

# Cystic fibrosis and neonatal screening

## Fibrose cística e a triagem neonatal

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### Abstract

*The clinical and diagnostic aspects of cystic fibrosis have been extensively reviewed, with an emphasis on neonatal screening. This systematic literature review involved a search for relevant contributions in the PubMed and SciELO databases. The first references to cystic fibrosis date to the Middle Ages. Cystic fibrosis is the most frequent autosomal recessive hereditary disease among Caucasians (1:2,000 to 3,500). More than 1,000 mutations lead to the disease, the most common being  $\Delta F508$ , with 70% prevalence among Canadian, Northern European, and American Caucasians and 23 to 55% prevalence among Brazilians. The basic defect is in chloride ion secretion. Cystic fibrosis screening has long been controversial, and after almost three decades, there are few nationwide programs (most are regional or local). However, the U.S. Centers for Disease Control and Prevention (CDC) has concluded that screening for cystic fibrosis is justified. The lack of a specific screening test and the ethnic heterogeneity of the Brazilian population pose challenges for neonatal screening.*

*Cystic Fibrosis; Neonatal Screening; Review*

### Historical background

According to a centuries-old European legend, children that tasted salty when kissed on the forehead were considered “enchanted” or “bewitched” and would soon die. The first references to possible carriers of the disease appeared in the literature in the 16<sup>th</sup> century, as autopsy reports. The first records of an autopsy performed on a “cursed” 11-year-old girl who presumably died of cystic fibrosis was performed in 1595 by Pieter Pauw, professor of Botany and Anatomy in Leiden, Netherlands, and included the first medical description of the related pancreatic lesions <sup>1</sup>.

In 1606, Alonso y de los Ruyzes de Fonteca, professor of medicine in Henares, Spain, reported that one’s fingers tasted salty after rubbing the forehead of these supposedly bewitched children <sup>1</sup>.

Nearly three centuries later, in 1905, Landsteiner described meconium ileus, relating it to exocrine pancreatic insufficiency. Three decades later, Fanconi (1936) referred to the disease as cystic fibromatosis with bronchiectases and recognized it as a disease entity independent of celiac disease <sup>1</sup>. At the same time, Dorothy Andersen, in 1938, described the disease in minute detail, with its clinical, anatomopathological, and epidemiological characteristics, referring to it as “cystic fibrosis of the pancreas” <sup>1</sup>.

In mid-1945, Farber believed that the cause of the disease was a generalized “state of thickening of secretions”, thus coining the term mu-

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coviscidosis<sup>2</sup>. Andersen and Hodges showed for the first time in 1946 that the disease had a genetic origin and resulted from an autosomal recessive mutation<sup>1</sup>.

The year 1948 witnessed a strong heat wave in New York, and a high incidence of “prostration” was observed in patients treated at Columbia Hospital. Based on careful observation by a young pediatrician, Paul di Sant’Agnese, children with prostration presented a diagnosis consistent with cystic fibrosis. He further observed that these children had abnormal sweat, with high concentrations of sodium and chloride<sup>3</sup>.

The sweat chloride test was standardized by Gibson & Cooke<sup>4</sup> in 1959 and is still considered the gold standard for diagnosing the disease.

In 1979, Crossley<sup>5</sup> demonstrated the increase in plasma trypsinogen (IRT, immunoreactive trypsin), thus providing a practical method that is still used for neonatal screening of cystic fibrosis.

In the early 1980s, Paul Quinton<sup>6</sup> and team began to unveil the basic defect in chloride ion secretion. Soon afterwards, researchers led by Francis Collins at the University of Michigan and Lap-Chee Tsui and Jack Riordan at the Hospital for Sick Children in Toronto located the gene for cystic fibrosis in the long arm of chromosome 7, which was cloned and sequenced<sup>7,8,9</sup>. This discovery allowed more accurate diagnosis of the disease and better understanding of the clinical disorders it causes and the impact of the deficiency on its protein product, the cystic fibrosis transmembrane conductance regulator (CFTR).

