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"Cystic Fibrosis and Sweat Test – Clinic and Genetic Characterization of a Portuguese Sample Non-Screened not included in the Neonatal Screening"- preliminary results

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ABSTRACT

Cystic fibrosis (CF) is the most common and lethal autosomal recessive disorder among the Caucasian population. Advances in medical care have extended the life expectancy of affected individuals to beyond 40-50 years, transitioning CF from a predominantly pediatric disease to a chronic, multi-system condition. In Portugal, neonatal screening for CF was introduced in 2013, becoming the primary diagnostic method. However, for cases not detected through neonatal screening, clinical presentation becomes vital for diagnosis and the sweat test (ST) remains the gold standard for CF diagnosis, particularly when sweat chloride levels are ≥60 mmol/L and two CFTR mutations are identified. Diagnosis becomes complex with chloride levels of 30-59 mmol/L. In 2015, intermediate chloride levels (30-59 mmol/L) were recognized for all ages, reclassifying previously 'unlikely CF' cases (30-40 mmol/L) to 'possible CF'. Despite advances, late diagnoses with significant lung impairment are still reported globally, affecting patient outcomes. Moreover, CFTR variants can lead to atypical CF manifestations. The phenotypic variability, particularly in the Portuguese population, suggests many undiagnosed cases and uncertain CFTR mutation impacts.

This study focuses on a sample of Portuguese individuals selected through three methods: analysis of all STs performed at Centro Hospitalar do Porto over seven years, identifying those with intermediate ST levels; identification of individuals followed at northern Portugal hospitals over five years with clinical suspicion of CF but without diagnosis; and analysis of bronchiectasis databases, selecting cases without iontophoretic ST determination or with intermediate ST levels. Inclusion required at least two STs performed by iontophoresis, with two intermediate and/or positive results necessary for final inclusion. Exclusion criteria included prior CF diagnosis, normal ST results by iontophoresis, other etiologic diagnoses of non-CF bronchiectasis, or neonatal diagnosis (post-October 2013 births). Participants completed a questionnaire to gather clinical and demographic data, fecal elastase quantification, spirometry, and CF serum genetic data (most common mutations, followed by sequencing if fewer than two mutations were detected). Data were encoded, stored in a database, and analyzed descriptively and correlatively, with statistical significance set at $p<0.05$.

This ongoing study faced limitations primarily due to the SARS/COV2 pandemic. Adjustments to the initial schedule were necessary due to increased clinical care demands and efforts to minimize exposure risk for eligible individuals. Initially, the study focused on the CHP Sweat Test Database. From 2010 to 2016, 1817 sweat tests were conducted at CHP, with 303 showing intermediate values; 150 of these were included in the final sample. The sample consisted of 150 individuals, with a median age of 13 years at the initial data collection (November 2020). Among these, 69.3% (n=103) were under 18 years, with a slight male predominance (n=81, 54%). The primary symptoms prompting sweat tests were difficult-to-control asthma (n=60, 40%), poor weight gain (n=27, 18%), bronchiectasis/pulmonary CT alterations (n=20, 13.3%), and recurrent pneumonia (n=11, 7.3%).

The genetic characterization of CFTR mutations/variants in a sample of Portuguese individuals will provide valuable insights into the frequency of specific mutations/variants and their correlation with phenotypic manifestations. It is important to note that this study is ongoing, and we eagerly await the final results.

