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Original Article

Long-term Outcomes of Pediatric CFSPID: A 15-Year Clinical and Genomic Study Across Newborn Screening Cystic Fibrosis Units

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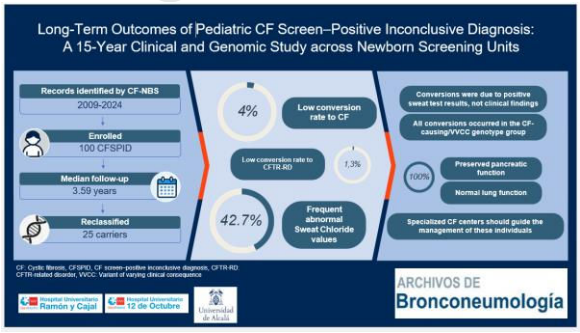
Graphical abstract



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ABSTRACT

Background

Newborn blood spot screening (NBS) for cystic fibrosis (CF) increasingly identifies infants with inconclusive results, classified as CF screen–positive inconclusive diagnosis (CFSPID). However, long-term outcome data remain limited.

Objective

To analyze the clinical and biochemical course of children with CFSPID.

Methods

We conducted a bi-center observational cohort study including all children designated as CFSPID through the Madrid NBS program from July 2009 through June 2024. Follow-up assessments included serial sweat chloride (SC) testing, respiratory and GI evaluations, spirometry from age 5 or older, nasopharyngeal cultures, and fecal elastase measurements.

Results

A total of 100 children were enrolled. After a median follow-up of 3.59 years [IQR, 1.73–5.43], 25% were reclassified as unaffected carriers following CFTR variant reinterpretation. The remaining 75 were categorized into 3 genotype groups: 66 with CF-causing (CFc)/Variant of Varying Clinical Consequence (VVCC), 4 with VVCC/VVCC, and 5 with CFc/Variant of Uncertain Significance (VUS). By the end of follow-up, 42.67% developed at least 1 intermediate or positive SC value. Three children (4%), all carrying the CFc/VVCC genotype, converted to cystic fibrosis (mean age at conversion, 4.23 years), and 1 child (1.3%) developed a CFTR-related disorder. Clinical signs were mild, with normal spirometry and full pancreatic sufficiency. No *Pseudomonas aeruginosa* isolates were detected.

Conclusions

Conversion from CFSPID to CF was rare (4%), but abnormal SC values were frequent (42.67%), supporting the need for structured, long-term monitoring. Continued follow-up in specialized CF centers is essential for early detection of disease progression.

Keywords: CFSPID; newborn screening; sweat chloride; cystic fibrosis; conversion; pediatrics.

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Abbreviations

- CBAVD: congenital bilateral absence of the vas deferens
- CF: Cystic fibrosis
- CFc: CF-causing variant
- CFTR: Cystic fibrosis transmembrane conductance regulator
- CFTR-RD: CFTR-related disorder
- CFSPID: CF screen–positive inconclusive diagnosis
- ECFS: European Cystic Fibrosis Society
- FEV_1: Forced expiratory volume in 1 second
- IRT: Immunoreactive trypsinogen
- IQR: Interquartile range
- MSSA: Meticillin-sensitive *Staphylococcus aureus*
- NBS: Newborn blood-spot screening
- nCFc: Non-CF-causing variant
- NPA: nasopharyngeal aspirate cultures
- *P. aeruginosa*: *Pseudomonas aeruginosa*
- PI: Pancreatic insufficiency
- PS: Pancreatic sufficiency
- PEx: Pulmonary exacerbation
- RP: Respiratory physiotherapy
- SC: Sweat chloride
- VUS: Variant of uncertain significance
- VVCC: Variant of varying clinical consequence

1. Introduction

Cystic fibrosis (CF) is the most common life-limiting autosomal recessive condition in the populations of European ancestry[1]. It results from pathogenic variants in both alleles of the *CFTR* gene, which encodes the CFTR chloride/bicarbonate channel responsible for epithelial salt and water transport. Loss or dysfunction of this channel leads to dehydration of mucosal surfaces in the respiratory, GI, and reproductive tracts, causing the disease multisystem phenotype[2].

Newborn blood-spot screening (NBS) enables presymptomatic detection of CF and is associated with earlier nutritional intervention, better pulmonary outcomes, and improved survival[3,4]. The Community of Madrid (Spain) implemented CF-NBS in July 2009 using a 3-tier protocol: first, measuring immunoreactive trypsinogen (IRT) from dried blood spots[5], with a threshold of 44 ng/mL; if exceeded, a 2nd IRT measurement is performed on the same sample, and if the mean exceeds 50 ng/mL, CFTR gene panel testing is conducted[6]. If at least 1 CFTR variant is identified, the screening is considered positive, and the infant is referred for a sweat chloride (SC) test. If no variants are found, a 2nd blood sample is collected, and an IRT level > 35 ng/mL is considered positive as well. Identified variants are classified according to CFTR2[7] and CFTR-France[8] as CF-causing (CFc), variants of varying clinical consequence (VVCC), variants of uncertain significance (VUS), or non-CF-causing (nCFc)[9]. SC testing completes the diagnostic pathway and yields 4 potential outcomes: false-positive screen, healthy carrier, confirmed CF, or an inconclusive category termed CF screen–positive inconclusive diagnosis (CFSPID) in Europe and CFTR-related