## Incidence

Cystic fibrosis incidence varies according to ethnic group, ranging from one in 2,000 to one in 3,500 Caucasians born in Europe, the United States, and Canada, and with the lowest incidence among Hispanics (1:8,400 births), African-Americans (1:15,000 births), and the Asian population of Hawaii (1:89,000 births)<sup>10</sup>.

Population studies in Brazil in the States of Paraná, Santa Catarina, Minas Gerais, and São Paulo report incidence rates of 1:9,520, 1:8,779, 1:9,115, and 1:8,403, respectively<sup>11,12,13,14</sup>.

The natural history of the disease has undergone major changes over the years. The first clinical descriptions considered it “fatal” in the first year of life. In the 1960s, mean survival was 10 years, increasing to 16 and 18 years, respectively, in the 70s and 80s; mean survival for patients followed by specialized reference centers is now 30 years, with many patients reaching 40 years of age<sup>2</sup>.

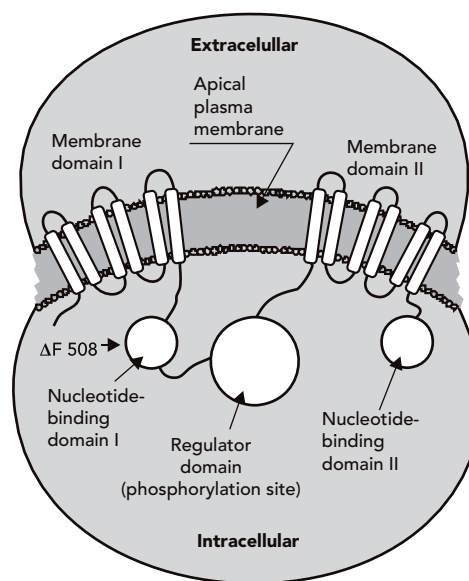
## The genetics of cystic fibrosis and the role of CFTR

Cystic fibrosis is an autosomal recessive hereditary disease associated with a gene located in the long arm of chromosome 7, locus q31. The gene consists of 250 Kb, presents 27 coding sequences (exons), and transcribes a long ribonucleic acid (mRNA) with 6.5 Kb and an ion transmembrane transport regulatory protein, or chloride channel regulator known as the cystic fibrosis transmembrane conductance regulator (CFTR)<sup>15</sup>.

Consistent with the characteristics of this gene family, the CFTR structure is comprised of five domains: two membrane-spanning domains (MSDs), two nucleotide-binding domains (NBD1 and NBD2), and a regulatory domain (RD) (Figure 1)<sup>16</sup>.

Figure 1

Structure of the cystic fibrosis transmembrane conductance receptor protein (CFTR).



The NBDs and RD are situated in the cytoplasm, while the MSDs form the main pore of the chloride channel. Both the RD and NBDs are involved in regulating the channel activity. The first stage consists of phosphorylation of the RD by a cAMP-dependent protein kinase. The NBDs

then bind and hydrolyze the ATP, providing energy that enables the channel to open and close. The absence of phosphate in the RD makes the channel impermeable to chloride ions.

The protein is made in the nucleus and undergoes a complex process before locating in the cell membrane. This process includes two glycosylation stages, in the endoplasmic reticulum and Golgi complex. This protein consists of 1,480 amino acids and has a molecular weight of 168,173 Da and functions as a cAMP-regulated chloride channel.

The protein, besides its principal function in chloride conductance, plays other roles, including participation in sodium absorption. In the lungs, hydration of mucus is maintained by chloride secretion and sodium absorption.

