Cystic Fibrosis Newborn Screening: Distribution of Blood Immunoreactive Trypsinogen Concentrations in Hypertrypsinemic Neonates

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Abstract The IRT screening test for the use in diagnosing newborns with CF has a high sensitivity but is not very specific resulting in a large number of screened positive infants found to have a normal sweat test. The aim of this study was to analyze the differences in b-IRT levels among different groups of newborns positive to NBS.

Population data included all b-IRT positive (>99th centile) neonates born in Lombardia from 2000 to 2007. The hypertrypsinemic newborns were divided into four groups, according to CF status (noncarrier, carrier, CFTR-RD, CF).

Among a total of 717,172 newborns screened within the study period, 7,354 newborns were found positive to

NBS and were included in the study. An overall statistically significant difference in b-IRT levels was found among the four groups ($p < 0.001$), while b-IRT values did not differ between noncarriers and carriers. b-IRT levels had a low predictive accuracy in correctly identifying the four different groups (c-index: 0.60), but the accuracy was high in discriminating between classic CF and carrier or noncarrier status in neonates positive to NBS. The IRT level on the initial blood specimen obtained at birth differs based on the CF genotype, although a wide range of individual variation may occur.

Abbreviations

b-IRT	Birth immunoreactive trypsinogen
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CFTR-RD	CFTR related disorder
MLPA	Multiplex ligation probe amplification assay
NBS	Newborn screening
OLA	Oligonucleotide ligation assay
PAP	Pancreatitis-associated protein

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Introduction

Cystic fibrosis (CF; MIM #219700) is the most frequent severe autosomal recessive disorder in the European population. In the Italian population, it affects about 1 in 3,100 births and approximately 1 in 30 individuals are heterozygous (Padoan et al. 2001). In other European countries, the incidence can be as high as 1 in every 2,000 births with Caucasians affected more frequently than

other ethnic groups (Bobadilla et al. 2002). Mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*/ABCC7; MIM #602421) induce a wide spectrum of clinical phenotypes from classic CF characterized by pancreatic insufficiency and positive sweat chloride values, to milder forms of the disease with pancreatic sufficiency, to *CFTR* related disorders (*CFTR*-RDs), where a diagnosis of CF cannot be established because the individual does not meet standard diagnostic criteria (Farrell et al. 2008; Castellani et al. 2008).

The disease is characterized by one or more of several features varying in severity, including a progressive decline of pulmonary function secondary to chronic lung infections, exocrine pancreatic insufficiency leading to malnutrition and growth impairment, liver disease, and decreased reabsorption of chloride from sweat. Pathological, functional, and imaging studies all support the presence in early life of significant abnormalities in the small airways in individuals who have CF (Tiddens et al. 2010).

Newborn infants with CF have raised levels of immunoreactive trypsinogen (IRT) in serum. Measurement of IRT in the first week of life has enabled CF to be included into existing newborn screening (NBS) blood spot protocols. A raised IRT in the first week of life (b-IRT) is a sensitive but not specific test for CF and a second sample within 6 weeks of life is used in some newborn screening protocols to avoid excessive numbers of sweat test (Castellani et al. 2009; Southern et al. 2007).

Following identification of the *CFTR* gene, DNA analysis for common CF-associated mutations has been increasingly used as a second tier test (Castellani et al. 2009).

However, the sensitivity of the IRT/DNA/IRT protocol is dependent on the frequency of common *CFTR* mutations in the population and on b-IRT cut-off level.

Advantages of DNA testing are that it can be undertaken on the original blood spot sample and can lead to the identification of two CF-causing mutations (Spence et al. 1993; Gregg et al. 1993; Larsen et al. 1994). A potential disadvantage of DNA analysis is carrier recognition (Parsons et al. 2003; Comeau et al. 2004).

Increased sweat chloride concentration remains an important diagnostic marker of CF, particularly when preliminary DNA analysis is negative or not fully informative, and sweat testing is an integral component of CF newborn screening (Parad et al. 2005).

The aim of this study was to analyze the differences in b-IRT levels among different groups of newborns positive to NBS, including classic CF, *CFTR*-RD, carriers and noncarriers to evaluate the predictive value of b-IRT.