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metabolic syndrome (CRMS) in the United States[10]. The most recent international consensus (2023) defines CFSPID as an asymptomatic infant with an abnormal CF-NBS result and either (i) SC < 30 mmol/L and 2 *CFTR* variants, at least 1 with unclear phenotypic consequences (VVCC or VUS), or (ii) SC 30–59 mmol/L with 1 or 0 CFc variants[10]. CFSPID may evolve to CFTR-related disorder (CFTR-RD) or CF if SC levels rise or symptoms develop, requiring regular specialist follow-up[9,11].

Longitudinal studies suggest that a minority of children with CFSPID subsequently meet diagnostic criteria for CF or CFTR-RD, whereas most remain clinically unaffected. However, individual disease trajectories are difficult to predict, and the resulting diagnostic uncertainty can impose a psychological burden on families, despite structured follow-up and genetic counselling[12].

Currently, no Spanish cohort has described the natural history of CFSPID. We, therefore, aimed to characterize the clinical course, SC progression, and genotype reinterpretation in a population-based cohort of children designated as CFSPID through the CF-NBS programme in the Community of Madrid over a 15-year period.

2. Methods

2.1 Study Design and Setting

We conducted a bi-center observational cohort study across the 2 CF-NBS reference units in the Community of Madrid (Spain): *Hospital Universitario Ramón y Cajal* and *Hospital Universitario Doce de Octubre*. The study population included all children categorized as CFSPID according to the European Cystic fibrosis Society (ECFS) criteria[10], born between July 2009 and June 2023, and identified through the regional IRT/DNA/2nd IRT algorithm. All cases underwent confirmatory SC testing.

The initial DNA screening panel was the *Elucigene CF-EU2v1*, along with an Iberian extension that included 12 additional variants common in Spanish and Hispanic populations[6], including the following VVCC: c.1210-34_1210-6TG[11]T[5] (5T;11TG), c.1210-34_1210-6TG[12]T[5] (5T;12TG), c.1210-34_1210-6TG[13]T[5] (5T;13TG), c.3454G>C (D1152H) and c.350G>A (R117H;7T). In the latter case, the newborn screening panel already provides information on the poly-T tract, confirming that R117H is associated with 7T and therefore classified as a VVCC (if associated with 5T, it would be considered CFc). Comprehensive *CFTR* gene analysis, including Sanger sequencing and multiplex ligation-dependent probe amplification (MLPA), was undertaken in cases with 0 or 1 variant detected by the screening panel.

In infants with a positive CF-NBS result and at least 1 *CFTR* variant (carriers), genetic counselling was offered to inform families about reproductive risk and further parental testing if desired. In these extended genetic studies, both parents were occasionally found to carry *CFTR* variants. In such cases, complete *CFTR* sequencing was performed in the infant to determine whether the second variant had also been inherited. It was through this pathway that some infants were secondarily diagnosed with CFSPID (*Figure 1*).

Variants were categorized using the CFTR2 and CFTR-France databases[7,8]. If the identified variant was not listed in either database, it was classified as a VUS.

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2.2 Data Collection and Clinical Definitions

Demographic and clinical data were updated until June 2024. Follow-up adhered to ECFS standards of care[11]. The study was approved by the local ethics committees, and written informed consent was obtained from parents or legal guardians for inclusion in hospital databases.

SC results were interpreted as normal (< 30 mmol/L), intermediate (30–59 mmol/L), or positive (≥ 60 mmol/L)[13]. Conversion to CF was defined as SC ≥ 60 mmol/L on 2 separate occasions or the development of a consistent CF phenotype[11]. Reclassification could also occur following updated variant interpretation. A diagnosis of CFTR-RD was assigned to symptomatic individuals who did not meet diagnostic criteria for CF[11].

Pulmonary exacerbations (PEx) were defined in accordance with CF Foundation criteria[14]. Lung function was assessed from age 5 years using forced expiratory volume in one second (FEV_1), expressed as z-scores according to Global Lung Initiative reference values[15,16]. Anthropometry and body mass index (BMI) were converted to World Health Organization z-scores. Pancreatic status was determined by faecal elastase measurement; values > 200 µg/g were considered indicative of pancreatic sufficiency (PS).

2.3 Statistical Analysis

Data distribution was assessed using the Shapiro–Wilk test. Non-parametric tests were applied when the assumption of normality was not met. Correlations were calculated using Spearman's coefficient, and group comparisons were performed with the Kruskal–Wallis test. Statistical significance was set at $p < 0.05$. Continuous variables are expressed as median (interquartile range, IQR) or mean ± standard deviation (SD), as appropriate.