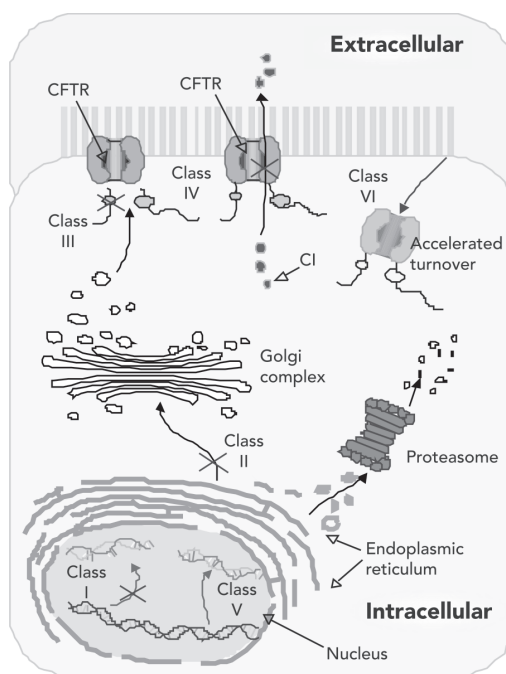
Homozygous mutations in the cystic fibrosis gene result in absence of activity or partial functioning of the CFTR and reduction in chloride excretion through the luminal epithelial surface. To maintain ionic neutrality, there is a compensatory inflow of sodium and water, leading to dehydration of the cell surface, with the formation of thickened mucus, characteristic of the disease<sup>17</sup>. Different forms of pathogenesis resulting from these alterations are present in the wide range of affected organs, including the pancreas, sweat glands, and respiratory, digestive, and reproductive tracts.

Currently, more than 1,000 mutations associated with the disease have been described<sup>16</sup>. These mutations are distributed in 6 classes, having as their basis the mechanisms that determine development of the disease: (a) class I causes a disorder in CFTR mRNA production and consequently a defect in the protein transcription; (b) class II results from a processing defect: the protein is synthesized, but the post-translational modifications do not occur properly, and there is no glycosylation of the protein, which is retained in the endoplasmic reticulum and degraded before reaching the membrane; (c) class III is associated with a defect in regulation of the protein, which is located correctly in the cell membrane, but does not respond to cAMP agonist stimuli in the RD, essential for opening of the chloride channel; (d) class IV involves a reduction in chloride conductance, whereby the chloride ions fail to move effectively through the channel, leading to a conductance defect; (e) class V results in "splicing" abnormalities in the CFTR (removal of non-coding sequences, introns, from the primary RNA transcript, by an mRNA processing enzyme complex), with a partial reduction in the number of functioning chloride channels and reduced amount of functional CFTR protein; and (f) class VI, resulting

from alterations in cell-surface CFTR stability (Figure 2)<sup>10,16,18</sup>.

Figure 2

Classification of the cystic fibrosis transmembrane conductance receptor (CFTR) mutations.



Modified from Rowe et al.<sup>16</sup>.

Mutations I, II, and III are associated with the more severe clinical forms, resulting in complete loss of chloride channel function, while mutations IV and V produce milder clinical manifestations, associated with altered conductance or reduction in the synthesis of normal CFTR<sup>19</sup>.

The most commonly described mutation in the United States, called  $\Delta F508$ , is characterized as a class II defect<sup>18</sup>. CFTR with this mutation presents a deletion of three base pairs, involving the loss of an amino acid, phenylalanine, at position 508 (exon 10). Thus, when the protein reaches the endoplasmic reticulum, the cell's quality control mechanism recognizes the protein as "misfolded" (altered spatial conformation) and degrades it soon after synthesis, before reaching the cell surface<sup>16</sup>.

The  $\Delta F508$  mutation is found in some 70% of Canadian, American, and Northern European Caucasian patients, decreasing in frequency in