Patients and Methods

Study Population

This study relies on the cohort of newborns screened for CF between 2000 and 2007 in Lombardia, a region located in North West Italy, with 9.7 million inhabitants and 90,000 average annual births. Although most of the inhabitants are of Caucasian origin, its population is relatively heterogeneous. Starting from the 1960s, a strong migration from Southern to Northern Italy occurred involving also the Lombardia region, and starting from the 1980s an increasing number of immigrants from other nations (Eastern Europe, Africa, Far East) also settled in this area. It was reported that 21.5% of deliveries in Lombardia was from non-Italian women in 2006 (Ministry of Welfare).

Ethnic data were not collected for NBS, and we did not apply a different protocol for different ethnic groups.

The study population data included all b-IRT positive (>70 ng/ml, >99th centile) neonates born in Lombardia over the above-mentioned 8-year period. Those with positive b-IRT were included in the analysis. All the newborn with birth weight less than 2,000 g were excluded to avoid false-positive results due to prematurity. In fact, IRT levels can be elevated in premature infants and infants cared for in Neonatal Intensive Care settings. In addition, all the subjects with b-IRT values above 500 ng/ml were considered outlier. According to our NBS protocol, all the b-IRT positive babies are followed up both for molecular and for biochemical analyses. Although no informed consent is required for newborn screening in Lombardia due to the fact that it is mandatory by the national law for the prevention of handicap, preventive information and opt-out option are offered to the parents.

Methods

b-IRT measurement was undertaken using time-resolved fluoroimmunoassay (PerkinElmer Autodelphia™ assay) in neonatal dried blood spots collected at 48th to 120th hour from birth. A second sample is usually obtained between the 20th and the 30th day from infants whose first result was higher than the cut-off value (99th centile). However, this second sample was not taken into account in this study because of the differences in the day of withdrawal and the fact that the predictive value of IRT dramatically decreases after the sixth week. All the neonates are systematically controlled for second sample IRT.

The cut-off for b-IRT was set at the centile of 99%, that is at 70 ng/ml. The laboratory employed a policy of internal quality control and participated in European external quality assessment (IRTIQAS, France).

Blood spots were used as a source of DNA for the polymerase chain reaction (PCR), followed by OLA. The number of CFTR mutations examined is 34 (OLA); this panel, used from 1998 to 2007, covers a median of 72% of CFTR mutations in the Italian population.

According to our NBS protocol based on two-step b-IRT evaluation, sweat test was performed on all neonates with one or two CF mutations and in all neonates with high second b-IRT sample. Sweat test was performed by iontophoresis of pilocarpine nitrate (Gibson and Cooke method) and sweat chloride was measured by coulometric analysis.

Sweat chloride values were considered positive if >60 mEq/l (classic CF); CFTR-RDs were characterized by sweat chloride values in the borderline range (30–60 mEq/l), carrier subjects by negative sweat chloride values (<30 mEq/l). The minimum amount of sweat considered reliable was 75 mg. All the positive sweat tests were repeated after 1 week from the first testing and all the borderline sweat tests were regularly repeated (once a year).

All newborns with positive sweat chloride values were sent to the CF center and, if one or no mutation in CFTR gene had been identified by NBS, they were extensively analyzed by sequencing of CFTR gene and multiplex ligation probe amplification assay (MLPA) (Paracchini et al. 2008).

The molecular genetics laboratory was already participating to the UK NEQAS for Molecular Genetics (CF blood spot).

Mutation Nomenclature

Mutation names are the legacy names according to the international consortium mutation database (www.genet.sickkids.on.ca/cftr), with the A of the ATG translation start codon numbered as +133 (cDNA sequence: GenBank NM_000492.3). Mutation names according to Human Genome Variation Society (HGVS) nomenclature are indicated in brackets at their first occurrence.

Statistical Analysis

Since b-IRT values were not normally distributed, differences in b-IRT values among the four CF groups (non-carriers, carriers, CFTR-RD, classic CF) were assessed by the nonparametric Kruskal–Wallis Test. Contrasts were then performed by comparing the groups two by two. Correction for multiple comparisons was made by Holm method (Holm 1979).

Predictive accuracy of b-IRT values was evaluated with the concordance statistics (c-index). A value of 1 denotes perfect concordance, while a value of 0.5 is no better than chance.

p -values <0.05 were considered statistically significant. The analyses were performed using SAS software, version 8.2.

Results

Among a total of 717,172 newborns screened within the study period, 7,354 newborns were found positive to NBS and were included in the study. This indicates a recall rate of 1.02%, corresponding to the established 99th centile cut-off.