3. Results

3.1 Cohort Characteristics

Since the implementation of CF-NBS in 2009, a total of 102 infants met the ECFS criteria for CFSPID (*Figure 1*). A total of 47 children were initially classified as CFSPID through CF-NBS: 44 with a CFc/VVCC genotype and 3 with VVCC/VVCC. An additional 50 children were first identified as carriers but were later reclassified as CFSPID after parental testing revealed a 2nd variant. This prompted full *CFTR* sequencing, which confirmed the following genotypes: 41 CFc/VVCC, 4 VVCC/VVCC, and 5 CFc/VUS. In another 5 CFc/VVCC early cases, the complete screening pathway could not be reconstructed.

Seven variants initially considered VVCC were later reclassified as nCFc: 5T;11TG, c.224G>A (R75Q), c.509G>A (R170H), c.1584G>A (1716G>A), c.1684G>A (V562I), c.2991G>C (L997F) and c.274-6T>C (406-6T>C). Therefore, 25 children (25%), 22 with CFc/VVCC and 3 with VVCC/VVCC, were reclassified as unaffected carriers. In addition, 2 children were excluded due to incomplete records. The final cohort, therefore, consisted of 75 children (57.3% female). Median age at first specialist assessment was 34 days [IQR 23–45], and median follow-up to June 2024 was 3.59 years [IQR 1.73–5.43].

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A total of 26 distinct VVCCs and 3 VUS (c.1775G>A (C592Y), c.1450C>T (H484Y), and c.2950G>T (D984Y) were identified. The most frequent genotype was c.1521_1523del (F508del)/5T;12TG (16%), followed by F508del/R117H;7T and F508del/D1152H, each found in 5.3% of children. All other genotypes occurred in one or two children, reflecting marked genetic heterogeneity.

3.2 Genotype-Based Subgroups

The 75 children who retained a CFSPID designation were stratified into 3 genotype subgroups (*Table 1*):

- Group #1 – CF-causing/VVCC (n = 66)
- Group #2 – VVCC/VVCC (n = 4)
- Group #3 – CFc/VUS (n = 5)

3.3 IRT and Sweat Chloride Evolution

Median initial IRT was 69.61 ng/mL [IQR, 56.8–84.52], with no significant differences across genotype groups ($p = 0.28$). IRT values did not distinguish children who later converted to CF (78 ng/mL vs 65.6 ng/ml, $p = 0.23$) and did not predict final SC category (< 30 mmol/L vs intermediate/positive, 62.76 ng/mL vs 69 ng/mL, $p = 0.88$).

Median first-month SC was 15.8 mmol/L [IQR 12–22], with no correlation with IRT ($p = 0.09$) and no difference across genotype subgroups ($p = 0.07$). Initial SC did not predict progression to intermediate/positive SC values ($p = 0.84$). At baseline, 9 children (12%) had intermediate sweat chloride values, all in group 1; 3 children had sweat chloride levels of 40 mmol/L or higher at baseline, and 1 of these later exceeded 60 mmol/L. By the end of follow-up, 32 of the 75 children (42.67%) had developed at least 1 intermediate or positive SC value (*Figure 2*). Among these, median SC increased by 2.2 mmol/L per year within the first 7 years of life, with the most marked increases within the first 3 years (+3, +8, and +15 mmol/L in years 1–3, respectively).

All conversions occurred in group #1, but differences in conversion rates between subgroups were not significant ($p = 0.71$).

3.4 Clinical and Microbiological Course

No cases of bronchiectasis or chronic rhinosinusitis were documented. Fourteen children received oral antibiotics for PEx, 6 experienced bronchiolitis, 6 developed pneumonia, 6 experienced wheezing, and 2 were diagnosed with asthma (*Table 1*). Nine children underwent spirometry; the median FEV_1 z-score at the last measurement was -0.28 SD [IQR, –1.15. +0.72].

Nasopharyngeal aspirate cultures were available for 52 children. *Haemophilus influenzae* was isolated in 61.5%, *Moraxella catarrhalis* in 53.9%, methicillin-sensitive *Staphylococcus aureus* (MSSA) in 51.9%, *Streptococcus pneumoniae* in 50%, and usual flora in 48.1% (*Table 1*). No *Pseudomonas aeruginosa (P. aeruginosa)* or non-tuberculous mycobacteria were detected.

Digestive signs were mild: 12 cases of constipation and 1 case of treated gastro-oesophageal reflux. No episodes of pancreatitis, failure to thrive, or dehydration occurred. Median BMI z-score

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at the index visit was -0.2 [IQR, -0.7, +0.4], and all children were PS. Abdominal ultrasound (n = 9) and liver elastography (n = 6; median 4.5 kPa [IQR, 3.8-5.4]) were normal.