Central and Southern Europe<sup>20</sup>. In Brazil, the frequency of this mutation, analyzed in the alleles of affected patients, varies from region to region: 49% in Rio Grande do Sul<sup>21</sup>, 55% in Santa Catarina<sup>22</sup>, 31.7 to 52% in São Paulo<sup>23,24,25</sup>, 39% in Paraná<sup>25</sup>, 32.6% in Minas Gerais<sup>13</sup>, 22.7% in Pará<sup>26</sup>, and 30.68% in the State of Rio de Janeiro<sup>27</sup>. A recent study<sup>22</sup> analyzed the frequency of this mutation in European descendants in five Brazilian States (Minas Gerais, São Paulo, Paraná, Santa Catarina, and Rio Grande do Sul) and observed that approximately 48% of the alleles in CF patients carried the  $\Delta F508$  mutation and estimated the prevalence of the disease at 1:7,576 live births in European-descendant Brazilians. Due to the country's extensive miscegenation, the study also showed a large regional variation, estimating the mutation's prevalence at 1:32,258 in children born in the State of São Paulo and 1:1,587 in Rio Grande do Sul.

The other mutations are rare (< 1%), with 10 of them showing a frequency of 2% to 10% in various populations, for example G542X (class I mutation), G551D (class III mutation), and R117H and A455E (class IV)<sup>16</sup>. In Brazil, the G542X mutation is found more frequently in the States of São Paulo and Minas Gerais (19.6% and 13.8% respectively) than in the States of Rio de Janeiro, Rio Grande do Sul, Santa Catarina, and Paraná (2.1, 4.9, 6.2, and 8.7%, respectively)<sup>28,29</sup>. In the States of Santa Catarina and Paraná, other rare mutations have been reported, like N1303K (4.5%), G85E, R334W, and R1162X (3.6%), 2183AA>G and W1282X (2.7%), and R553X (1.8%)<sup>30</sup>. In the State of Rio de Janeiro, among the rare mutations, 3120+1G→A was found in 3.7% of the alleles<sup>29</sup>. Cabello et al.<sup>31</sup> explain the higher frequency of this mutation, 3120+1G→A, as the result of the population's ethnic composition in the State of Rio de Janeiro, with a higher proportion of African-descendants. Meanwhile, the G551D mutation, occurring in less than 1% in the State of Rio de Janeiro, was found in 3% of the alleles studied in the State of Pará<sup>26</sup>.

### Clinical manifestations of cystic fibrosis

Cystic fibrosis is characterized by a wide heterogeneity of manifestations, but the classical triad consists of chronic lung disease, pancreatic insufficiency, and high sodium chloride levels in sweat.

The lung is affected by chronic infections, accounting for the disease's high morbidity and early mortality.

The chloride transport defect leads to increased sodium absorption, decreased water

secretion, and greater reabsorption of periciliary fluid. The result is decreased fluid volume on the airways surfaces, with the formation of thick, viscous mucus, ineffective for mucociliary transport, leading to secondary infection and inflammation<sup>10,22</sup>.

Alterations in airways surface liquid and mucociliary clearance mean that host chemotaxins (interleukin-8) and bacteria in the airways lead to intense migration of neutrophils in the lungs. The presence of abundant neutrophils in the airways causes the release of elastase and large amounts of DNA, resulting from their breakdown, making the mucus even more viscous and leading to the appearance of proinflammatory substances<sup>32</sup>.

In cystic fibrosis, inflammation occurs early, precedes infection, and predisposes to colonization and infection of the airways, creating a vicious cycle that is a major cause of lung function decline in these patients<sup>33</sup>.

Colonization of the airways by opportunistic bacteria causes irreversible tissue damage in CF patients. The most frequently involved bacteria are: *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Staphylococcus aureus*, and *Haemophilus influenzae*, which adhere readily to airways secretions and are not eliminated effectively<sup>19</sup>. Some bacteria adapt to the medium, making it difficult to eradicate them. One example is *P. aeruginosa*, which adapts to the pulmonary micro-environment through the formation of macrocolonies (or biofilms) and the production of a capsular polysaccharide that inhibits penetration by antimicrobial agents, thereby giving the lung a mucoid appearance<sup>16</sup>.

