients carrying one CF mutation in *trans* with mutations associated with a wide phenotypic spectrum due to their large phenotypic variability;
- absence of mutation or presence of only one mutation (CF, CFTR-RD or mutation of unknown pathogenic potential) in patients with a sweat chloride test between 30 and 59 mmol/L: the diagnosis for CF cannot be excluded

(Recommendation #1). Cystic fibrosis might therefore be associated with the presence of one or two rare variants undetected with mutation analyses. This type of risk remains a rare occurrence. In France, only seven CF alleles out of 2320 (0.3%) could not be identified following extended genetic analysis [17], making the diagnostic sensitivity 99.7%;

- presence of one mutation of unknown pathogenic potential in *trans* with a CF or CFTR-RD mutation or presence of two mutations of unknown pathogenic potential: the possibility of developing CF cannot be excluded regardless of a negative or intermediate sweat chloride test. Few such cases have been identified in France. Until now, 64 mutations with unknown pathogenic potential have been characterized following an extended genetic analysis in 6.3% of newborns carrying two mutations [17]. The US screening algorithm also includes this situation [28]. However, it must be pointed out that American diagnostic guidelines have been developed based on the database of CF mutations validated in the Clinical and functional translation of CFTR project (CFTR2) [29]. At this point, it is important to keep in mind that this database is currently being built with only 322 mutations listed to date.

Therefore, French and American screening algorithms differ in terms of their genetic approach. First, the French screening algorithm does not exclusively rely on the CFTR2 database but also covers international databases and data from large cohorts reported in the literature. In fact, the pathogenic potential of a number of variants not yet validated by CFTR2 can be documented in published clinical observations or functional explorations, or in the French database CFTR-France [30]. An example of the benefit of this complementary approach is illustrated in the analysis of the 79 newborns screened in the Canadian program with an initial inconclusive diagnosis for CF [9]. Nine of these children were ultimately reclassified as having CF, including seven based only on their genotype or on genotype and a positive sweat chloride test. According to the French screening algorithm that considers extensive data retrieved from the literature, five children would have been diagnosed with CF very early on, carrying mutations that cause CF or a variable phenotype (c.200C > T [P67L] or c.617T > G [L206W]). An even more striking example is the California Newborn Screening Program that conducts CFTR sequencing in newborns with hypertrypsinemia and one common mutation prior to the sweat chloride test [31]. This kind of approach is responsible for numerous cases of inconclusive diagnosis (1.5 inconclusive diagnoses for 1 CF diagnosis) [16]. Since July 2007, out of 1855 children diagnosed with a suspicion of CF, 244 carried a variant of unknown pathogenic potential according to CFTR2, 56 of which had never been identified. Only 25 patients (10%) were reclassified as having CF based on the sweat chloride test or pancreatic insufficiency [13,16]. In this group, nine patients had two CF mutations: eight carried a very rare mutation and one had a variant reported in the literature but not validated by CFTR2. Therefore, based on the French algorithm, these patients could have been promptly classified and appropriate treatment could have been initiated early on. A second major difference between the French and American screening algorithms is that CFTR2 does not consider CFTR-RD or Group B mutations because the data are only collected from patients with CF or with a suspicion of CF. For simplification purposes, CFTR-RD mutations have been classified either as mutations of varying clinical consequences or as mutations not associated with CF, considered similar to nonpathogenic variants with no clinical relevance.

Finally, we believe that the penetrance of mutations needs to be taken into account for an optimized genetic approach. Incomplete penetrance of variants remains a challenge to interpretation. One of the most remarkable mutations is c.350G > A (R117H), which is frequently identified in newborns with hypertrypsinemia in association with c.1521_1523del (F508del) (11% of the cases in Canada [9], 26% in the US [7], and 7.2% in France [17]). Although this mutation has been detected in France in only seven of the 1160 newborns who screened positive with a sweat chloride test, it was found in 98 of the 184 newborns with an inconclusive diagnosis for CF, 50% of the cases [17]. Identification of this mutation substantiates the need for searching for the splice variant c.1210-12 T (T5 variant), which might aggravate the impact of c.350G > A (R117H) when it is located on the same chromosome (in *cis*) [32] (Recommendation #7). A large French collaborative study compiling data from all phenotypes associated with this mutation and epidemiological findings has shown that this mutation, when located in *cis* with the normal splicing variant c.1210-12T (T7 variant) [7] and combined with the common CF mutation c.1521_1523del (F508del), was expected to be found mainly in asymptomatic individuals (97% of cases) and patients presenting with very mild disease (3% of the cases), manifesting as, for example, isolated infertility, or more rarely, another single-organ disease, and exceptionally lung disease observed in classic forms of CF (one infant with CF out of 3650 individuals expected to carry this genotype) [33]. No correlation has been found between sweat chloride levels and the severity of the disease [32–35]. Based on the data from this French collaborative study, the mutation c.350G > A (R117H) associated with the T7 splicing variant has been reassigned to the "CFTR-related disease" category (Group B of CFTR-RD mutations) but with a very low penetrance of 0.03% [33]. In contrast, the US CFTR2 database categorizes this mutation as being of variable pathogenic potential, without considering its low penetrance. Consequently, the assessment of the pathogenic potential of mutations should take into account their level of penetrance based on observations, not only in patients with CF but also in adult patients presenting a single-organ disease as well as asymp-

Table 4
Summary of main studies in infants with inconclusive cystic fibrosis diagnosis following newborn screening.