3.5 Conversion to Cystic fibrosis

Three of the 75 children (4%) converted to CF due to persistently positive SC values (*Table 2*). Conversion occurred at ages 0.8, 3.2, and 8.7 years. All 3 from group #1 and demonstrated rising SC trajectories (*Figure 2*); 2 developed early-onset respiratory symptoms, and the 3rd remained largely asymptomatic apart from one PEx (*Table 2*).

3.6 Conversion to CFTR-Related Disorder

One child with intermediate sweat chloride values (30–38 mmol/L) was reclassified as having a CFTR-related disorder because of recurrent pulmonary exacerbations and chronic upper airway infection beginning at 3.5 years of age, despite preserved lung function and pancreatic sufficiency (Table 2).

4. Discussion

4.1 Biological Markers of Progression

In agreement with former studies, IRT was not predictive of disease progression in CFSPID[17]. In contrast, SC dynamics proved informative: converters had higher initial SC levels and steeper yearly increases, which is consistent with comparative studies with higher conversion rates in those with intermediate SC at birth and the notion that early chloride trends may reflect subclinical CFTR dysfunction[18-20]. In our cohort, 2/9 (22.22%) of infants with baseline SC > 30 mmol/L progressed to CF, within the 8–35% range reported across European cohorts[17.21]. Abnormal SC values were observed in 42.67% of the cohort. The pronounced inter-individual variability of SC trajectories supports a personalised monitoring approach over fixed testing schedules[22].

4.2 Genotype–Phenotype Correlations

Most children carried a CFc/VVCC genotype, consistent with the detection bias of IRT/DNA-based algorithms. Although all converters belonged to this group, phenotypic expression was heterogeneous, limiting genotype-only prognostication[10.11,21].

The high prevalence of 5T;12TG and R117H;7T, both eligible for CFTR modulators in the United States, raises clinically relevant questions for minimally symptomatic European children. A conversion rate between 10-20% in individuals with 5T;12TG has been published, and our prior work specifically addressing this group showed 42.8% intermediate SC values[19.22-24]. For R117H;7T, earlier reports documented bronchiectasis in up to 29%, pancreatic insufficiency (PI) up to 3% and congenital bilateral absence of the vas deferens (CBAVD) in up to 57%[25.26]. Similarly, in our cohort, it is the most clinically relevant variant. These findings underline the potential utility of structured, non-invasive surveillance (eg, lung clearance index, low-dose chest CT, liver elastography) to guide timely therapeutic decisions. However, such strategies carry ethical and economic implications, particularly regarding the risk of over-medicalization in asymptomatic individuals.

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Rare alleles, such as c.14C>T (P5L) and D1152H, were also present in the cohort; both have previously been associated with respiratory morbidity. P5L has been associated with intermediate SC levels, bronchiectasis, occasional PI, and dehydration[7.8,27.28]. D1152H confers an estimated 27% risk of conversion to CF and 7–28% risk of CFTR-RD (eg, pancreatitis, CBAVD)[17.29]. These examples illustrate the wide phenotypic spectrum of CFTR dysfunction and emphasise the need for flexible, phenotype-informed care pathways.

Of note, the ongoing reinterpretation of genetic data led to the downgrading of 7 VVCCs to nCFc, reclassifying 25% of children as carriers[7.8], which highlights the dynamic nature of variant interpretation and its crucial role in clinical management.

4.3 Clinical Outcomes and Microbiology

Most children remained clinically stable, with preserved growth and lung function, which is consistent with other CFSPID cohorts[30]. Fourteen required antibiotics for PEx, yet no bronchiectasis, chronic rhinosinusitis, or *P. aeruginosa* colonisation was observe, which contrasts with some series reporting early *P. aeruginosa* isolation among CFSPID patients[15.23,24]. Mild GI symptoms were infrequent, and all participants maintained PS throughout follow-up.

4.4 Strengths and Limitations

Strengths of the study include its population-based design, multicentER setting, and the longest follow-up of CFSPID children reported in Spain. Additional advantages include consistent classification criteria, standardised SC protocols, and comprehensive genotyping supported by internationally recognised databases.

Limitations include: (i) small numbers in the VVCC/VVCC and CFc/VUS groups, limiting subgroup analyses; (ii) incomplete early diagnostic data for 5 participants; (iii) the retrospective nature of some data points; and (iv) potential underreporting of mild clinical events during the COVID-19 pandemic.

4.5 Implications and Future Directions

Our findings support an individualized, long-term surveillance model that integrates genotypic, biochemical, and functional data. This approach can help identify children at genuine risk of progression, while minimising unnecessary interventions in stable carriers.

From a therapeutic and public health perspective, the increasing availability of CFTR modulators for borderline genotypes (eg, 5T;12TG and R117H;7T) underscores the need for clear guidance on their use in asymptomatic individuals. Screening programs must also prepare for broader genetic panels, dynamic reinterpretation of variants, and increased multidisciplinary involvement.

Future research should prioritize national CFSPID registries, cost-effectiveness studies of monitoring tools, and prospective trials to determine when CFTR modulators may benefit asymptomatic or borderline cases.