Pancreatic insufficiency is already present at birth in some 75% of individuals with CF, in 80 to 85% by the end of the first year of life, and in some 90% by adulthood<sup>34</sup>. The pancreas displays a decrease in the formation of pancreatic juice and bicarbonate due to the non-functioning of the chloride channels in ductal epithelial cells; without ductal fluid, there is no enzyme transport. Due to obstruction of the pancreatic canaliculi, the enzymes secreted in the acini fail to reach the duodenum, leading to deficient digestion and malabsorption of macro and micronutrients<sup>34</sup>. Voluminous, fatty, pale feces with a characteristic odor result from the deficient digestion, finally involving more or less severe protein-calorie malnutrition due to loss of nutrients through the feces. In addition, a process of pancreatic autodigestion begins which can last for months or years; in some patients, the insulin-producing beta cells in the islets of Langerhans are also destroyed, leading to the emergence of diabetes mellitus<sup>35</sup>.

The sweat glands of CF patients do not generally present obstruction or significant mor-

phological alterations, but they do display abnormalities in sodium chloride homeostasis. In the sweat glands of normal individuals, the first secretion produced in the glandular annulus is modified as it crosses the duct, before emerging on the skin's surface. Under normal conditions, sodium (followed by chloride ions) is rapidly reabsorbed from the ductal lumen, through the apical sodium channels and the CFTR. In CF patients, the lack of CFTR functioning restricts chloride reabsorption, thus limiting the amount of salt that can be reutilized. Since there is no other route for effective chloride reabsorption in the duct, sodium is also poorly absorbed, and the sweat emerging on the skin's surface has a high salt content<sup>16</sup>.

Men with cystic fibrosis are frequently sterile due to obstruction of the vas deferens<sup>16</sup>. In the female reproductive tract, the gene's expression varies in the cervical epithelium and Fallopian tubes and is high in the endometrium. Women with CF ovulate normally but experience difficulty in conceiving due to thick cervical mucus, which acts as a barrier against sperm cells.

### Neonatal screening for cystic fibrosis

The implementation of neonatal screening for cystic fibrosis involves great controversy, considering questions as to the benefits of early diagnosis of the disease, the various protocols for its detection, and the costs involved<sup>36</sup>.

In 1979 in New Zealand, Crossley et al.<sup>5</sup> introduced the testing of immunoreactive trypsin (IRT) for CF screening, developing a method that allowed measurement in dried blood and therefore universal screening for cystic fibrosis. This test is still the basis for CF screening protocols.

Trypsinogen is a precursor for pancreatic enzyme, the concentration of which is usually persistently high in the blood of newborns with cystic fibrosis, even in cases where there is still pancreatic sufficiency<sup>37</sup>. This increase results from the pancreatic fibrosis displayed by the majority of individuals with CF even in the intrauterine period, leading to a reflux of pancreatic enzymes into the circulation and increased IRT levels.

Crossley et al.<sup>5</sup> used a radioimmunoassay with polyclonal antibodies to measure IRT on Guthrie cards. This method was later improved with the introduction of an enzymatic immunoassay with monoclonal antibodies and performed on ELISA plates, leading to increased sensitivity and shorter processing time<sup>38</sup>.

Various screening protocols are based on the IRT test as a preliminary tool for CF screening. These protocols have advantages and disadvan-

tages. The choice depends on various factors, like cost, collection time, and the specific genetic background in each region to be screened<sup>38</sup>. Almost universally, screening tests do not detect all affected children, and few cases will be detected clinically after a negative neonatal test.

### Currently used protocols

- **IRT/IRT**

The protocol initially adopted for screening programs was the two-stage IRT. When the first IRT (IRT1) is high, it is necessary to draw a second blood sample for new IRT measurement (IRT2); if the second test is also high, the sweat test is performed for the definitive diagnosis. This protocol was initially adopted in various countries, including Australia, Austria, Belgium, France, Italy, England, New Zealand, and the United States.

Travert analyzed the results for 2 million children screened by the IRT/IRT protocol in 15 laboratories, with four different testing systems, three of which by radioimmunoassay. Of the 730 children with cystic fibrosis, 6.4% were not diagnosed by the screening test. The positive predictive value (PPV) varied from 3% to 10% for the first IRT test and was around 52% for the second test. There was large variability between laboratories, partially explained by the different antibodies used at the time<sup>38</sup>.