Study	Definition	Screening	Study design	Follow-up (years)	Inconclusive diagnosis (*n*)	Conclusive diagnosis for CF (*n*)	Ratio inconclusive/ conclusive	% of infants with inconclusive diagnosis and *P. aeruginosa* positive
[8] USA	At most 1 CF mutation and ST between 30 and 59 mmol/L 2 mutations (at most 1 CF) and ST < 30 mmol/L	IRT/DNA (test panel for targeted mutations)	Multicenter, prospective	10	1,540	309	5:2	11
[9] Canada/Italy	1 CF mutation and ST between 30 and 59 mmol/L 2 mutations (at most 1 CF) and ST < 30 mmol/L 0 mutation, and ST between 30 and 59 mmol/L	IRT/DNA (test panel for targeted mutations)	Multicenter, prospective, control case	3	82	80	From 1.4:1 to 2.9:1 depending on the CF center	12
[13,16,31] USA (California)	2 mutations (at least 1 non CF) and ST < 60 mmol/L	IRT/DNA	Multicenter, prospective	1	533	20	1:1.5	UND
[15] USA (Wisconsin)	At most 1 CF mutation and ST between 30 and 59 mmol/L 2 mutations (at most 1 is CF) and ST < 30 mmol/L	IRT/DNA (test panel for targeted mutations)	Retrospective Crosssectional	21	57	300	5:1	7

CF: cystic fibrosis; ST: sweat chloride test; IRT: immunoreactive trypsinogen; UND: undetermined.

tomatic subjects carrying two mutations. In light of these results, the c.350G > A (R117H) mutation was ultimately excluded from the newborn screening test panel on 1 January 2015 (http://www.afdphe.org/sites/default/files/bilan_activite_2012.pdf). Newborns carrying this mutation will nevertheless be diagnosed through extended genetic analysis in cases where one targeted mutation is identified and the sweat chloride test is > 30 mmol/L. These patients will require the regular clinical follow-up detailed below, as these individuals might in rare instances develop symptoms indicative of CF or a single-organ pathology (Recommendation #14) [35]. The exclusion of mutation R117H from the screening panel allowed for a reduction in the number of infants with an uncertain diagnosis in comparison with infants diagnosed with having CF, from 1/6.3 in 2014 to 1/9 in 2015 (A.F.D.P.H.E 2015 activity report, http://www.afdphe.org). These findings underscore the challenge of comparing results from different studies when the screening algorithms and methodology used to classify mutations differ. Table 4 illustrates the impact of disparities between screening algorithms and mutation classification on overall conclusions and highlights the importance of conducting standardized international epidemiological studies.

5. Functional diagnostic approach (Recommendations #3, and #8–12)

In those patients for whom the CF diagnosis remains equivocal, CFTR functional explorations may provide further helpful information to firmly establish a diagnosis (Fig. 1). A repeat sweat chloride test within 3 months of the initial measurement is critical due to great variability around the 30-mmol/L cut-off value (~20%) (Recommendation #3) [36]. Any change in the sweat test is of particular importance to conclusively rule out the diagnosis when chloride values are < 30 mmol/L and at most one mutation (except mutations typically associated with a negative sweat test) has been identified, or in contrast to confirm it when chloride levels are > 60 mmol/L (Recommendations #9 and 10). Therefore, it is imperative to reassess the sweat testing at 12 months, and a confirmatory repeat at 6 and 24 months is also recommended (Recommendation #8) because these results might modify and clarify the diagnosis. In the above-mentioned French study, the 3-year follow-up was able to identify six children out of 14 for whom the sweat chloride concentrations increased over time above 60 mmol/L [4], and in the Canadian study the sweat test became positive at 21 months in four children out of nine [9]. Yet, there is no evidence for a direct correlation between elevated chloride levels at the initial assessment and a higher risk of a sweat test turning positive [4,8,9].

In cases where repeated sweat tests remain within the intermediate range, functional analyses measuring CFTR activity might help clarify the diagnosis (Recommendations #11 and 12). Studies have shown that absence or dysfunction of the CFTR protein resulted in abnormal airway epithelial ion transport. The assessment of CFTR function is based on in vivo pharmacological studies measuring nasal potential difference (NPD) or ex vivo in rectal biopsies perfused in an Ussing chamber under short-circuit current conditions, on occasion in combination with intestinal organoid analysis [37]. A superficial biopsy is obtained without sedation. The procedure is virtually painless due to the absence of sensory fibers in the rectum. There are no complications except in case of thrombocytopenia and hemostatic abnormalities (systematically screened by performing blood count and coagulation tests prior to the biopsy) [38]. Additionally, a novel methodology to analyze sweat production in response to a specific CFTR stimulation in the acinus of the sweat gland appears to accurately measure CFTR protein function [39]. Unfortunately, this approach requires complete immobilization of the patient for at least 20 min, which is practically impossible with a newborn. Agonist- or antagonist-mediated pharmacodynamic modulation of transepithelial potential difference in vivo or ex vivo proves to be useful in understanding pathophysiological consequences from *CFTR* mutations (Figs. 2 and 3).

