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LETTER TO THE EDITOR

Impact of *CFTR* large deletions and insertions on the clinical and laboratory severity of cystic fibrosis: a serial case report

Dear editor,

Cystic fibrosis (CF OMIM: #219700) is an autosomal recessive disorder caused by pathogenic variants in the *CFTR* (*Cystic Fibrosis Transmembrane Conductance Regulator*).[1] Among the 2,106 variants described in *CFTR*, large deletions or insertions are considered rare [59 (2.80%)].[2] The identification of large alterations in the *CFTR* is challenging and might result in wrong diagnosis, indicating false-negative in carriers of rare variants that are potentially severe.[3,4] Thus, the implementation of additional techniques in the CF diagnosis workflow becomes necessary, which includes the use of Multiplex Ligation Probe Amplification (MLPA) to identify chromosome rearrangements, deletions, and insertions.[3] So we aimed to describe the genetic profile of large deletions or insertions in *CFTR* identified using MLPA and to describe its influence on CF patients' phenotype in a referral center.

Five CF patients (chloride over 60 mEq/L in two sweat tests) presenting at least one pathogenic variant in the *CFTR* characterized as a large deletion or insertion were included after the study approval by the Ethics Committee (#78192216.2.0000.5404). The caregivers of the CF patients who participated in our study signed the consent to publish patients' data. The screening of the pathogenic variants in the *CFTR* was carried out as previously desbribed.[5] The following markers were described: patients' age at diagnosis; ethnic group; spirometry, classified according to the forced expiratory volume (FEV_1) at different levels of obstruction: mild ($\geq$70%), moderate (60-69%), moderately severe (50-59%), severe (35-49%), and very severe ($<$35%); Shwachman-Kulczycki score graded as excellent (86-100), good (71-85), mild (56-70), moderate (41-55), and severe ($\leq$40)[6]; immunoreactive trypsinogen; and sweat test results. The microbiological evaluation was carried out for the colonization by 11 microorganisms. In addition, the comorbidities and medication used by the patients were described.

All the patients had one identified variant, c.1521_1523delCTT (F508del; p.Phe508del). The MLPA technique also identified four variants considered large deletions or insertions, namely, *CFTR*dele7-18, *CFTR*dup6b-16, *CFTR*dele14b+*CFTR*dup9, and *CFTR*dele16-20. The variant *CFTR*dele16-20 was identified in two patients. The *CFTR* genotype, race, and diagnostic tests were described (Table 1), as well as the comorbidities, Shwachman-Kulczycki score, microorganism profile, and medication used by the patients (Table 2).

In our cohort, four patients were self-declared Caucasians, and one was of mixed race; four of them were female. Two patients were diagnosed when they were five months old; two were two months old; and one was one month old. The Shwachman-Kulczycki score varied distinctly for each participant. All participants were colonized by *Pseudomonas aeruginosa* and *Staphylococcus aureus*, while unequal colonization by other microorganisms was observed in the patients. All participants used inhaled antibiotics, mucolytic agents, nutritional supplements, and pancreatic enzymes; four patients used bronchodilator and one used inhaled corticosteroid. In addition, all patients in our study cohort had pancreatic insufficiency (Table 2).

Since the *CFTR* pathogenic variants present different effects on the phenotype, it seems relevant to optimize the detection method to avoid inaccurate and/or delayed diagnosis.[7,8] In such contexts, the MLPA technique implementation in the CF diagnosis should be optimized.[7] For instance, Atag et al. (2019) evaluated 250 CF patients that presented 80 genetic distinct variants in the *CFTR* and, out of those, five (*CFTR*dele2, *CFTR*dele4-11, *CFTR*dele5-10, *CFTR*dele12, and *CFTR*dele19-21) were characterized as large deletions and occurred in 16 CF patients. Large deletions were associated to the worst pulmonary phenotype, pancreatic insufficiency and liver involvement.[8] The same findings were reported by Martins et al. (2019) who reported the presence of a severe phenotype with pancreatic insufficiency and infection by *P. aeruginosa*[9] in five patients with large deletions or insertions in the *CFTR*.

The identification of all types of *CFTR* variants, including large deletions and insertions, should be one of the main points to be considered in the patients' differential diagnosis.[4] For example, in a study carried out in Serbia, twenty-two different *CFTR* variants were identified in the population studied, evidence of high heterogeneity. Most of these variants had not been reported in neighboring countries, possibly due to the use of commercial tests for CF diagnosis in those places, which did not include the MLPA technique. Due to the use of different molecular analysis techniques,

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Table 1 Description of genotype, race, and diagnostic tests results in cystic fibrosis patients in the presence of *CFTR* large deletions or insertions.