The test's PPV and specificity depend on the cutoff point, and most programs adopt a cutoff that results in a 0.3-1.0% recall rate. The cutoff points can be fixed or vary between assays, using percentages<sup>38</sup>.

Since IRT levels decrease over time, some screening programs adopt a lower cutoff, around 50ng/ml, for the second IRT test<sup>39</sup>.

With the IRT/IRT protocol, approximately one in 200 children requires a second IRT<sup>38</sup>, causing great anxiety in the parents. According to the Wisconsin program in the United States, fetal distress accounts for 25% of the false-positive results<sup>40</sup>. Other factors include intestinal atresia, congenital infections, and trisomies 13 and 18<sup>38,41</sup>.

Approximately 95% of children with cystic fibrosis are detected with this protocol, but with a high percentage of false-positive results.

- **IRT/DNA**

Identification of the CFTR gene in 1989 and of the most frequent mutation,  $\Delta F508$ , in some populations allowed performing efficient neonatal screening with a single blood sample. IRT is measured in routine neonatal screening samples, and



those with high levels are processed for the analysis of mutations, using DNA isolated from blood eluted from the same filter paper.

When two mutations are found, the diagnosis of cystic fibrosis is confirmed; when one mutation is found the patient must undergo the sweat test, since the individual may merely be a carrier of one of the CF genes or may present cystic fibrosis if there is a second mutation that was not investigated by the genetic test used. When no mutation is detected, the probability of cystic fibrosis is low, with no need for the sweat test.

Using this strategy, a group of Australian researchers<sup>42</sup> observed better specificity and PPV, fewer false-positives, and a 96% detection rate.

An analysis of nine years of experience in CF screening in Wisconsin also showed an improvement in the screening results with the inclusion of this protocol. Initially, with the IRT/IRT protocol, the sensitivity, specificity, and PPV were 87%, 99.9%, and 12.5%, respectively. With the new method, the sensitivity increased to 94%, besides allowing earlier identification of affected children and fewer false-positives, besides not requiring a second IRT test and allowing the benefit of genetic counseling. In 2002, neonatal screening for cystic fibrosis in Wisconsin began analyzing 25 types of mutations, increasing the diagnostic sensitivity to 99% and the diagnosis of affected individuals from 41% (investigating one mutation) to 64%<sup>43</sup>.

This program's costs were also evaluated and proved similar to those of other well-established disorders, like phenylketonuria (US\$ 3.64) and hypothyroidism (US\$ 4.42), while the estimated cost for molecular analysis was approximately US\$ 4.00 per newborn<sup>43</sup>.

- **IRT/DNA/IRT**

This protocol combined a first IRT test with DNA analysis for the  $\Delta F508$  mutation and a second sample for IRT when the child had a mutant allele. Pollitt et al.<sup>44</sup>, using this methodology, observed a reduction of 92% in the number of recalls for a second IRT test, as compared to the IRT/IRT protocol, and a reduction of 80% in recalls for the sweat test.

#### Other approaches

- **PAP/IRT**

Pancreatitis-associated protein (PAP) is elevated in the blood of children with cystic fibrosis<sup>44</sup>. Sarles et al.<sup>45</sup> studied the combined use of measuring PAP and IRT. In the limited number of children they tested, they concluded that this proto-

col has good sensitivity and high specificity and can preclude performing the genetic test, which requires informed consent according to the legislation in many countries.

- **Meconium lactase**

Italian researchers have studied the measurement of meconium lactase as an additional test associated with IRT and/or DNA analysis. Based on their studies, they postulated that performing IRT at birth, associated with the lactase test and analysis of three mutations, led to higher sensitivity and a considerable increase in specificity, principally in genetically diverse populations<sup>46</sup>. This protocol requires two different samples and is not currently applied elsewhere.