CFTR function is typically evaluated in highly specialized CF centers. Nasal potential difference measurements have been shown to have both high positive and high negative predictive values. Supporting the important diagnostic value of NPD are documented cases of newborns with abnormal NPD and borderline sweat test that became abnormal over time and who ultimately developed clinical features suggestive of CF, while other infants with a negative NPD and a sweat test that eventually normalized never presented any signs of CF [4]. Short-circuit current measurements on rectal biopsies are a valuable tool for ex vivo investigation of Cl^- transepithelial transport whose homeostasis is essential to the fluidity of serous secretions in the lungs. This procedure does not require the cooperation of the subject, which is of critical importance in newborns, and allows for the testing of multiple agonists and antagonists. Moreover, distal intestinal epithelium, due to its high density in CFTR channels, can reveal electrophysiological abnormalities that otherwise cannot be detected on the nasal epithelium.

A diagnosis of CF can be ruled out when these functional analyses are within the normal range. Therefore, simple CF carriage can be concluded when a single CF mutation has been identified. If a mutation of unknown pathogenicity is found in *trans* with a known CF mutation, it can be concluded that the variant is likely nonpathogenic. When a functional abnormality is detected (Fig. 3), CFTR-mediated residual activity might often be observed, while patterns similar to those recorded in patients with a classic form of CF remain rare.

All these observations clearly illustrate a shift in the diagnostic paradigm of CF that can no longer be interpreted only as positive or negative (CF mutation: presence or absence) but