Introduction

Cystic fibrosis (CF) is the most frequent and lethal autosomal recessive condition in the Caucasian population. Estimates indicate that children currently born with the disease have an average life expectancy beyond 40–50 years, making this no longer an almost exclusively pediatric condition. CF is a multisystemic condition, caused by mutations on the CF transmembrane conductance regulator (CFTR) gene on chromosome 7, which encodes for an ion channel protein. Over 2000 variants of the CFTR gene have been reported to date, being F508del the most common. Neonatal screening of CF has been developed and implemented in Portugal in 2013, and since then this represents the main diagnostic procedure for the condition. When considering CF diagnosis beyond the neonatal screening setting, signs and symptoms at the time of clinical presentation become crucial. Compared with individuals presenting only with atypical features, those with multiple typical CF manifestations at presentation will most likely have underlying CFTR alterations as an explanation for the observed phenotype. The sweat test (ST) is the gold standard for CFTR assessment and for the diagnosis of CF, allowing for an almost unequivocal diagnosis when the levels of chloride in sweat are equal to or greater than 60 mmol/L and two genetic mutations are present. However, diagnosis is more challenging when chloride values between 30–59 mmol/L are found in the ST. In 2015, the "*Diagnosis Consensus Conference Committee*" considered chloride levels between 30–59 mmol/L as intermediate for all age groups. As a result, patients with clinical suspicion of CF and chloride levels between 30 and 40 mmol/L, which were previously classified as 'unlikely CF', are now considered 'possible CF' according to current guidelines. Reports of late diagnoses of the disease are still described worldwide, often with bronchiectasis and/or severely compromised lung function, with impact on patients' prognosis. On the other hand, CFTR gene variants may contribute to less frequent clinical manifestations of CF. Patients with bronchiectasis have a higher incidence of CFTR mutations, including variants reported to have a reduced impact in CFTR function. Overall, the phenotypic variability of this condition, and particularly in the Portuguese population, suggests that a large number of cases remain undiagnosed and that there are several CFTR alterations with uncertain phenotypic impact.

The main objective of this paper is to reveal the preliminary results of a study which aims to: aims of this work are to: (i) characterize, both clinically and genetically, a sample of Portuguese individuals with intermediate levels in the ST, according to recently published guidelines; (ii) diagnose CF cases in the Portuguese population not covered by neonatal screening and characterize them clinically and genetically; and (iii) reference all cases identified in the study to CF Reference Centers, precluding disease progression and mitigating associated morbidity/mortality by starting appropriate treatment.

Material and Methods

The planned study consists of an observational study of a sample of individuals from the Portuguese population selected in three different ways: analysis of every ST performed at Centro Hospitalar do Porto (CHP) over a period of 7 years and selection of samples with intermediate ST levels; identification of individuals followed at hospitals from the northern region of Portugal over a period of 5 years with clinical suspicion of CF but without CF diagnosis; analysis of bronchiectasis databases and selection of cases without iontophoretic ST determination or with intermediate ST levels. Only cases with, at least, two STs performed by iontophoresis (previously to study entry or during the study, if necessary) are being considered for study inclusion and only those with two intermediate and/or positive ST results are being be included in the final study sample. Cases with a CF diagnosis prior to study entry, with a normal ST result as determined by iontophoresis, with other etiologic diagnoses of non-CF bronchiectasis, or covered by neonatal diagnosis (i.e. born after october 2013) are excluded from the study. A study questionnaire is being given to all study participants, with the purpose of retrieving clinical and

demographic data, fecal elastase quantification, spirometry determination, and CF serum genetic data (by analysis of most common mutations, followed by sequencing when less than two mutations are detected). Retrieved data is being encoded and database stored; final descriptive statistical analyses will be performed, as well as statistical correlation analyses between variables. Statistical significance will be set at $p<0.05$.

Detailed Description – Material and Methods

SAMPLE DEFINITION:
The final study sample will be obtained by three main approaches (**Figure 1**). Only the first of the following stages has been completed:

- **Analysis of sweat tests (ST) performed at CHP by pilocarpine iontophoresis technique:**

Identification of individuals with an intermediate sweat chloride value at the ST(s) according to new diagnostic criteria (chloride 30–59 mmol/L) through evaluation of all STs performed at CHP over the last 7 years (from 01/01/2010 to 31/12/2016). Development of a database for analysis (CHP Sweat Test Database). All cases with at least one sweat test with intermediate chloride values were contacted and invited to participate in the study. Cases with only one sweat test with an intermediate chloride value repeated the test; if test result was again intermediate (chloride 30–59 mmol/L) or the ST was positive, such cases were included in the study sample.