The baseline characteristics of the population positive to NBS are presented in Table 1. 45.1% of the newborn infants were females and 54.9% were males. The mean birth weight (Standard Deviation) was 3277 g (461 g). Five-hundred-and-twenty-three infants were excluded because of birth weight lower than 2,000 g. The remaining hypertrypsinemic newborns ($n = 7,354$) were divided into four groups, according to the CF status from 0 to 3, respectively: CF 0 (normal sweat test and no CFTR mutations – non carrier), CF 1 (normal sweat test and one CF mutation – carrier), CF 2 (borderline sweat test and 1 CF causing mutation or 2 CFTR variants), CF 3 (positive sweat test and 2 CF causing mutations – CF).

In the study population, 36 neonates were reporting having meconium ileus at birth. Sweat test was performed

Table 1 Baseline characteristics of study population

Variable	CF = 0 No CFTR mutation	CF = 1 1 CFTR mutation, negative ST	CF = 2 1 or 2 CFTR mutation, borderline ST	CF = 3 2 CFTR mutations, positive ST	All ($N = 7,354$)
Birth [$N(\%)$]	6,708	480	46	120	7,354
Gender [$N(\%)$]					
M	3,039 (91%)	210 (6%)	20 (1%)	60 (2%)	3,329
F	3,669 (91%)	270 (7%)	26 (1%)	60 (1%)	4,025
Birth weight (mean $\pm$ SD)	3,277 $\pm$ 461	3,319 $\pm$ 464	3,337 $\pm$ 475	3,097 $\pm$ 446	3,277 $\pm$ 461

ST sweat test

Table 2 b-IRT (ng/ml) values according to CF status in 7,354 newborn infants (Lombardia region, Italy, 2000–2007)

CF group	Median IRT ng/ml (range)	Comparison with CF = 0	Comparison with CF = 1	Comparison with CF = 2
0	80 (70–494)			
1	80 (70–290)	0.19		
2	95 (70–212)	<0.0001	<0.0001	
3	159 (70–371)	<0.0001	<0.0001	<0.0001

$p < 0.0001$

Fig. 1 Distribution of b-IRT by CF group. The x-axis reports categories of b-IRT values, the y-axis reports the percentage of subjects in each category. Black arrows represent the two peaks of the CFTR-RD distribution

on these neonates independently from IRT. Among them, 10 had negative b-IRT; of these, four neonates were CF affected, and six had negative sweat chloride value. Among the 26 neonates with meconium ileus and positive b-IRT, 24 were CF-affected and two had negative sweat chloride value. No specific follow-up was applied for negative sweat tests. We identified 28 CF subjects with meconium ileus on a total of 120 CF (23.3%). Considering the fact that our center missed some cases of meconium ileus because it was not notified to us, our results are supported by literature data showing that meconium ileus occurs in 16–20% of CF newborns (Dorfman et al. 2009).

One-hundred-and-sixty-six CF neonates were identified in the population, resulting in an incidence of 1:4,320, with an expected CF carrier incidence of 1:33.

In the population of hypertrypsinemic newborns, 480 carriers were identified, resulting in an incidence of 1:15.3.

b-IRT values among the four groups are presented in Table 2. The median (range) b-IRT was 80 ng/ml (70–494) for the non carrier group (CF 0); 80 ng/ml (70–290) for the carrier group (CF 1); 95 ng/ml (70–212) for the CFTR-RD

group (CF 2) and 159 ng/ml (70–371) for the classic CF group (CF 3).

An overall statistically significant difference was found among the four groups ($p < 0.0001$). By comparing groups two by two, we found that b-IRT values did not differ between CF 0 and CF 1, while the difference in b-IRT was statistically significant among all the other comparisons, even after correction for multiple testing ($p < 0.0001$) (Table 2). As shown in Fig. 1, the noncarrier and the carrier groups showed similar b-IRT values, while CF and CFTR-RD subjects had higher b-IRT values. However, the overlapping area among the four groups is wide, giving a low predictive accuracy of b-IRT in correctly identifying patient group (c-index: 0.60).

Table 3 shows the allele frequencies of the subjects with CFTR-RD according to different b-IRT values range. Twenty-nine subjects had b-IRT values between 70 and 90 ng/ml, 17 subjects between 91 and 130 ng/ml and 8 subjects >130 ng/ml. In the cohort of 54 CFTR-RD patients, we identified mutations/variants in 66 alleles using a commercial kit (66/108; 61%); in 33 alleles, mutations/variants were identified after sequencing of the whole gene

Table 3 Allele frequencies in CFTR-RD infants according to different b-IRT values (cut-off value 99th centile).