#### Cystic fibrosis screening around the world

There are few nationwide neonatal screening programs for cystic fibrosis implemented around the world. Most of the existing programs are regional.

According to an evaluation of the situation with CF screening in various countries by Southern & Littlewood<sup>47</sup> in 2003, Canada, Spain, Estonia, Finland, Greece, Israel, Ireland, Jordan, Mexico, Netherlands, Norway, Portugal, Slovak Republic, Sweden, and Switzerland did not have national screening programs for the disease. In other countries, pilot studies had been performed, but the decision was made not to implement the program. The latter included Denmark, which opted for pre-conception screening, and Germany and the Netherlands, which initially used the meconium test, with high false-positive and false-negative rates, resulting in the non-introduction of a national program. Meanwhile, other countries like Australia, Austria, Belgium, France, Italy, New Zealand, and Poland launched their programs in the years 1981, 1988, 1988, 1988, 1973, 1981, and 1999, respectively.

In 2003, in England, six centers performed screening, with coverage of 22% of newborns, and the national program was implemented in 2004. In the United States at the time, six States (Colorado, Massachusetts, New Jersey, New York, Wyoming, and Wisconsin) had implemented their own programs, and three (Connecticut, Montana, and Pennsylvania) did partial screening<sup>47</sup>.

A recent publication by the European Cystic Fibrosis Foundation<sup>39</sup> reported on the current situation with CF screening in Europe. Twenty-six centers participated in this evaluation: seven from England, 12 from Italy, three from Spain, and one each from Austria, France, Poland,

and Czech Republic, while in France and Austria neonatal screening is centralized in a single laboratory. These services have been working with CF neonatal screening for an average of ten years (varying from nine months to 31 years), screening 1,600,000 children/year and detecting some 400 cases/year. Median age at diagnosis is reported at 37 days (32-50). This was the first detailed report on CF screening in Europe, and the results showed heterogeneous protocols, varying from the sweat test after the first IRT to those involving IRT1/IRT2/DNA, IRT1/DNA/IRT2, IRT1/protein in meconium/IRT2, IRT1/sweat chloride test. Nineteen programs use DNA testing after the first IRT. The median number of mutations analyzed is 31.

In the United States, 34 of the 50 States currently perform screening for cystic fibrosis (<http://genes-r-us.uthscsa.edu/>, accessed on 03/Jan/2008).

In Brazil, CF neonatal screening was the object of a ruling by the Ministry of Health in 2001. The screening test is the IRT, with investigation of the  $\Delta F508$  mutation being available for diagnostic confirmation. The public program is implemented in routine neonatal screening in the States of Santa Catarina, Paraná, and Minas Gerais. In other States it has been performed in some private laboratories.

In Paraná, CF screening began in 2001, using the IRT/IRT protocol and with the sweat chloride test for diagnostic confirmation. The first report on the experience with screening in Paraná involved 456,982 newborns from September 2001 to April 2004 and found 4,028 (0.9%) children with the first IRT higher than 70ng/ml. Of these, 3,815 (94.7%) appeared for the second IRT, which was altered in 478 (12.8%), while 48 (0.01%) had the diagnosis confirmed by the sweat chloride test (cutoff of 50mMol/l). These results showed a high false-positive rate in the first IRT, and the incidence of cystic fibrosis was 1:9,520 newborns in the State <sup>11</sup>.

In the State of Santa Catarina, CF neonatal screening was evaluated for the period from October 2000 to December 2005, using as the protocol the IRT test from the fourth to seventh day of life, with a cutoff of 70ng/ml, with the second test performed up to 30 days of life and three samples of sweat chloride for the definitive diagnosis. During this period 386,183 children were screened; of these, 3,902 (1%) had an altered first IRT and 181 (0.05%) an altered second IRT. Forty-four cases of cystic fibrosis were diagnosed, for an incidence of 1:8,776. All the children with CF were white, 11 homozygous and 14 heterozygous for the  $\Delta F508$  mutation. Mean age at diagnosis was 64 days <sup>12</sup>.