- **Information retrieved from level II/III hospitals from the North region of Portugal:**

Telephone or electronic email contact with hospitals/ medical departments from the North region of Portugal explaining the project and identifying individuals followed at level II and III hospitals over the last 5 years with clinical findings compatible with CF (identified by ICD9 diagnostic coding) and without CF diagnosis. All individuals identified will be contacted and invited to participate in the study. Individuals will initially perform a ST; if test result is normal, individuals will be excluded from the study; if test result is positive or intermediate, individuals will perform a second ST. Individuals with two positive (chloride ≥ 60 mmol/L), one positive and one intermediate (chloride 30–59 mmol/L), or two intermediate ST results will be included in the study sample.

- **Data from the North region of Portugal included in the European Bronchiectasis Registry (EMBARC):**

Medical departments/units from the North region of Portugal responsible for introduction of patient data on EMBARC will be contacted and invited to participate in the study. Cases with a ST performed by the internationally recommended method will be selected. All cases with an identified (non-CF) cause of bronchiectasis will be excluded; cases with a normal ST result (according to new recommendations) or two positive STs will also be excluded. The remaining cases (without etiologic diagnosis, without ST results, or with a ST performed by other methods) will perform an initial ST; if test result is normal, individuals will be excluded from the study; if test result is positive or intermediate, the ST will be repeated. All cases with two positive (chloride ≥ 60 mmol/L), one positive and one intermediate, or two intermediate ST results will be included in the study sample.

All cases with a CF diagnosis before study entry and born after October 21st of 2013 (CF neonatal screening start date in Portugal) will be excluded from the final sample.

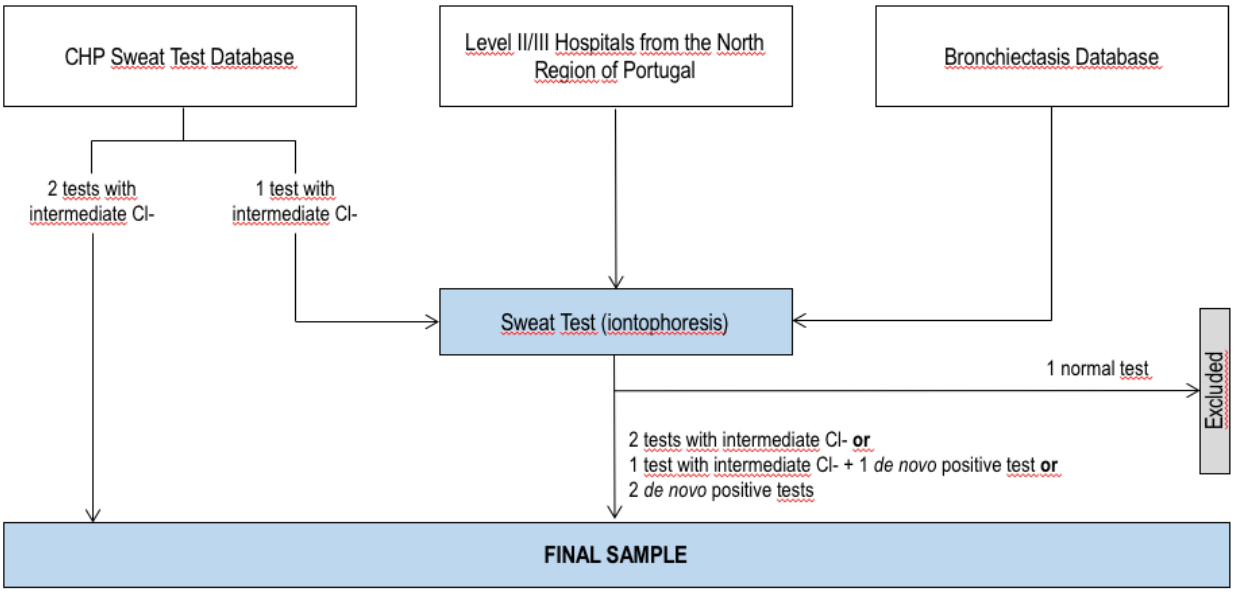


Figure 1. Study sample determination

Clinical and analytic assessment of the study sample

All individuals who consent to participate in the study are being given a questionnaire for clinical/ demographic characterization and are performing faecal elastase quantification, spirometry determination, and CF serum genetic analysis of common mutations. If two CFTR mutations are not identified, sequencing will be performed. All variants/ variant combinations, or mutations identified will be classified according to expected clinical and functional impact, according to *CFTR2* database.