4.6 Final conclusions

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This study provides the first population-based description of CFSPID in Spain, summarizing 15 years of experience from the Madrid NBS program and incorporating updated genetic and clinical data.

Conversion to CF was rare (4%), and only 1 child (1.3%) developed a CFTR-related disorder. All conversions occurred in children carrying a CFc/VVCC genotype, confirming the prognostic relevance of CFc alleles.

Although most children remained clinically stable, 42.7% developed at least 1 intermediate or positive SC value, reinforcing the need for structured follow-up during early childhood.

In conclusion, these results support the integration of genotype-based risk assessment into routine follow-up protocols and highlight the importance of specialized CF centers in guiding management and early detection of disease progression.

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Conflicts of interest
None declared.

Artificial Intelligence Involvement
No part of the manuscript was prepared with the aid of artificial intelligence tools.

Contributors' Statement
Dr. Morales and Dr. Lamas conceived, designed, and coordinated the study. Dr Morales interpreted all collected data, prepared the tables, and drafted the manuscript. Dr. Blitz, Dr. Vicente, Dr. Luna, Dr. Salcedo, Dr. Tabares, and Dr. Gascón collected the clinical data from CFSPID patients followed at the Madrid cystic fibrosis centers. Mr Boutry performed the statistical analyses. All authors critically revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

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Institutional Review Board Statement
The study was conducted in full compliance with the principles outlined in the Declaration of Helsinki and was approved by Hospital Universitario Ramón y Cajal Ethics Committee (protocol v1.2, 15 December 2020; approval date: 9 March 2022).

Informed Consent Statement
Written informed consent was obtained from all participants involved in the study.

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Data Availability Statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request, as stated in the informed consent.

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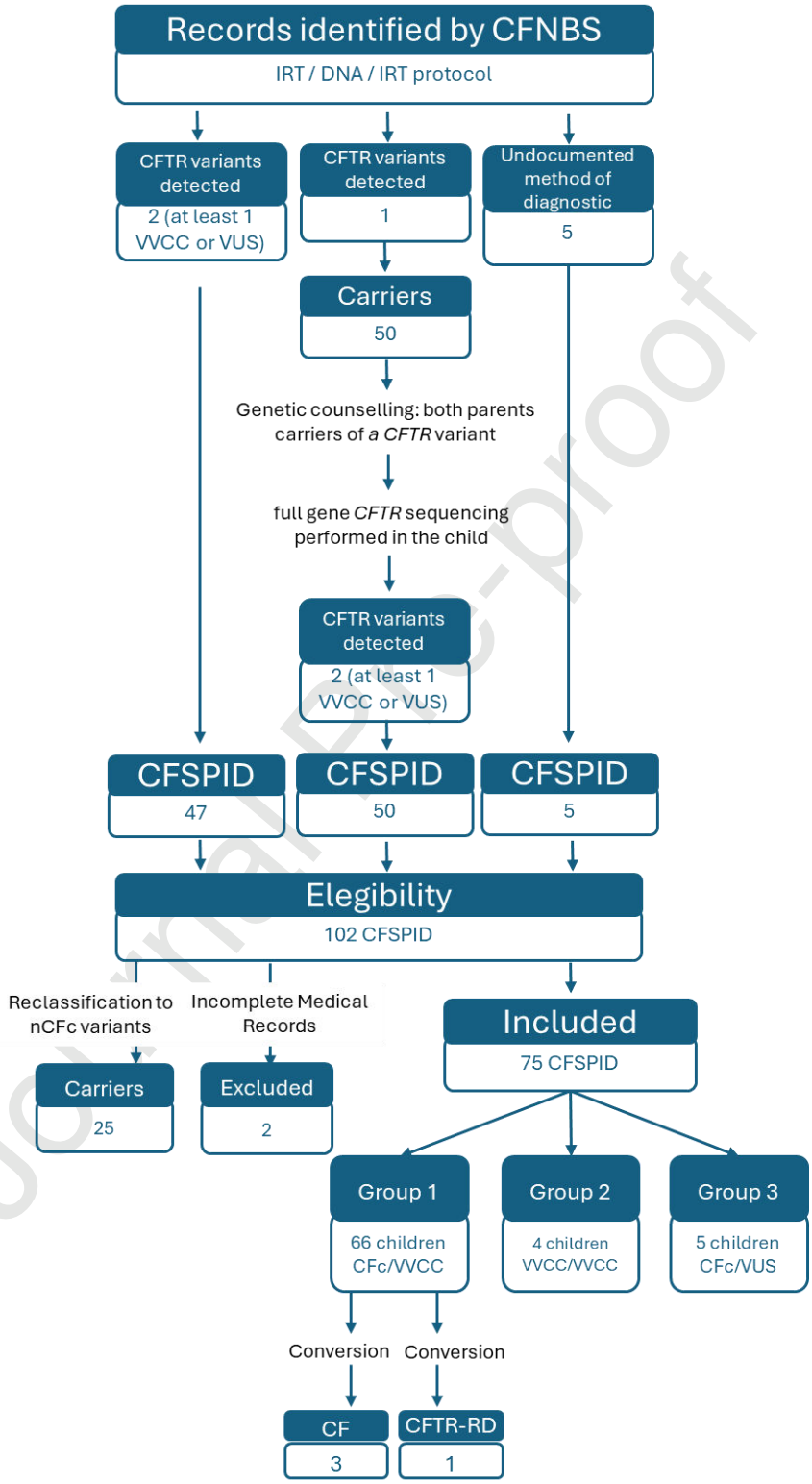
Figure 1. PRISMA flow diagram of the study showing eligible children and their distribution across diagnostic groups