CFTR-RDb-IRT 70–90 ng/ml29 subjects		CFTR-RDb-IRT 90–130 ng/ml17 subjects		CFTR-RDb-IRT > 130 ng/ml8 subjects	
	Mutated alleles/ tot alleles (%)		Mutated alleles/ tot alleles (%)		Mutated alleles/ tot alleles (%)
F508del	13/58 (22.41)	F508del	6/34 (17.65)	F508del	3/16 (18.75)
5T-TG12	11/58 (18.97)	5T-TG12	3/34 (8.82)		
		D1152H	3/34 (8.82)	D1152H	5/16 (31.25)
L997F	4/58 (6.90)				
R117H 7/7	3/58 (5.17)	R117H 7/7	3/34 (8.82)		
UN	2/58 (3.45)	UN	6/34 (17.65)	UN	2/16 (12.50)
		G542X	3/34 (8.82)		
		P5L	2/34 (5.88)		
				1717-1 G>A	2/16 (12.50)
N1303K,G542X,R1066H, G576A, 2789 + 5 G>A	2/58 (3.45)	2183AA>G, G1349D, N1303K, R347P, S1455X, H199R, D614G, R334W	1/34 (2.94)	2183AA>G, G1244E, R117H 7/7, R1158X	1/16 (6.25)

UN unknown

F508del; p.Phe508del; c.1521_1523delCTT

5T-TG12; c.1210-34TG[12]T[5]

L997F; p.Leu997Phe; c.2991 G>C

R117H; p.Arg117His; c.350 G>A

N1303K; p.Asn1303Lys; c.3909 C>G

G542X; p.Gly542X; c.1624 G>T

R1066H; p.Arg1066His; c.3197 G>A

G576A; p.Gly576Ala; c.1727 G>C

2789+5 G>A; c.2657 + 5 G>A

D1152H; p.Asp1152His; c.3454 G>C

P5L; p.Pro5Leu; c.14 C>T

2183AA->G; p.Lys684SerfsX38; c.2051_2052delAAinsG

G1349D; p.Gly1349Asp; c.4046 G>A

R347P; p.Arg347Pro; c.1040 G>C

S1455X; p.Ser1455X; c.4364 C>G

H199R; p.His199Arg; c.596A>G

D614G; p.Asp614Gly; c.1841A>G

R334W; p.Arg334Trp; c.1000 C>T

1717-1 G- > A; c.1585-1 G>A

G1244E; p.Gly1244Glu; c.3731 G>A

R1158X; p.Arg1158X; c.3472 C>T

(31%); and 9 alleles remained unidentified (8%). Eighteen patients presented two mutations/variants after DNA analysis by commercial kit. None of the CFTR-RD subjects were of non-Caucasian origin.

Discussion

b-IRT is still the unsurpassed biochemical marker for NBS for CF. Even if the specificity of an initial positive b-IRT

test is not elevated, and therefore the positive predictive value of b-IRT between the 48th and the 120th hour from birth is low (3–10%) (Travert 1988), the sensitivity is considered to be high (Wilcken 1993).

The integration of IRT measurement, molecular strategy, and correct execution of sweat test is presently considered the only acceptable strategy (high sensitivity and specificity) for NBS for CF.

Since the introduction in 1983 of evaluation of IRT in dried blood spots collected on Guthrie cards (Crossley

et al. 1979), successive pilot studies on NBS have been implemented in the Lombardia region. In the first period (1983–1992), the screening program was an IRT/IRT two-step strategy. Subsequently, an IRT/DNA/IRT strategy was adopted starting from 1993 to September 1998, with testing for the F508del allele in all the hypertrypsinemic newborn infants. This strategy improved the sensitivity (detection rate) of the program (Padoan et al. 1999). After September 1998, the DNA analysis was implemented by using an oligonucleotide ligation assay (OLA), which allowed the identification of the 34 most common mutations in the CFTR gene. The second b-IRT (recall test at 25th to 30th day) is maintained because of the high genetic heterogeneity within the regional newborn population (Italian and non-Italian).

The established IRT cut-off in our protocol is the 99th centile. Based on these results, we are now evaluating the possibility to use a strategy based on progressive IRT cut-off values.

Using this approach, we found an incidence of CF of 1:4320, with an expected incidence of CF carriers of 1:33. In contrast, in our population of hypertrypsinemic newborns, the incidence of CF carrier is higher (1:15), due to the fact that we selected neonates with hypertrypsinemia. Our data are not in disagreement with what reported by Castellani et al. (2005) and Lecoq et al. (1999).