Data analysis

Retrieved data will be encoded and stored in a database of the statistical software IBM SPSS v24®. Descriptive statistical analyses will be performed, through estimation of frequencies and median and standard deviations of variables under analysis. Statistical correlations between study variables will be investigated in order to determine association or dependency relations between them, by using different statistical tests (Chi-square, Fisher, Mann-Whitney, Kruskal-Wallis and Student's t tests) according to their nature and to the type of sample. All tests will be performed at a significance level of 0.05.

Ethical considerations

This study has received authorization by the CHP Health Ethics Committee. Individuals' participation is voluntary and all study participants (or their legal representatives, in cases of children and adolescents aged <18 years old) are properly informed (by oral and written way) of the study objectives and procedures. Individuals aged ≥18 years are asked for written informed consent to participate and informed consent is obtained from legal representatives in cases of individuals aged <18 years old. Individuals aged between 12 and 18 years are further asked written informed consent to participate. Data confidentiality is fully assured for all study participants and legal representatives. Retrieved data is being stored in a secure and confidential database by assigning a code to each participant, using the statistical software IBM SPSS v24®. Only the study investigator has access to the participants' codes, assuring anonymity and preserving their identity. The same code is subsequently used in the electronic registration of each participant, as a way to secure their identity. Only the study investigator has access to the match between each code and individuals' identification and this information will only be used in cases when participants require a prompt referral to CF Reference Centers, including de *novo* diagnoses.

Results

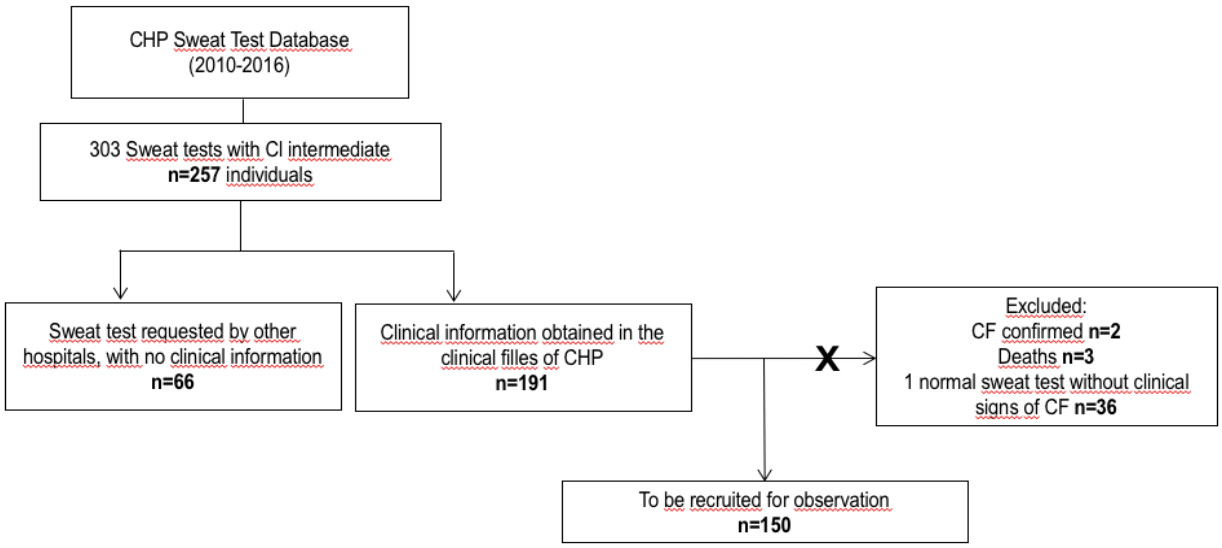
This is an ongoing study and limitations were essentially related to the SARS/COV2 pandemia. Changes had to be made to the initially planned schedule due to increased demand for clinical care activities, integration into the first team responsible for assisting hospitalized patients with SARS/COV2 infection in the Pediatrics Department and also to minimize the risk of exposure for the potentially eligible individuals. For this reason, and in an initial phase, this study focused on the "CHP Sweat Test Database" (left side of figure 1).