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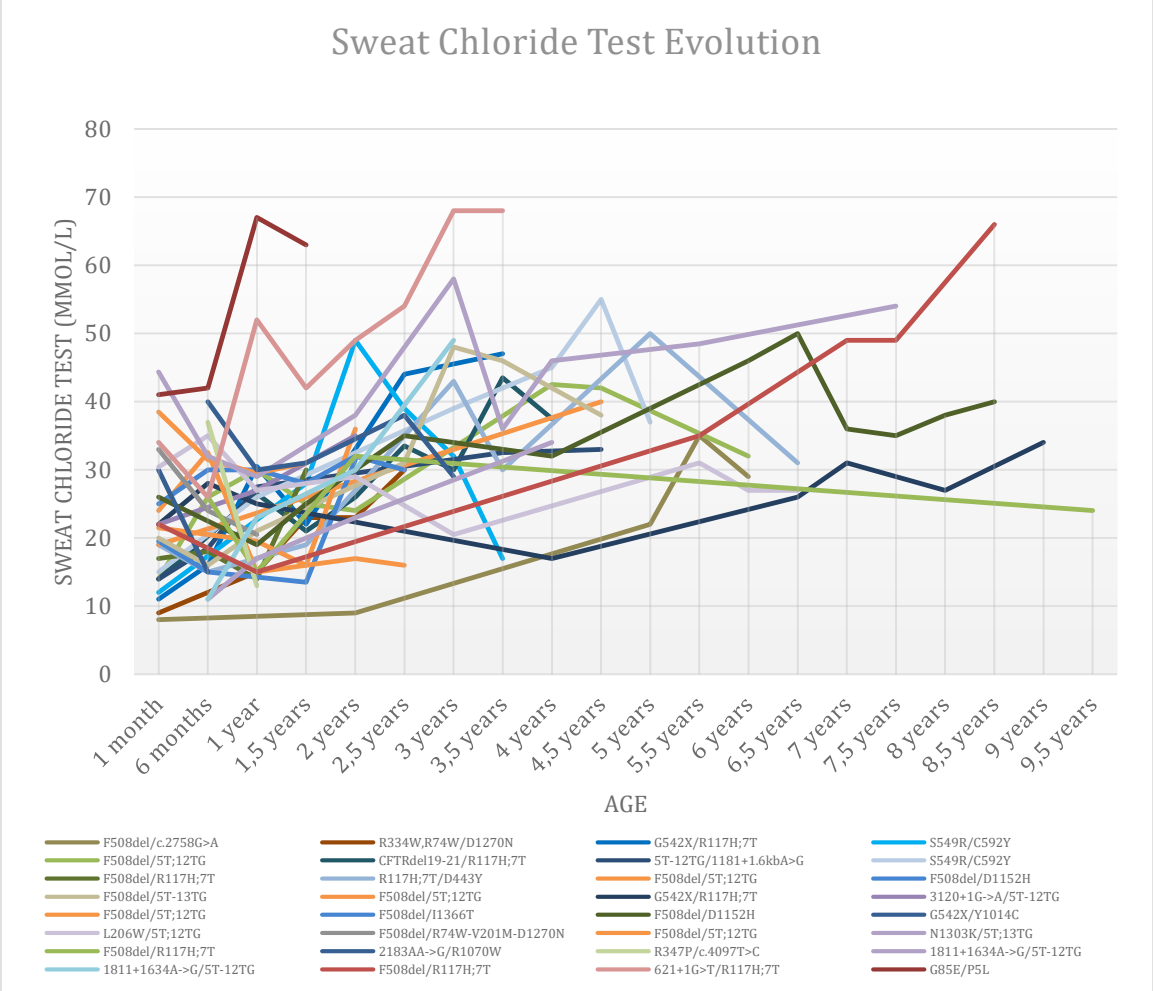
CF, Cystic fibrosis; NBS, Neonatal Bloodspot Screening; IRT, immunoreactive trypsinogen; CFSPID, CF screen positive inconclusive diagnosis; NCFc, Non-CF-causing variant; CFc, CF-causing variant; VVCC, variant of varying clinical consequence; VUS, variant of uncertain significance; CFTR-RD, Cystic fibrosis transmembrane regulator related disorder.