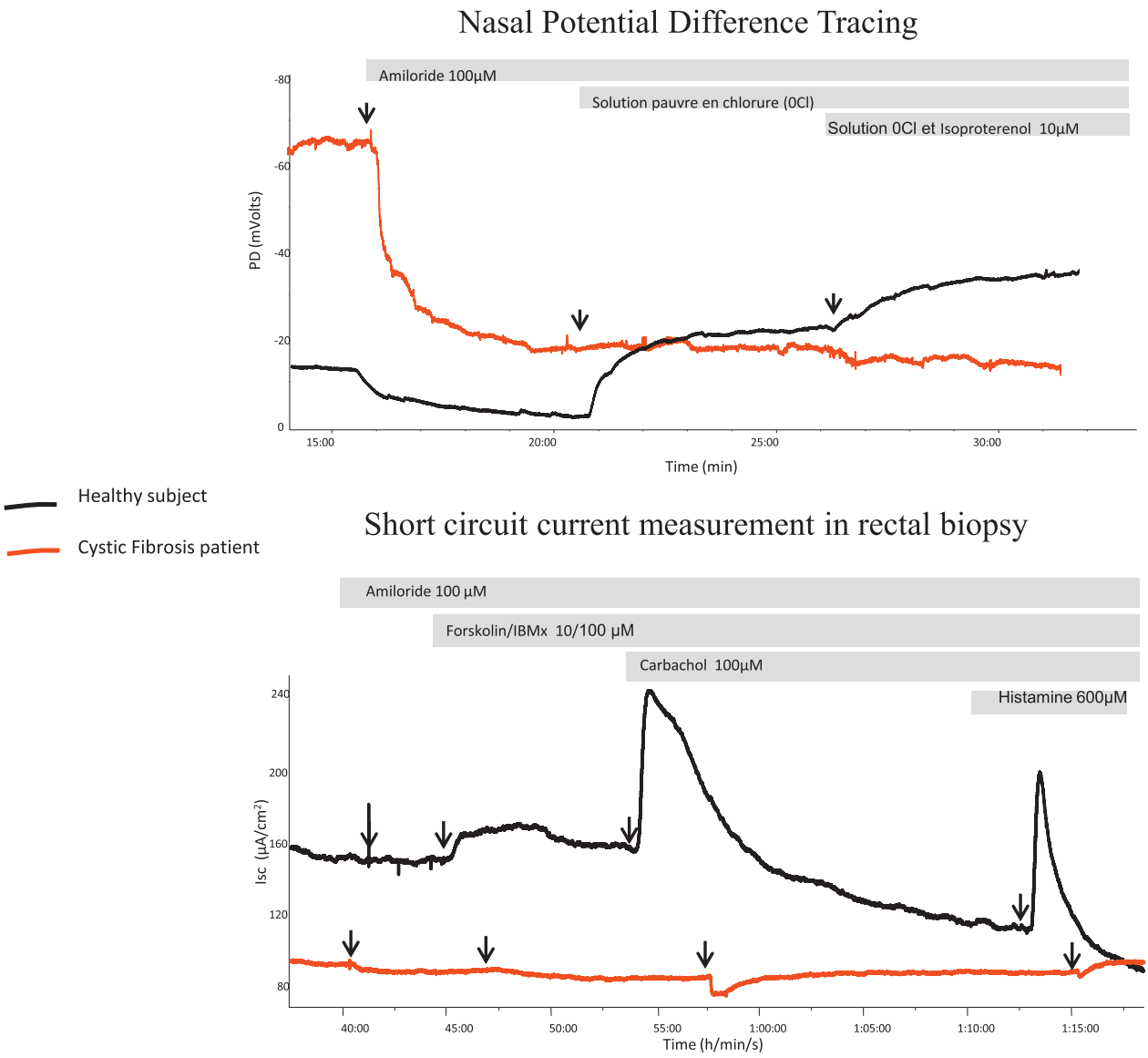

Figure 2. Epithelial functional analyses. Profiles of transepithelial nasal potential difference (NPD) (upper panel) and short-circuit current (SCC) on rectal biopsy (lower panel) in a healthy subject and a patient diagnosed with a classic form of CF. These tests have been standardized at the CF European network level (http://www.ecfs.eu). Upper panel. The NPD is measured between the airway surface and the subcutaneous space. A very fine exploration catheter filled with a solution that conducts an electrical signal is placed against the nasal mucosa to measure nasal mucosa potential. Another electrode is placed on the skin to measure cutaneous potential. While the infant is comfortably installed in its mother's arms, nasal mucosa is continuously perfused with solutions to evaluate (*cystic fibrosis transmembrane conductance regulator* [CFTR]) pharmacodynamic response. Chloride ion (Cl^-) efflux causes hyperpolarization (Δ oCl^-/Isoproterenol). Any CFTR dysfunction stimulates sodium ion (Na^+) reabsorption (increased response to amiloride and more negative baseline values) and inhibits Cl^- reabsorption (absence of repolarization). Lower panel. Transepithelial short-circuit current is typically evaluated on rectal biopsy and measures the transepithelial potential resulting from Cl^- transport as well as the current (SCC) needed to cancel it. A positive SCC indicates a flow of negative charges from the serosa to the mucosa. In practice, the rectal biopsy must be very superficial (confined to the epithelium whenever possible). A piece of epithelium is inserted in an Ussing chamber consisting of two compartments delimiting an opening where the tissue is placed. The ability of rectal epithelium to secrete Cl^- is investigated in response to: (1) cAMP, specific CFTR activator after treatment with forskolin (adenylyl cyclase activator, an enzyme that synthesizes cAMP) and IBMX (3-isobutyl-1-methylxanthine), which inhibits cAMP degradation (Δ forskolin); (2) calcium after exposure to carbachol (cholinergic pathway; Δ carbachol); (3) and histamine (cAMP synthesis results from G protein activation following coupling to histamine receptor H2). In rectal epithelium from a healthy subject, Cl^- secretion leads to repolarization. In contrast, the response is blunted or inverted in epithelium with CFTR dysfunction. H: hour; min: minute; s: second.