Between 2010 and 2016, 1817 sweat tests were performed at CHP, 303 of which had intermediate values, and 150 of these were selected for the final sample (figure 2).

We obtained a final sample consisting of 150 individuals who, at the time of initial data collection (November 2020), had a median age of 13 years (minimum 1 and maximum 12), 69.3% (n=103) of whom were under 18 years of age, with a slight predominance of the male gender (n=81, 54%).

The main symptoms that prompted the sweat test were difficult-to-control asthma (n=60, 40%), poor weight gain (n=27, 18%), bronchiectasis/pulmonary CT alterations (n=20, 13.3%), and recurrent pneumonia (n=11, 7.3%).

Figure 2



Discussion

Cystic fibrosis (CF) is the most frequent and lethal autosomal recessive condition in the Caucasian population. Affecting 1 in every 4000 newborns in the U.S., it has an even higher prevalence is some European countries.[1] Over the last years, disease-associated mortality has decreased exponentially. Estimates indicate that children currently born with the disease have an average life expectancy over 40–50 years of age, making it no longer an almost exclusively pediatric condition.[2,3]

Despite significant advances in the knowledge of this condition, late diagnoses persist due to its phenotypic variability.[3]

It is a multisystemic condition, caused by mutations on the CF transmembrane conductance regulator (CFTR) gene on chromosome 7, which encodes for an ion channel protein. Over 2000 variants of the CFRT gene have been reported to date, being F508del the most frequent.[1,3]

Clinical presentation is diverse and depends on which mutations are present. Classically described clinical diagnostic criteria include meconium ileus, steatorrhea, failure to thrive, recurrent respiratory tract infections, persistent cough, bronchiectasis, digital clubbing, sinusitis, and nasal polyps. Disease symptoms, severity, and prognosis depend on several factors, including gene mutations, microbiologic

agents identified in respiratory secretions, nutritional status, and even patient's socio-economic context. CF mostly affects the lungs, what greatly contributes to the disease morbidity/mortality. Dysfunction of the CFTR gene expression in epithelial cells of the respiratory tract originates an ion transport defect responsible for subsequent mucus thickening. Due to this, respiratory secretions become more susceptible to microorganism proliferation, causing infections, inflammation, and tissue injury.[2-4]

The diagnosis of CF is easily established when the condition has a typical clinical presentation, namely when symptoms develop during the first months of life. Clinical suspicion is confirmed when levels of chloride in sweat are equal to or greater than 60 mmol/L in the sweat test (ST). Since the discovery of the CFTR gene, it became evident that the diagnosis of CF is not always straightforward, as several diseases are associated with mutations in this gene. Individuals with two genetic mutations may present with mild symptoms only manifesting during adolescence or adulthood, or with isolated signs or symptoms, as infertility or pancreatitis.[3,9] Consequently, due to its considerable phenotypic variability, CF demands a broad and comprehensive clinical approach, preferably by health professionals with a significant clinical experience and expertise in the condition.[2]

Initially, diagnostic suspicion was based exclusively on the phenotype, through identification of characteristic signs and symptoms. With the introduction of neonatal screening, this became the preferential (and most commonly performed) diagnostic procedure.[2] Although it has been performed in several countries for several years now, in Portugal CF screening started in october 2013 through a national pilot study supported by *Unidade de Rastreio Neonatal, Metabolismo e Genética* of the National Health Institute Doutor Ricardo Jorge (INSA).

