# A Low-Cost and Simple Genetic Screening for Cystic Fibrosis Provided by the Brazilian Public Health System

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Cystic fibrosis newborn screening was implemented in Brazil by the Public Health System in 2012. Because of cost, only 1 mutation was tested - p.Phe508del. We developed a robust low-cost genetic test for screening 11 CFTR gene mutations with potential use in developing countries. *(J Pediatr 2018;■■:■■-■■).*

Cystic fibrosis (CF-OMIM #219700) is an autosomal recessive genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene (cytogenetic location: 7q31.2), which affects sodium, chloride, and bicarbonate homeostasis in epithelial cells.[1] This disease is highly prevalent ranging from 1 in 900 to 1 in 25 000 live births worldwide.[2] In Brazil, the incidence of cystic fibrosis is estimated to be 1 in 7576 live births.[3]

The normal newborn screening protocol used in Brazil is performed in sequential steps according to the age of newborns. First, an immunoreactive trypsinogen (IRT) dosage is conducted before 30 days of life. Borderline values (≥70 ng/mL) require a second collection. After this age, sweat test measures are carried out.[4] However, these tests may be imprecise, leading to false-positive, false-negative, or even uncertain results.[5,6] For this reason, clinical evaluations and conventional genetic tests (p.Phe508del analysis) may be requested.[4]

The p.Phe508del mutation is the most frequent, responsible for more than 48% of cystic fibrosis alleles in the population of Southern Brazil[7] and 69.8% around the world (available at http://cftr2.org.). However, 2023 mutations have already been described in the *CFTR* gene (available at http://genet.sickkids.on.ca/). A wide clinical variability of cystic fibrosis has been attributed to this high number of mutations.[8] The expense of introducing a molecular diagnosis in public health centers, as a confirmatory test, still constitutes a barrier in developing countries.

Genetic testing can be combined with biochemical and clinical evidence, leading to a more accurate diagnostic approach. In 2012, cystic fibrosis newborn screening of the p.Phe508del mutation was implemented in Rio Grande do Sul, Brazil through Brazil's public health system (Sistema Único de Saúde [SUS]). Because of the cost, only 1 mutation was screened. To improve this situation, public Brazilian institutions (Universidade Federal do Rio Grande do Sul, Hospital Materno Infantil Presidente Vargas, and Centro de Desenvolvimento Científico e Tecnológico) signed an agreement to create a more accurate complementary test for cystic fibrosis diagnosis to be implemented through SUS. Thus, our aim was to develop a robust inexpensive genetic test, SNaPshot, for screening simultaneously 11 *CFTR* gene mutations. In Brazilian newborn screening, it has been as used a complementary method to evaluate inconclusive results.

SNaPshot is a fast method with a minimum of hands-on time suited for the rapid screening of a large number of samples and multiple genetic variants in the same reaction.[9]

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| *CFTR* | Cystic fibrosis transmembrane conductance regulator |
| ddNTP | Dideoxynucleotide |
| IRT | Immunoreactive trypsinogen |
| PCR | Polymerase chain reaction (PCR |
| SUS | Sistema Único de Saúde |
| Wt | Wild type |

## Methods

A total of 34 DNA samples from newborns screened by the Reference Service in Neonatal Screening of Rio Grande do Sul, where there was suspicion of cystic fibrosis were analyzed. The patients came from several regions of the state of Rio Grande do Sul and were screened for *CFTR* mutations using the newly optimized CFTR SNaPshot assay. The criteria for subject selection were 2 increased IRT values, borderline or an abnormal sweat test, and molecular testing for p.Phe508del (Wild type [Wt]/Wt or p.Phe508del/Wt) and previously genotyped using commercial genotyping kits Inno- LiPA *CFTR*19 and Inno- LiPA *CFTR*17+Tn (Innogenetics, Ghent, Belgium). As a negative control, a total of 45 DNA samples from the DNA databases of the Reference Service of newborns screening were tested.