Description: This figure presents the PRISMA flow diagram illustrating the inclusion process of all children diagnosed with CFSPID through CF-NBS in the Community of Madrid (Spain), detailing exclusions, reclassifications, and final diagnostic groups within the cohort.

Figure 2. Longitudinal evolution of sweat chloride values in children with intermediate or positive results



Description: This figure illustrates the individual trends of sweat chloride values (SC) throughout time in 32 children within the CFSPID cohort who developed intermediate (30–59 mmol/L) or positive (≥60 mmol/L) results during follow-up. The figure captures semiannual/annual progression patterns. Most children with abnormal SC values developed them within the first three years of life, with some cases showing marked early increases associated with subsequent

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conversion to CF. This visualization underscores the heterogeneity in SC progression in CFSPID, reinforcing the importance of longitudinal monitoring in identifying individuals at higher risk for progression.

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Table 1. Clinical characteristics of children with CFSPID, stratified by genotype group.

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	CFc/ VVCC	VVCC/VVCC	CFc/VUS
CFSPID children (n = 75)(%)	66 (88%)	4 (5.33%)	5 (6.67%)
Female (%)	36 (54.54%)	4 (100%)	3 (60%)
Detailed *CFTR* Genotypes (% of 75)	F508del/5T;12TG (16%) F508del/R117H;7T (5.33%) F508del/D1152H (5.33%) 1811+1.6kbA->G/5T;12TG (4%) F508del/I1366T (4%) F508del/F1052V (2.67%) G542X/R117H;7T (2.67%) Other combinations (52.01%)	5T;12TG/5T;12TG (1.33%) 5T;12TG/R117H;7T (1.33%) R117H;7T/D443Y (1.33%) D1152H/I1366T (1.33%)	S549R/C592Y (2.67%) F508del/H484Y (2.67%) F508del/D984Y (1.33%)
Median age at the end of the study, years (IQR)	3.58 [1.71 – 4.87]	8.18 [5.51 – 9.6]	4.19 [1.51 – 5.25]
CF-NBS diagnosis by the initial panel (%)	30 (45.45%)	2 (50%)	0 (0%)
Median IRT, ng/mL (IQR)	69.86 [56.24-87.1]	71.13 [65.06 – 95.56]	65 [59.15 – 73.31]
SC results mmol/L (%): - Positive - Intermediate - Negative - Median of the first SC [IQR]	 3 (4.54%) 26 (39.39%) 37 (56.06%) 17 [12 – 23.62] (9 in intermediate range)	 0 (0%) 1 (25%) 3 (75%) 11.5 [10.25 -13.75]	 0 (0%) 2 (40%) 3 (60%) 15 [12.5 – 15]
Respiratory symptoms (%)	PEx (19.7%) bronchiolitis (9.09%) wheezing (6%) pneumonia (6.06%) asthma (3.03%)	PEx (25%) wheezing (25%)	pneumonia (40%) wheezing (20%)
Bronchial colonization isolations (%)	*H. influenzae* (43.94%) *M. catarrhalis* (39.39%) MSSA (37.88%) Usual flora (34.85%) *S. pneumoniae* (34.85%) *S. pyogenes* (7.58%) *H. parainfluenzae* (4.54%) *A.fumigatus complex* (4.54%) *E. coli* (4.54%) K. pneumoniae (4.54%) *C. propinquum* (3.03%) *S. maltophilia* (1.51%) *MRSA* (1.51%) *Candida Albicans* (1.51%)	*MSSA* (25%) *S. pneumoniae* (25%) *H. influenzae* (25%)	*S. pneumoniae* (40%) *H. influenzae* (40%) MSSA (20%) *M. catarrhalis* (20%) Usual flora (20%)
Best FEV_1 *z-score*, median [IQR]	0.12 [-0.41, +1] (n = 9)	1.76 [+0.19, +3.33] (n = 2)	0.39 (n = 1)
Digestive symptoms (%)	constipation (13.64%) gastroesophageal reflux (1.5%)	constipation (50%)	constipation (20%)
Pancreatic sufficiency	100% (n = 48)	100% (n = 1)	100% (n = 3)
Last BMI *z-score* [IQR]	0.16 [-0.5, +0.5]	0.6 [-0.12, +1.62]	1.8 (n = 1)
Hospitalizations for respiratory or digestive reasons	bronchospasm (3.03%) pneumonia (3.03%) gastroenteritis (3.03%) bronchiolitis (1.51%)		pneumonia (20%)

CF, Cystic fibrosis; CFTR, Cystic fibrosis transmembrane conductance regulator; CFSPID, CF screen positive inconclusive diagnosis; CFc, CF causing variant; VVCC, variant of varying clinical consequence; VUS, variant of uncertain significance; IQR, Interquartile range; CF-NBS, CF newborn blood-spot screening; IRT, Immunoreactive trypsinogen; SC, Sweat chloride test; PEx, Pulmonary exacerbations; *H. influenzae,*