The ST is the most commonly used diagnostic tool for CFTR function assessment. It quantifies the amount of chloride in sweat by pilocarpine iontophoresis, which was described for the first time by Gibson & Cooke in 1959[5] and remains the gold standard for CF diagnosis.[6] Sweat is collected through the Macroduct® system, according to recommendations by the main international reference centers. The test is easily performed and takes between 20 to 30 minutes.[2] Results may unequivocally indicate CF (for instance, if sweat chloride is ≥60 mmol/L and two CFTR mutations are found). The challenge is bigger when intermediate values are found in the ST (sweat chloride between 30−59 mmol/L).[4]

Diagnostic criteria for CF were revised in 2015 in the '*US CF Foundation Diagnosis Consensus Conference*' and included the latest evidences concerning neonatal screening methods and recommendations for nonscreened populations (**Table 1**).[1,4]

When considering CF diagnosis outside of neonatal screening, clinical recognition of characteristic signs and symptoms becomes crucial (**Table 2**).[1,4] An individual with multiple typical features of CF (including bronchiectasis, nasal polyps, pancreatic insufficiency) has a higher probability of CFTR dysfunction as an explanation for the observed phenotype than an individual with atypical clinical manifestations only (including isolated symptoms, as chronic cough, sputum without bronchiectasis and/or recurrent pancreatitis).[4]

In 2008, a sweat chloride value ≥60 mmol/L in the ST was considered diagnostic for CF (positive ST) and values from 30 to 59 mmol/L in infants aged less than 6 months, or from 40 to 59 mmol/L in individuals aged over 6 months, were considered intermediate levels (intermediate ST). In 2015, the "*Diagnosis Consensus Conference Committee*" classified values from 30 to 59 mmol/L as intermediate for all age groups (**Table 3**). The fact that some individuals have a diagnosis of CF and sweat chloride values <60 mmol/L may be largely explained by mutations in the CFTR gene that do not translate in the total loss of chloride channel function. To determine the clinical and functional impact of CFTR mutations, the *Clinical and Functional Translation of CFTR*" (CFTR2) was developed.[4,7]

Table 1. 2015 *CF Foundation Diagnosis Consensus Conference* guidelines for diagnosis of Cystic Fibrosis in nonscreened populations

Statement numbers*	*Consensus statements*	Comentários
25.	For individuals presenting with CF symptoms, the same diagnostic criteria recommended for the screened population for sweat chloride testing, CFTR genetic analysis, and CFTR functional testing should be used to confirm a CF diagnosis.	This represents a change in cut-off values used in the sweat test. In previous statements, a sweat chloride level <40 mmol/L indicated 'non-CF'; currently, this level was replaced by <30 mmol/L.
26.	The diagnosis of CFTR-related disorder has been defined as a monosymptomatic clinical entity (CBAVD/pancreatitis/bronchiectasis) associated with CFTR dysfunction that does not fulfil the diagnostic criteria for CF.	Requires an international effort to characterize this clinical entity.
27.	Clinicians should avoid the use of terms like classic/nonclassic CF, typical/atypical CF, delayed CF, because these terms have no harmonized definition and could be confusing for families or caregivers.	The *European CF Society Diagnostic Network Working Group* has kept the terms '*nonclassic*' or '*atypical*' CF.

CBAVD, congenital bilateral absence of the vas deferens.
*Adapted from Farrell et al.[1]

Table 2. Signs and symptoms of cystic fibrosis

	Frequent Presentation	Infrequent Presentation*
Family history	Sibling or parent with CF	Parent of child with CF
Perinasal sinuses	Chronic sinusitis, nasal polyposis	-
Respiratory system	Bronchiectasis, recurrent or chronic lower respiratory tract infection (particularly by *Pseudomonas*)	ABPA, non-tuberculosis mycobacteria infection, asthma, chronic obstructive pulmonary disease
GI/Lumen	Meconium ileus, distal intestinal obstruction	Abnormal intestinal motility, rectal prolapse
GI/Hepatobiliary	Pancreatic insufficiency, recurrent pancreatitis	Hepatic enzyme elevation, ecchymosis, cirrhosis, prolonged neonatal jaundice, liposoluble vitamin deficiency (may manifest as anemia, edema, night blindness and skin rash)
Reproductive system	Congenital bilateral absence of the vas deferens (CBAVD)	Female infertility