In Minas Gerais, CF screening began in July 2003. From the beginning of the program until April 2005, 455,755 newborns were screened, 53 of whom were identified with cystic fibrosis (incidence of 1:9,115). Mean age at diagnosis was 51.4 days <sup>13</sup>.

In the State of São Paulo, a pilot study for implementation of CF screening tested 33,600 infants from the North, Northeast, and West of the State and showed a similar incidence to that of other States of Brazil, estimated at 1:8,403. Mean age at diagnosis in this study was 69 days, and of the four children diagnosed through this screening, three already presented overt symptoms of the disease when the diagnosis was confirmed <sup>14</sup>.

The benefits of early treatment related to improvement in the child's nutritional status and growth are unquestionable <sup>48</sup>. However, this evidence is less clear when analyzing the evolution of the pulmonary disease and long-term survival <sup>37,49,50</sup>. However, detecting the disease in its asymptomatic stage can provide a valuable opportunity to collect epidemiological data and obtain better understanding of the natural history of the disease <sup>51</sup>. In addition, in relation to *P. aeruginosa* infection (the principal risk factor for morbidity and mortality), early diagnosis can serve to test the efficacy of preventive therapeutic strategies and could help improve prognosis <sup>51,52</sup>.

However, although CF screening has been available for nearly three decades, there are few nationwide programs, with the majority implemented at the regional or local level. The reasons for this include uncertainties as to the long-term benefits and the lack of a definitive screening test <sup>50,53,54</sup>. Since cloning of the CF gene in 1989 <sup>7,8,9</sup> and the availability of molecular testing for diagnosing the disease, other questions have arisen: (i) Are the benefits of early diagnosis for the child's growth and nutrition sufficient to justify screening? (ii) Would detecting carriers of the mutation, when used for diagnosis, produce some harm for these individuals? (iii) Approximately 5% of the children identified through screening present a borderline sweat test (concentrations of 30-40mmol/L) and more "benign" mutations. How many of these individuals will have health problems and require treatment for the disease?

However, analyzing all these issues, in 2003 the U.S. Centers for Disease Control and Prevention (CDC) published guidelines for cystic fibrosis screening <sup>55</sup>. The report concluded that CF screening is justified on grounds of evidence of moderate benefit and low risk of harm, despite acknowledging that the individual States should assess their available resources and the concurrent public health priorities.

## Resumo

*Aspectos clínicos e diagnósticos da fibrose cística são revistos de modo abrangente, com ênfase na triagem neonatal. Esta revisão sistematizada da literatura envolveu busca de contribuições relevantes nos bancos de dados PubMed e SciELO. Referências sobre fibrose cística existem desde a Idade Média. É a doença hereditária autossômica recessiva mais freqüente em caucasianos (1:2.000 a 3.500). Mais de mil mutações levam à doença, a mais comum:  $\Delta F508$  (prevalência: 70% em caucasianos canadenses, americanos e norte-europeus; de 23 a 55% em brasileiros). O defeito básico ocorre na secreção do íon cloro. Sua triagem é assunto polêmico e apesar de estar disponível há quase três décadas, por meio de diferentes protocolos, poucos programas de abrangência nacional existem. Entretanto, o Centers for Disease Control and Prevention, dos Estados Unidos, afirma que o rastreamento neonatal para fibrose cística é justificado. A falta de um teste específico e a heterogeneidade étnica da população brasileira dificultam sua triagem neonatal.*

*Fibrose Cística; Triagem Neonatal; Revisão*

## Contributors

R. Rodrigues conducted the data analysis and drafted the manuscript. C. S. Gabetta, K. P. Pedro, F. Valdetaro, and M. I. M. Fernandes participated in the critical analysis of the manuscript. P. K. R. Magalhães contributed to the data analysis and drafting of the manuscript. J. N. Januário contributed to the organization and critical revision of the manuscript. L. M. Z. Maciel participated in the data analysis and drafting of the manuscript.

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