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Haemophilus influenzae; M. catarrhalis, Moraxella catarrhalis; MSSA*, Meticillin sensitive Staphylococcus aureus;* S. *pneumoniae, Streptococcus pneumoniae; S. pyogenes, Streptococcus pyogenes; H. parainfluenzae, Haemophilus parainfluenzae; A. fumigatus complex, Aspergillus fumigatus complex; E. coli, Escherichia coli; Klebsiella pneumoniae, K. pneumoniae; C. propinquum, Corynebacterium propinquum; S. maltophilia, Stenotrophomonas maltophilia; E. cloacae, Enterobacter cloacae; MRSA, Methicillin-resistant Staphylococcus aureus;* FEV_1, forced expiratory volume in one second;

Description: This table summarizes the clinical, biochemical, and genetic characteristics of children diagnosed with CFSPID in the Community of Madrid (Spain), stratified into 3 groups according to genotype: (1) Cystic fibrosis causing variant (CFc) with a Variant of varying clinical consequence (VVCC), (2) compound heterozygosity for VVCCs, and (3) CFc with a Variant of uncertain significance (VUS). Data include sex distribution, age at first visit, sweat chloride (SC) categories during follow-up, respiratory and digestive symptoms, pancreatic status, lung function, and microbiological findings, illustrating the heterogeneity within the cohort. "Intermediate" SC refers to values of 30–59 mmol/L during follow-up. Pancreatic sufficiency was defined by fecal elastase > 200 µg/g. Pulmonary exacerbations (PEx) include episodes requiring antibiotic treatment.

Table 2. Clinical and diagnostic features of CFSPID children who converted to Cystic fibrosis (CF) or CFTR-Related disorder (CFTR-RD)

Conversions	Case #1 CF	Case #2 CF	Case #3 CF	Case #4 CFTR-RD
Genotype	*G85E/P5L* (CFc/VVCC)	*621+1G>T/R117H;7T* (CFc/VVCC)	*F508del/R117H;7T9T* (CFc/VVCC)	*F508del/D1152H* (CFc/VVCC)
Year of Birth	2022	2019	2015	2015
Gender	Male	Female	Male	Female
IRT (ng/mL)	97.2	78 / 47.9	44.8	43.8
Initial SC (mmol/L)	38 / 44	34	22	26
SC progression	Intermediate at 1st month Positive since 0.8 yrs	Intermediate at 1st month Positive since 3.2 yrs	Intermediate at 5 yrs Positive at 8.7 yrs	Intermediate since 2.5 yrs
Clinical Symptoms	Recurrent PEx from 0.8 years	Dry cough from 1.5 years 1 PEx/year	One PEx at 2.7 yrs	Persistent cough and recurrent PEx since 3.5 yrs
Respiratory Therapy	RP since 9 months	RP from the age of 2	Not required	RP, ICS, chronic azithromycin, inhaled ampicillin
Microbiology	*MSSA* *K. pneumoniae* *H. influenzae* *M. catarrhalis* *A. fumigatus*	*C. propinquum* *M. catarrhalis* *H. influenzae* *S. pneumoniae*	*MSSA*	*MSSA* *H. influenzae*
FEV_1 z-score	Not available	Not available	–1.89 to –0.19 SD	+2.5 SD

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Pancreatic Status	Sufficient	Sufficient	Sufficient	Sufficient
Digestive Findings	Stable	GERD Constipation	BMI -1.9 SD at 14 months, normalized later	Constipation, dysphagia, abdominal pain
Last BMI z-score	-0.82	-0.24	-0.18	-1.1
Hospitalizations	None	None	None	None

CF, Cystic fibrosis; CFTR-RD, Cystic fibrosis transmembrane conductance regulator related disorder; CFc, CF causing variant; VVCC, variant of varying clinical consequence; IRT, Immunoreactive trypsinogen; SC, Sweat chloride test; PEx, Pulmonary exacerbations; RP, Respiratory physiotherapy; *MSSA, Meticillin sensitive Staphylococcus aureus; Klebsiella pneumoniae, K. pneumoniae; H. influenzae, Haemophilus influenzae; M. catarrhalis, Moraxella catarrhalis*; *A. fumigatus complex, Aspergillus fumigatus complex; C. propinquum, Corynebacterium propinquum; S. pneumoniae, Streptococcus pneumoniae*; FEV_1, forced expiratory volume in one second; GERD, gastroesophageal reflux; BMI, body mass index; SD, standard deviations.

Description: This table illustrates detailed clinical, biochemical, and genetic characteristics of the 4 children within the CFSPID cohort who converted to cystic fibrosis (CF) (n = 3) or CFTR-Related Disorder (CFTR-RD) (n = 1) during follow-up. It includes genotype, age at conversion, sweat chloride evolution, pancreatic status, respiratory symptoms, microbiological findings, and anthropometric data, illustrating the heterogeneity of clinical progression in this subgroup. Conversion to CF was defined by two sweat chloride values ≥ 60 mmol/L, while conversion to CFTR-RD was based on persistent isolated respiratory symptoms without meeting CF diagnostic criteria.

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