Others	Hyponatremic dehydration, failure to thrive	Pseudo-Bartter syndrome, *aquagenic wrinkling of skin*, digital clubbing

ABPA, *allergic bronchopulmonary aspergillosis*; CBAVD, *congenital bilateral absence of the vas deferens*; GI, gastrointestinal
* many of the infrequent manifestations are not rare in CF patients; however, they are not frequent as isolated manifestations.
Adapted from Farrell et al[1] and Sosnay et al.

Table 3. Sweat Test result interpretation

Result	Chloride value
Normal	Chloride <30 mmol/L
Intermediate	Chloride 30–59 mmol/L
Positive	Chloride >60 mmol/L

Adapted from Farrel et al.[1]

Individuals with mild symptoms often present with intermediate ST values (30–59 mmol/L).[9]

Consequently, individuals with clinical findings compatible with CF and ST values from 30 to 40 mmol/L, which were previously classified as 'unlikely CF', are now classified as 'possible CF', according to current guidelines.[4]

Reports of late diagnoses of CF continue to be described worldwide, often with associated bronchiectasis and/or severely impaired lung function, with an impact on disease prognosis.[8,4] The diagnosis is more straightforward in individuals with major mutations and classical symptoms, including presence of the most prevalent mutation, F508del.[3]

On the other hand, mutations on the CFTR gene may contribute to the development of bronchiectasis and other less frequent clinical manifestations in CF. Indeed, the incidence of CFTR mutations is higher in individuals with bronchiectasis, including mutations with a typically residual impact on CFTR function. Similar mutational variants are also found in patients with pancreatitis, chronic sinusitis, and bilateral absence of the *vas deferens*.[4]

It is also interesting to observe that CFTR expression and function can show some degree of impairment in airway epithelia in other conditions, like COPD and cigarette smoke[10].

Hence, in an era of new modulatory therapies with proven benefit and significant impact on patients' quality of life, it is very relevant to investigate the presence of CFTR dysfunction, identify the corresponding genotype, and determine if specific mutation(s) is(are) responsible for a specific phenotype.[4]

An accurate diagnosis is key to start appropriate treatment as soon as possible. This has been acknowledged to have an impact on the reduction of morbidity/mortality and improvement of patients' quality of life. Therapeutic options have substantially improved, not only through the development of better and more directed antibiotics and less invasive administration routes, but also of drugs that seek to correct the CFTR dysfunction.[2]

In an era of new therapies with proven benefit for patients with this condition, the determination of individuals' genotype and its correlation with phenotype is very relevant.[4]

The identification of CFTR was the basis for the development of life changing treatments that can restore the protein disfunction that characterise CF. Nevertheless, several studies documented that a wide spectrum of CFTR variants can cause different types of CFTR dysfunction, traditionally classified in classes I to VI/VII.[10]

Few studies have sought to make the genotype-phenotype correlation for different CFTR mutations/variants in the Portuguese population, particularly in cases with clinical findings compatible with CF and intermediate ST values.

The phenotypic variability of CF, and particularly in the Portuguese population, suggests, not only the presence of undiagnosed cases, but also the existence of cases with different degrees of CFTR dysfunction with unknown clinical impact.[4] Diagnosis and characterization of individuals not included in the neonatal screening program, born in the country before october 2013 and with clinical findings compatible with CF, may represent a significant challenge. Our preliminary results regarding phenotypic variability in the presence of CFTR protein dysfunction are consistent with what has been described in the literature.