In this study, a wide population of newborns positive to NBS was divided into four groups, according to CF status: noncarrier, carrier, CFTR-RD, classic CF. Although an overall statistically significant difference in b-IRT values in dried blood spot was found among the four groups, we found that b-IRT has a low predictive accuracy in correctly identifying the four different groups (c-index: 0.60). On the other hand, the accuracy of b-IRT is high in discriminating between classic CF or CFTR-RD and carrier or noncarrier neonates positive to NBS.

Interestingly, we found no difference in b-IRT levels between noncarrier and carrier hypertrypsinemic newborns, and the molecular strategy proved to be necessary for this propose.

As shown in Fig. 1, the distribution of b-IRT values in CFTR-RD group (CF 2) shows two peaks (black arrows), one overlapping the distribution of noncarrier (CF 0) and carrier (CF 1) groups, the other placed between the first peak and the peak corresponding to classic CF (CF 3).

With regard to allele frequencies according to different b-IRT range values in subjects with CFTR-RD, the 5T-TG12 allele (c.1210-34TG[12]T[5]) presents high frequency (18.9%) in subjects with b-IRT values between 70 and 90 ng/ml, while its frequency decreases in subjects with b-IRT between 100 and 130 ng/ml (10.7%) and it is

not present in the group with b-IRT values higher than 130 ng/ml (Table 3).

These data confirm that most T5 CFTR alleles will in general result in a CFTR-related disorder, such as Congenital Bilateral Absence of the Vas Deferens (CBAVD) or chronic idiopathic pancreatitis (Castellani et al. 2008) and not classic CF.

On the other hand, D1152H (c.3454 G>C) frequency is above 30% in the group of subjects with b-IRT >130 ng/ml, 8.8% in the group with b-IRT 90–130 ng/ml, while this variant is not present in the group with b-IRT 70–90 ng/ml. In our population, subjects compound heterozygous for D1152H and a CF causing mutation, show b-IRT levels intermediate between non-CF (CF 0, CF 1) and CF subjects (CF 3). According to the literature (Mussaffi et al. 2006), clinical diagnosis may be difficult for these subjects because they often do not meet current diagnostic criteria, but they may develop CF disease in adulthood. Therefore, these patients may benefit from a regular clinical follow-up to detect earlier signs of the disease.

L997F (c.2991 G>C) compound heterozygous subjects presented b-IRT levels comparable to noncarriers or carriers. These data are supported by recent data (Derichs et al. 2005) providing evidence that L997F is not a CF disease-causing mutation, but rather a CFTR gene mutation without obligatory association with CFTR-related disease (Padoan et al. 2002) or alternatively a functional genetic polymorphism.

CFTR-related newborns are usually healthy in the first years of life, presenting little or no sign of disease, the long-term consequences may be variable, and some of them may over time develop features associated with CF.

Conclusion

These results suggest that elevated neonatal b-IRT concentrations are connected with sweat chloride level, and possibly influenced by CFTR genotype. CF neonates with CFTR-RD mutations have less increased b-IRT than neonates with mutations causing a severe clinical phenotype. Despite neonatal b-IRT concentrations may be considered as one of the phenotypic differences associated with different genotypes, a wide range of individual variation may occur. Further studies concerning this issue are needed.

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Synopsis

The IRT level on the initial blood specimen obtained in neonates at birth differs based on the CF genotype, although a wide range of individual variation may occur.