The study was approved by the Ethical Committee of the Hospital Materno Infantil Presidente Vargas, and the parents

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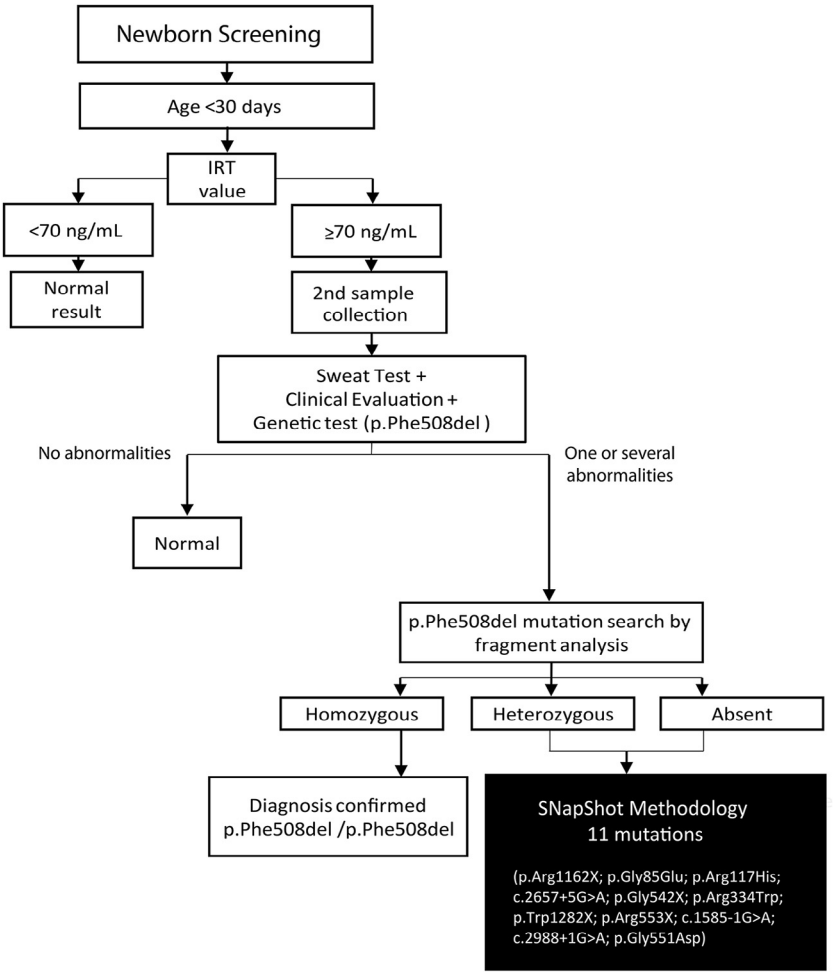


**Figure 1.** Representation of the flowchart of newborn screening performed in the state of Rio Grande do Sul. The white boxes represent the actual genetic screening for cystic fibrosis in Brazil. Our SNaPshot strategy as complementary screening for cystic fibrosis is located in the black box.

of the subjects gave written informed consent for the participation in the study. A flowchart of neonatal screening performed in this work is represented in **Figure 1**.

### Mutations Selection

The mutations that comprise the panel (p.Arg1162X, p.Gly85Glu, p.Arg117His, c.2657 + 5G>A, p.Gly542X, p.Arg334Trp, p.Trp1282X, p.Arg553X, c.1585-1G>A, c.2988 + 1G>A, and p.Gly551Asp) were selected according to their previously described potential relevance in Southern Brazil (state of Rio Grande do Sul) and in the Brazilian population among the 2000 mutations that have previously been described.[7,10-13] In addition, the most frequent mutations in European populations (populations mainly from Italy, Spain, Portugal, and Germany) stored in the cystic fibrosis mutation database "CFTR1" (http://www.genet.sckkids.on.ca/cftr) were considered for inclusion in the panel because of the great migration of European people to Southern Brazil. Mutations were also considered if they were present in the commercial test.

A second cystic fibrosis mutation database "CFTR2" (http://www.cftr2.org) was also used. This database collects the most recent information on newly discovered *CFTR* gene mutations, classifies them as disease-causing, neutral, or mutations of varying clinical consequences, and provides clinical information on specific mutation combinations.

### Primer Design and Validation: Multiplex Polymerase Chain Reaction and SNaPshot

The primers design of the multiplex polymerase chain reaction (PCR) and the SNaPshot (**Tables I** and **II**; available at www.jpeds.com) was performed using Primer3 software v.0.2 (Whitehead Institute, Cambridge, UK; http://bioinfo.ut.ee/primer3-0.4.0/), and the specificity was confirmed in silico using BLAST software (http://blast.ncbi.nlm.nih.gov) and experimentally by Sanger sequencing, where each amplicon was sequenced on an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, California), according to the



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