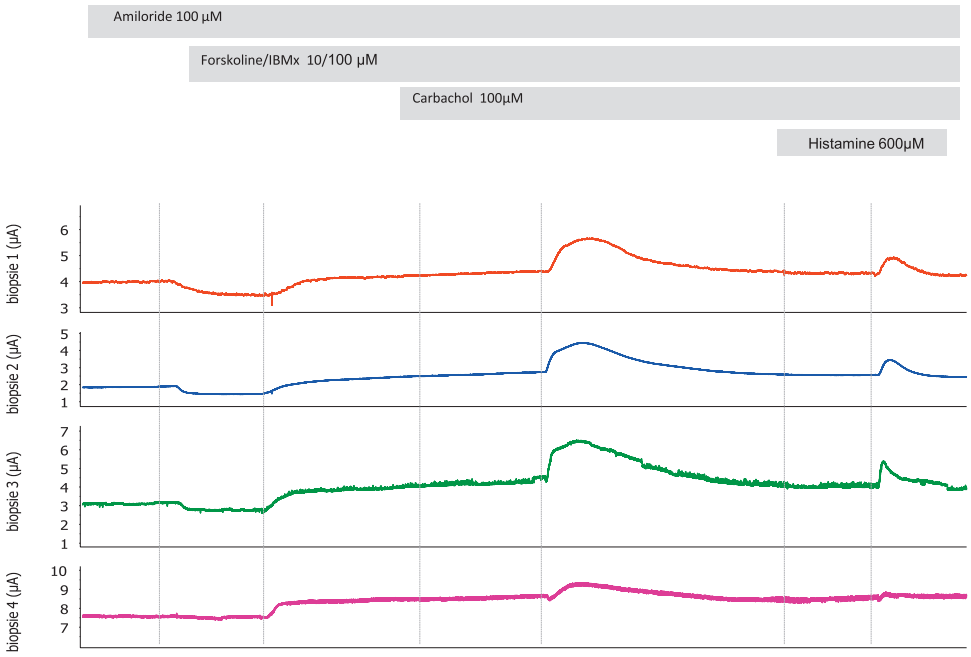

Figure 3. Short circuit current profiles on rectal biopsy in an infant with hypertrypsinemia and inconclusive diagnosis for CR at newborn screening. At 1 month of age, the infant presented with persistent hypertrypsinemia at 80 µg/L, intermediate sweat chloride values (40–48 mmol/L) and only one CF mutation among the most common ones (c.1000C > T [R334 W]). Short-circuit current patterns on four rectal biopsies show Cl^- secretion in response to various CFTR activators (carbachol, forskolin, histamine) but to a lesser extent than in the age-matched healthy subject. These results are indicative of residual CFTR activity. An extended *CFTR* gene analysis identified a second mutation with unknown pathogenic potential: c.3083T > G (M1028R). Upon epithelial functional analyses, it was concluded that the infant had a genotype associated with CFTR dysfunction at risk for developing CF. The newborn had yearly follow-up visits at the CF center and regular check-ups every 3 months with his pediatrician. He presented twice with persistent congestion and superinfection with *Staphylococcus aureus* requiring chest physiotherapy by the age of 3. CF: cystic fibrosis; CFTR: *cystic fibrosis transmembrane conductance regulator*.

should instead be approached as a continuum of CFTR dysfunction and thereby be defined in terms of risk of developing clinical symptoms of CF (probability of the risk depending on the severity of the dysfunction). The follow-up of this cohort of patients will be crucial to determining the threshold of residual activity that protects against developing a typical form of CF disease as well as the positive predictive value depending on the type of epithelium affected (nasal or intestinal epithelium, acinar sweat glands). This should warrant the creation of a specialized patient registry.

6. Ethical implications

Although genetic and functional techniques are growing increasingly sophisticated, diagnosis for CF remains significantly challenging to rule out or confirm. This diagnostic dilemma raises major ethical considerations in terms of potential therapeutic benefits (early detection of a yet asymptomatic infant) and harm (identifying as at risk of CF an infant who might never develop any clinical feature of the disease). It might then be tempting for healthcare providers to promptly initiate active care for these individuals as if they had CF. The primary objective for neonatal screening is to minimize the likelihood of missing the diagnosis of patients at significant risk for CF. Yet, members of this working group point out that labeling those children who, for the most part, will never develop clinical symptoms, has deleterious effects and might be a source of anxiety for families. The parent–child relationship might be affected and the child's perception of himself and his future might be undermined if treated as a sick person [40–42]. This might lead to an overinterpretation of minor health problems such as asthma or constipation. Moreover, genetic uncertainty is likely to impact professional decisions (military, pilot training) and the work environment, insurance and loans, life partners, and future pregnancies. Initiation of medical care may induce adverse consequences such as phobia related to needles, unjustified radiation, and risk of nosocomial infections. In addition, any subsequent pregnancy in parents of children with a CF diagnosis raises the difficult issues of prenatal screening and termination of pregnancy. Taking into account all these considerations, a newborn with an inconclusive diagnosis of CF should not systematically qualify for long-term illness. Each situation needs be discussed on a case-by-case basis (Recommendation #23). Also, genetic counseling must be offered to parents, ideally prior to any future

pregnancy, to thoroughly discuss the implications of diagnosis uncertainty (Recommendation #18).

Furthermore, labeling these children might be highly detrimental. Associating them with a defined clinical entity carries a potential for stigmatization, whereas most of them will never develop the disease [41,42]. The authors of the guidelines recommend follow-up visits at extended intervals in CF centers as the most consensual approach (Recommendations #24 and 25). Follow-up will be carried out with the primary care physician who will be informed of the uncertain diagnosis, the specificities of clinical management, and the need to promptly refer the patient to the CF center if symptoms suggestive of CF develop. This recommendation contrasts with the management advocated by the American guidelines, which suggest active surveillance, for instance, a monthly follow-up during the first few months and every 3 months thereafter, as for a patient with CF [43,44]. With this compromise that embraces uncertainty [45,46], the child can have access to care whenever specific clinical signs emerge prior to the development of complications, while patients who do no present any symptoms benefit from annual follow-up visits. This preferred option of watchful waiting for the management of these patients sends a message of vigilance to the family without categorizing the subjects as having a disease. This can only be done if a comprehensive explanation is provided to the parents with honesty and integrity about the risks of developing the disease along with diagnostic and therapeutic considerations. Based on the authors' personal experience, this strategy always creates a positive perception in parents from which they can build solid foundations for the future.

7. Initial assessment and care management initiation (Recommendations #15–22)

Clinical follow-up should be set up at the CF center in order to detect any symptom suggestive of the development of CF requiring the patient to be reassigned the diagnosis of CF (Table 5). Any undetermined diagnosis justifies an initial assessment to screen for conditions indicative of CF, particularly, presence of bacterial pathogens in bronchial secretions, exocrine pancreatic insufficiency (fecal elastase test), or hepatomegaly. The objective is to detect any clinical feature suggestive of CF for which an appropriate care should be initiated as early as possible. This type of evaluation must be performed in a specialized CF center at 3, 6, and 12 months and yearly thereafter for a clinical assessment and bronchial secretion bacteriological analysis (Recommendations #24 and #25).