References

- Bobadilla JL, Macek M Jr, Fine JP, Farrell PM (2002) Cystic fibrosis: a worldwide analysis of CFTR mutations—correlation with incidence data and application to screening. *Hum Mutat* 19:575–606
- Castellani C, Picci L, Scarpa M, Dechecchi MC, Zanolla L, Assael BM, Zacchello F (2005) Cystic fibrosis carriers have higher neonatal immunoreactive trypsinogen values than non-carriers. *Am J Med Genet A* 135:142–144
- Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P et al (2008) Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 7:179–196
- Castellani C, Southern KW, Brownlee K, Dankert Roelse J, Duff A, Farrell M et al (2009) European best practice guidelines for cystic fibrosis neonatal screening. *J Cyst Fibros* 8:153–173
- Comeau AM, Parad RB, Dorkin HL, Dovey M, Gerstle R, Haver K et al (2004) Population-based newborn screening for genetic disorders when multiple mutation DNA testing is incorporated: a cystic fibrosis newborn screening model demonstrating increased sensitivity but more carrier detections. *Pediatrics* 113:1573–1581
- Crossley JR, Elliott RB, Smith PA (1979) Dried-blood spot screening for cystic fibrosis in the newborn. *Lancet* 1:472–474
- Derichs N, Schuster A, Grund I, Ernsting A, Stolpe C, Körtge-Jung S et al (2005) Homozygosity for L997F in a child with normal clinical and chloride secretory phenotype provides evidence that this cystic fibrosis transmembrane conductance regulator mutation does not cause cystic fibrosis. *Clin Genet* 67:529–531
- Dorfman R, Li W, Sun L, Lin F, Wang Y, Sandford A et al (2009) Modifier gene study of meconium ileus in cystic fibrosis: statistical considerations and gene mapping results. *Hum Genet* 126:763–778
- Farrell PM, Rosenstein BJ, White TB, Accurso FJ, Castellani C, Cutting GR et al (2008) Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation Consensus Report. *J Pediatr* 153:S4–S14
- Gregg RG, Wilfond BS, Farrell PM, Laxova A, Hassemer D, Mischler EH (1993) Application of DNA analysis in a population-screening program for neonatal diagnosis of cystic fibrosis (CF): comparison of screening protocols. *Am J Hum Genet* 52:616–626
- Holm S (1979) A simple sequentially rejective multiple test procedure. *Scand J Stat* 6:65–70
- Larsen J, Campbell S, Faragher EB, Gotz M, Eichler I, Waldherr S et al (1994) Cystic fibrosis screening in neonates—measurement of immunoreactive trypsin and direct genotype analysis for delta F508 mutation. *Eur J Pediatr* 153:569–573
- Lecoq I, Brouard J, Laroche D, Férec C, Travert G (1999) Blood immunoreactive trypsinogen concentrations are genetically determined in healthy and cystic fibrosis newborns. *Acta Paediatr* 88:338–341
- Mussaffi H, Prais D, Mei-Zahav M, Blau H (2006) Cystic fibrosis mutations with widely variable phenotype: the D1152H Example. *Pediatr Pulmonol* 41:250–254
- Padoan R, Seia M, Giunta A, et al. (1999) Audit of neonatal screening program for cystic fibrosis: 13th NACFC, Seattle. *Pediatr Pulmonol* (Suppl 19):213
- Padoan R, Pardo F, Giglio L, Bossi A (2001) Assemblea dei Direttori dei Centri. Regional differences in the incidence of cystic fibrosis in Italy. *Ital J Pediatr* 27:876–886
- Padoan R, Bassotti A, Seia M, Corbetta C (2002) Negative sweat test in hypertrypsinaemic infants with cystic fibrosis carrying rare CFTR mutations. *Eur J Pediatr* 161:212–215
- Paracchini V, Seia M, Coviello D, Porcaro L, Costantino L, Capasso P et al (2008) Molecular and clinical features associated with CFTR gene rearrangements in Italian population: identification of a new duplication and recurrent deletions. *Clin Genet* 73:346–352
- Parad RB, Comeau AM, Dorkin HL, Dovey M, Gerstle R, Martin T et al (2005) Sweat testing infants detected by cystic fibrosis newborn screening. *J Pediatr* 147:S69–S72
- Parsons EP, Clarke AJ, Bradley DM (2003) Implications of carrier identification in newborn screening for cystic fibrosis. *Arch Dis Child Fetal Neonatal Ed* 88:F467–F471
- Southern KW, Munck A, Pollitt R, Travert G, Zanolla L, Dankert-Roelse J (2007) ECFS CF Neonatal Screening Working Group. A survey of newborn screening for cystic fibrosis in Europe. *J Cyst Fibros* 6:57–65
- Spence WC, Paulus-Thomas J, Orenstein DM, Naylor EW (1993) Neonatal screening for cystic fibrosis: addition of molecular diagnostics to increase specificity. *Biochem Med Metab Biol* 49:200–211
- Tiddens HA, Donaldson SH, Rosenfeld M, Paré PD (2010) Cystic fibrosis lung disease starts in the small airways: can we treat it more effectively? *Pediatr Pulmonol* 45:107–117
- Travert G (1988) Analysis of worldwide experience of neonatal screening for cystic fibrosis by measurement of blood immunoreactive trypsin. In: Travert G (ed) *Mucoviscidose: dépistage neonatal et prise en charge precoce*. CHRU de Caen, Caen, pp 1–23
- Wilcken B (1993) Newborn screening for cystic fibrosis: its evolution and a review of the current situation. *Screening* 2:43–62