Conclusions and final comments

The genetic characterization of CFTR mutations/variants in a sample of individuals from the Portuguese population will enable a better understanding of the frequency of certain mutations/variants in the referred population and their correlation with phenotypic manifestations. An accurate CF diagnosis, even in advanced stages of the disease, has the potential to reduce or even prevent irreversible organ damage and to start proper conventional and/or new treatments, with a significant impact on patients' morbidity/mortality and prognosis. Despite recent progresses, challenges and questions encountered at the time of diagnosis should not delay treatment, as it is associated with substantial benefits for patients. The present study may represent an additional contribute to standardise diagnostic criteria and phenotypic characterization of the population not covered by neonatal screening, particularly in cases with intermediate ST values.
It is important to note that this study is still ongoing, and we eagerly await the final results.

Beyond CF, we hope this work could provide further insights into the role of CFTR in other mucostatic airway diseases, such as COPD, leading to more specific and effective therapies for these common lung diseases.

Conflict of Interest Statement:

None

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None

References

1. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, Howenstine M, McColley SA, Rock M, Rosenfeld M, Sermet-Gaudelus I, Southern KW, Marshall BC, Sosnay PR. Diagnosis of Cystic Fibrosis: Consensus Guidelines from the Cystic Fibrosis Foundation. J Pediatr. 2017 Feb; 181S:S4-S15.e1. doi: 10.1016/j.jpeds.2016.09.064. Erratum in: J Pediatr. 2017 May;184:243. doi: 10.1016/j.jpeds.2017.02.028. PMID: 28129811.

2. Royal Brompton & Harefield NHS Foundation Trust. Clinical guidelines for the care of children with cystic fibrosis. 7th edition. Available on: www.rbht.nhs.uk/childrencf. United Kingdom, 2017.

3. Bush A, Bilton D, Hodson M. Hodson and Geddes' cystic fibrosis.

4. Sosnay PR, White TB, Farrell PM, Ren CL, Derichs N, Howenstine MS, Nick JA, De Boeck K. Diagnosis of Cystic Fibrosis in Nonscreened Populations. J Pediatr. 2017 Feb;181S:S52-S57.e2. doi: 10.1016/j.jpeds.2016.09.068. PMID: 28129813.

5. Gibson LE, Cooke RE. A test for concentration of electrolytes in sweat in cystic fibrosis of the pancreas utilizing pilocarpine by iontophoresis. Pediatrics. 1959 Mar;23(3):545-9. PMID: 13633369.

6. Clinical and Laboratory Standards Institute (CLSI). Sweat testing: sample collection and quantitative chloride analysis; approved guideline – third edition. CLSI document C34-A3 (ISBN 1-56238-713-8). Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2009.

7. Sosnay PR, Salinas DB, White TB, Ren CL, Farrell PM, Raraigh KS, Girodon E, Castellani C. Applying Cystic Fibrosis Transmembrane Conductance Regulator Genetics and CFTR2 Data to Facilitate Diagnoses. J Pediatr. 2017 Feb;181S: S27-S32.e1. doi: 10.1016/j.jpeds.2016.09.063. PMID: 28129809.

8. Nick JA, Nichols DP. Diagnosis of Adult Patients with Cystic Fibrosis. Clin Chest Med. 2016 Mar;37(1):47-57. doi: 10.1016/j.ccm.2015.11.006. Epub 2015 Dec 23. PMID: 26857767.

9. Goubau C, Wilschanski M, Skalická V, Lebecque P, Southern KW, Sermet I, Munck A, Derichs N, Middleton PG, Hjelte L, Padoan R, Vasar M, De Boeck K. Phenotypic characterisation of patients with intermediate sweat chloride values: towards validation of the European diagnostic algorithm for cystic fibrosis. Thorax. 2009 Aug; 64(8):683-91. doi: 10.1136/thx.2008.104752. Epub 2009 Mar 23. PMID: 19318346.

10. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. Eur Respir J. 2014 Oct;44(4):1042-54. doi: 10.1183/09031936.00228013. Epub 2014 Jun 12. PMID: 24925916.