There is no scientific evidence relevant to the initiation of therapy. Therefore, the working group concluded that preventive management was the best recommended approach, as it is in newborns with CF, considering the risk of developing lung disease as an important prognostic factor in disease progression. Close follow-up during the 1st year of life is recommended to identify classic forms of CF with early clinical manifestations. In this respect, newborns must receive an annual influenza vaccination in addition to their routine childhood immunization schedule (Recommendations #19 and 20). This statement is based on data from the general population showing that infectious complications and rates of hospitalization are higher in infants under 3 years of age at greater risk for respiratory illness [47]. Influenza vaccine can be administered to the newborn as early as 6 months of age [21]. Despite considerable heterogeneity among studies precluding any meta-analysis, chest physical therapy remains an integral part of the respiratory management in infants with a diagnosis of CF [21,48]. However, chest physiotherapy is not systematically indicated in newborns with an inconclusive CF diagnosis. Specific studies conducted in infants are scarce and in the absence of clinical pulmonary manifestations, physiotherapy does not constitute a postulate in the prevention of respiratory complications by means of airway clearance. Of course, the decision needs to be revised upon manifestation of clinical respiratory abnormalities (Recommendation #21). When sweat chloride is $\geq$ 30 mmol/L, sodium supplementation must be increased when there is a risk for excessive perspiration and dehydration, i.e., at high ambient temperatures or in subjects with fever (Recommendation #22). This supplementation should be adjusted based on the urinary Na/K ratio [19].

Table 5
Clinical features suggesting a diagnosis for CF.

Steatorrhea
Weight and height stagnation
Decreased fecal elastase
Constipation
Intestinal occlusion
Rectal prolapse
Recurrent bronchitis
Identification of bacterial pathogens in the sputum (*S. aureus*, *P. aeruginosa*, *S. maltophilia*)
Chronic cough
Severe or recurrent chronic obstructive pulmonary diseases
(Hyper)-secretory asthma
Hepatomegaly
Acute cardiac insufficiency
Dehydration

8. Clinical follow-up (Recommendations #25–29)

Follow-up visits at the CF center must segregate CF patients to prevent crossed infections as implemented for newborns with typical forms of CF [19] (Recommendation #26). Between two

scheduled visits, the appearance of symptoms indicative of CF, such as recurrent bronchial congestion, steatorrhea, or weight loss should lead to reevaluation at the CF center (Table 5).

This consensus is specific to the first 2 years of life but does not give any indication about the overall length of the follow-up. Beyond the scope of this consensus, members of the working group propose that a specialized follow-up should be maintained for the first 6 years of life. It is currently impossible to predict how long the concept of risk assessment for CF can be a substitute for symptomatic diagnosis. This notion is likely to be specific to each patient within a multifactorial context. Only the collection of clinical data provided by longitudinal follow-up of patients in conjunction with regular updating of genetic data and their interpretation will be instrumental in drawing a definitive conclusion. Bacteriological monitoring of bronchial secretions is currently the only paraclinical test to be consistently performed. In the above-mentioned Canadian study, *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were identified in 12 and 5% of newborns with inconclusive diagnosis, respectively [9], which is more frequent than in the general population (3% for *P. aeruginosa*, and 1–3.6% for *S. maltophilia*) [49]. Even though these bacteria can be occasionally found in healthy children's throats [50], and for a reason that remains unknown, they are more frequently found in patients with uncertain diagnosis, and their recurrence or persistence argues strongly in favor of CF. The vast majority of these infants present pancreatic sufficiency, and fecal elastase remains stable throughout the follow-up [12]. This marker therefore has a low specificity due to variability [9].

9. Conclusion

These recommendations taken overall are an attempt to standardize management practices in French CF centers for a meaningful, adapted, and ethical follow-up of infants with an inconclusive diagnosis for CF following newborn screening. Only a standardized follow-up of such cohorts of patients and longitudinal data collection will make it possible to establish prognostic factors and characterize relevant biomarkers to clearly discriminate forms that will ultimately be false positive from those that will progress towards a typical form of the disease or an associated single-organ disease. In the immediate future, these situations must be outlined clearly to parents and the pediatrician in charge must be unequivocally involved for optimal care of these patients.

More generally, this type of situation illustrates a striking shift in the diagnostic paradigm of genetic diseases. Whereas the diagnosis was previously made based on a dichotomous classification, it should be based on the assessment of the probability that the genotype affects the risk of progression, with symptoms that may appear secondarily, or even never develop, potentiated by environmental factors or other genetic factors.

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Disclosure of interest

The authors declare that they have no competing interest.