Marker		**BSD**	**JDQS**	**EVFM**	**JVQS**	**ECM**
CFTR Genotype	Allele 1	F508del	F508del	F508del	F508del	F508del
	Allele 2	*CFTR*dele7-18	*CFTR*dele16-20	*CFT*Rdup6b-16	*CFTR*dele16-20	*CFTR*dele14b and CFTRdup9
Race		Caucasian	Caucasian	Caucasian	Mixed race	Caucasian
IRT (ng/mL)*		204/131	86/138	262/354	159/147	260/410
Sweat chloride ion (mEq/L)*		89/92	95/92	95/90	110/106	116/128

F508del; p.Phe508del = c.1521_1523delCTT; IRT: immunoreactive trypsinogen; *CFTR*: Cystic Fibrosis Transmembrane Regulator.
* , first and second dosages were demonstrated.

Table 2 Comorbidities, Shwachman-Kulczycki score and medications in cystic fibrosis patients in the presence of *CFTR* large deletions or insertions.

Marker	**BSD**	**JDQS**	**EVFM**	**JVQS**	**ECM**
Comorbidities					
Nasal polyposis	Yes	No	No	No	No
Meconium ileus	No	No	No	No	Yes
Pancreatic insufficiency	Yes	Yes	Yes	Yes	Yes
Liver involvement	No	No	Yes	No	Yes
Growth deficit	No	Yes	No	Yes	Yes
Persistent respiratory symptom	No	No	No	No	Yes
Metabolic disorder	No	No	Yes	No	No
Shwachman-Kulczycki score (age, months)	54	18	71	104	138
General activity	20	25	25	20	25
Physical examination	20	25	25	20	25
Nutrition	25	25	20	15	25
Thorax X-ray	10	20	20	20	25
Total score	75	95	90	75	25
Score classification	Good	Excellent	Excellent	Good	Excellent
Bacteria					
Pseudomonas aeruginosa	Yes	Yes	Yes	Yes	Yes
mucoid *Pseudomonas aeruginosa*	No	Yes	No	No	No
Staphylococcus aureus	Yes	Yes	Yes	Yes	Yes
Streptococcus pneumoniae	No	Yes	No	No	No
Stenotrophomonas maltophilia	No	Yes	No	No	Yes
Haemophilus influenzae	No	No	Yes	Yes	No
Klebsiella pneumoniae	No	No	Yes	No	No
Escherichia coli	No	No	Yes	No	No
Moraxella catarrhalis	No	No	No	Yes	Yes
Acinetobacter baumannii	No	No	No	No	Yes
Burkholderia cepacia complex	No	No	No	No	No
Bronchodilator					
Short-acting β_2-agonist	No	No	No	Yes	Yes
Long-acting β_2-agonist	Yes	No	No	Yes	Yes
Anticholinergic	Yes	Yes	No	No	No
Inhaled corticosteroid	No	No	No	No	Yes
Inhaled antibiotic					
Colomycin	No	No	Yes	No	Yes
Tobramycin	Yes	Yes	Yes	Yes	Yes
Mucolytic					
Dornase alfa	Yes	Yes	Yes	Yes	Yes
N-Acetylcysteine	Yes	No	No	No	No
Saline solutions					
0.9%	Yes	No	No	Yes	Yes
3%	Yes	No	No	Yes	No
Oral medication					

Table 2 (*Continued*)

Marker	BSD	JDQS	EVFM	JVQS	ECM
Comorbidities					
Azithromycin	No	Yes	Yes	Yes	Yes
Ibuprofen	No	No	No	Yes	No
Corticosteroid	No	No	No	No	Yes
Proton pump inhibitors	No	No	Yes	No	No
H_2 Blockers	Yes	Yes	Yes	No	No
Ursodeoxycholic acid	Yes	No	No	No	Yes
Pancreatic enzymes	Yes	Yes	Yes	Yes	Yes
Nutritional supplement	Yes	Yes	Yes	Yes	Yes
P. aeruginosa eradication treatment	Yes	Yes	No	Yes	No

an increase from 54.45% to 72.8% was observed in the effectiveness rate to identify the *CFTR* genotype.[10]

The description of clinical manifestations along with the identification of large deletions or insertions in the *CFTR* pointed out a more severe phenotype of these patients in our serial case report. And, although younger patients do not present some symptoms, there is still great potential for developing them in the future. Currently, there is no corrective therapy for *CFTR* large deletions or insertions, due to the difficulties of modulating the impact of these large deletions and insertions in the gene expression mechanisms.[11]

In conclusion, our study identified four genetic variants of the type *CFTR* large deletions and insertions, which were characterized by their low genotypic and diagnostic frequency. Two participants presented the same variant, while the variants identified in the other three participants were unique. The identification of large deletions and insertions through a broader genetic analysis is very important for CF diagnosis, since those variants, despite being rare, might be associated with the disease higher severity phenotypes.

Author contribution

All authors approved the manuscript and agreed with its submission to the journal. Also, all authors wrote and revised the manuscript.

Data availability: The complete data collected to perform the study is presented in the manuscript.

Conflict of interest

None.

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