References

[1] Le dépistage néonatal systématique de la mucoviscidose en France : état des lieux et perspectives après 5 ans de fonctionnement. http://www.has-sante.fr/portail/./2009-04/rapport.mucoviscidose_en_fr; accessed 25 November 2016.

[2] Farrell PM, Rosenstein BJ, White TB, et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: cystic fibrosis foundation consensus report. J Pediatr 2008;153:S4–14.

[3] Massie J, Clements B, Australian paediatric respiratory group. Diagnosis of cystic fibrosis after newborn screening: the Australasian experience – 20 years and five million babies later: a consensus statement from the Australasian Paediatric Respiratory Group. Pediatr Pulmonol 2005;39:440–6.

[4] Sermet-Gaudelus I, Girodon E, Roussel D, et al. Measurement of nasal potential difference in young children with an equivocal sweat test following newborn screening for cystic fibrosis. Thorax 2010;65:539–44.

[5] De Boeck K, Wilschanski M, Castellani C, et al. Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006;61:627–35.

[6] Parad RB, Comeau AM. Diagnostic dilemmas resulting from the immunoreactive trypsinogen/DNA cystic fibrosis newborn screening algorithm. J Pediatr 2005;147(Suppl.):78–82.

[7] Ren CL, Desai H, Platt M, et al. Clinical outcomes in infants with cystic fibrosis transmembrane conductance regulator (CFTR) related metabolic syndrome. Pediatr Pulmonol 2011;46:1079–84.

[8] Ren CL, Fink AK, Petren K, et al. Outcomes of infants with indeterminate diagnosis detected by cystic fibrosis newborn screening. Pediatrics 2015;135:e1386–92.

[9] Ooi CY, Castellani C, Keenan K, et al. Inconclusive diagnosis of cystic fibrosis after newborn screening. Pediatrics 2015;135:e1377–2185.

[10] Massie RJ, Curnow L, Glazner J, et al. Lessons learned from 20 years of newborn screening for cystic fibrosis. Med J Aust 2012;196:67–70.

[11] Groves T, Robinson P, Wiley V, et al. Long-term outcomes of children with intermediate sweat chloride values in infancy. J Pediatr 2015;166. 1469-74.e1-3.

[12] Fitzgerald D, Van Asperen P, Henry R, et al. Delayed diagnosis of cystic fibrosis in children with a rare genotype(delta F508/R117H). J Paediatr Child Health 1995;31:168–71.

[13] Salinas DB, Sosnay PR, Azen C, et al. Benign outcome among positive cystic fibrosis newborn screen children with non-CF-causing variants. J Cyst Fibros 2015;14:714–9.

[14] Bombieri C, Claustres M, De Boeck K, et al. Recommendations for the classification of diseases as CFTR-related disorders. J Cyst Fibros 2011;10(Suppl. 2):86–102.

[15] Levy H, Levy H, Nugent M, et al. Refining the continuum of CFTR-associated disorders in the era of newborn screening. Clin Genet 2016;89:539–49.
[16] Kharrazi M, Yang J, Bishop T, et al. Newborn screening for cystic fibrosis in California. Pediatrics 2015;136:1062–72.
[17] Audrézet MP, Munck A, Scotet V, et al. Comprehensive CFTR gene analysis of the French cystic fibrosis screened newborn cohort: implications for diagnosis, genetic counseling, and mutation-specific therapy. Genet Med 2015;17:108–16.
[18] Munck A, Delmas A, Audrézet MP, et al. The French cystic fibrosis newborn screening: optimization of the programme by a centralized tracking process. Abstract 26. In: 39th European cystic fibrosis conference; 2016.
[19] Munck A, Mayell SJ, Winters V, et al. Cystic fibrosis screen positive, inconclusive diagnosis (CFSPID): a new designation and management recommendations for infants with an inconclusive diagnosis following newborn screening. J Cyst Fibros 2015;14:706–13.
[20] Sermet-Gaudelus I, Munck A, Rota M, et al. Recommandations françaises pour la réalisation et l'interprétation du test de la sueur dans le cadre du dépistage néonatal de la mucoviscidose. Arch Pediatr 2010;17:1349–58.
[21] Sermet-Gaudelus I, Couderc L, Vrielynck S, et al. Recommandations nationales pour la prise en charge du nourrisson dépisté atteint de mucoviscidose. Consensus de la fédération des centres de ressources et de compétences de la mucoviscidose. Arch Pediatr 2014;21:654–62.
[22] Ludwig B. Predicting the future: have you considered using the Delphi methodology? J Extension 1997;35:1–4, http://www.joe.org/joe/1997october/tt2.html.
[23] Castellani C, Cuppens H, Macek Jr M, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. J Cyst Fibros 2008;7:179–96.
[24] Highsmith WE, Burch LH, Zhou Z, et al. A novel mutation in the cystic fibrosis gene in patients with pulmonary disease but normal sweat chloride concentrations. N Engl J Med 1994;331:974–80.
[25] Lebecque P, Leal T, De Boeck C, et al. Mutations of the cystic fibrosis gene and intermediate sweat chloride levels in children. Am J Respir Crit Care Med 2002;165:757–61.
[26] Feldmann D, Couderc R, Audrezet MP, et al. CFTR genotypes in patients with normal or borderline sweat chloride levels. Hum Mutat 2003;22:340.
[27] Duguépéroux I, De Braekeleer M. The CFTR 3849+10kbC->T and 2789+5G->A alleles are associated with a mild CF phenotype. Eur Respir J 2005;25:468–73.
[28] Ren C. US diagnostic guidelines for cystic fibrosis. In: European cystic fibrosis society diagnostic network meeting; 2016.
[29] Sosnay PR, Siklosi KR, Van Goor F, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. Nat Genet 2013;45:1160–7.
[30] Bareil C, Thèze C, Béroud C, et al. UMD-CFTR: a database dedicated to CF and CFTR-related disorders. Hum Mutat 2010;31:1011–9.
[31] Prach L, Koepke R, Kharrazi M, et al. Novel CFTR variants identified during the first 3 years of cystic fibrosis newborn screening in California. J Mol Diagn 2013;15:710–22.
[32] Massie RJ, Poplawski N, Wilcken B, et al. Intron-8 polythymidine sequence in Australasian individuals with CF mutations R117H and R117C. Eur Respir J 2001;17:1195–2000.
[33] Thauvin-Robinet C, Munck A, Huet F, et al. The very low penetrance of cystic fibrosis for the R117H mutation: a reappraisal for genetic counselling and newborn screening. J Med Genet 2009;46:752–8.
[34] Jézéquel P, Dubourg C, Le Lannou D, et al. Molecular screening of the CFTR gene in men with anomalies of the vas deferens: identification of three novel mutations. Mol Hum Reprod 2000;6:1063–7.
[35] O'Sullivan BP, Zwerdling RG, Dorkin HL, et al. Early pulmonary manifestation of cystic fibrosis in children with the DeltaF508/R117H-7T genotype. Pediatrics 2006;118:1260–5.
[36] Vermeulen F, Le Camus C, Davies JC, et al. Variability of sweat chloride concentration in subjects with cystic fibrosis and G551D mutations. J Cyst Fibros 2016. pii: S1569-993.
[37] De Boeck K, Kent L, Davies J, et al. CFTR biomarkers: time for promotion to surrogate end-point. Eur Respir J 2013;41:203–16.
[38] Derichs N, Sanz J, Von Kanel T, et al. Intestinal current measurement for diagnostic classification of patients with questionable cystic fibrosis: validation and reference data. Thorax 2010;65:594–9.
[39] Gonska T, Ip W, Turner D, et al. Sweat gland bioelectrics differ in cystic fibrosis: a new concept for potential diagnosis and assessment of CFTR function in cystic fibrosis. Thorax 2009;64:932–8.
[40] Grob R. Is my sick child healthy? Is my healthy child sick? Changing parental experiences of cystic fibrosis in the age of expanded newborn screening. Soc Sci Med 2008;67:1056–64.
[41] Massie J, Gillam L. Uncertain diagnosis after newborn screening for cystic fibrosis: an ethics-based approach to a clinical dilemma. Pediatr Pulmonol 2014;49:1–7.
[42] Chmiel JF, Drumm ML, Konstan MW, et al. Pitfall in the use of genotype analysis as the sole diagnostic criterion for cystic fibrosis. Pediatrics 1999;103:823–6.
[43] Cystic fibrosis foundation, Borowitz D, Parad RB, et al. Cystic fibrosis foundation practice guidelines for the management of infants with cystic fibrosis transmembrane conductance regulator-related metabolic syndrome during the first 2 years of life and beyond. J Pediatr 2009;155(Suppl.):106–16.
[44] Ren CL, Parad R, Borowitz D. Indeterminate cystic fibrosis newborn screening results. Pediatr Pulmonol 2015;50:209–10.
[45] The power of embracing uncertainty; author Jamie Holmes explains the value of not knowing; scientific magazine, 13 octobre 2015. http://www.scientificamerican.com/./the-power-of-embracing-uncertainty.
[46] Heisenberg W. Physics and philosophy. The revolution in modern science. New York: Harper & Row Publishers, Inc; 1962.
[47] Patria MF, Tagliabue C, Longhi B, et al. Influenza vaccination in children at high risk of respiratory disease. Ther Adv Vaccines 2013;1:21–31.
[48] Hardy KA, Wolfson MR, Schidlow DV, et al. Mechanics and energetics of breathing in newly diagnosed infants with cystic fibrosis: effect of combined bronchodilator and chest physical therapy. Pediatr Pulmonol 1989;6:103–8.
[49] Rosenfeld M, Bernardo-Ocampo C, Emerson J, et al. Prevalence of cystic fibrosis pathogens in the oropharynx of healthy children and implications for cystic fibrosis care. J Cyst Fibros 2012;11:456–7.
[50] Carlson D, McKeen E, Mitchell M, et al. Oropharyngeal flora in healthy infants: observations and implications for cystic fibrosis care. Pediatr Pulmonol 2009;44:497–502.