



## CASE REPORT

## Rare CFTR large deletions: a series of cases

*Grandes deleções raras no CFTR: uma série de casos*

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### Abstract

**Aims:** this paper aims to describe diagnosis and follow-up of patients affected by the Cystic Fibrosis (CF) manifestations and *CFTR* large deletions. For this, we performed a retrospective analysis of medical records, including genotyping and retrospective follow-up of clinical and lung function data. Electronic and printed medical records of patients followed at a referral outpatient clinic in CF were evaluated.

**Case description:** we found that three patients had large deletions in the *CFTR* gene, being two of them heterozygous (heterozygous with deletion on exons from 2 to 3, and heterozygous for deletions on exons from 25 to 27) and one of them homozygous (homozygous for the deletions on exons from 19 to 21). One patient had a false negative result in complete genetic sequencing. All three received standard treatment for CF. Two patients died from CF pulmonary complications. Therefore, false negatives findings in *CFTR* sequencing for the diagnosis of CF are rare but may be more frequent in patients with large deletions.

**Conclusions:** *CFTR* large deletions are associated with severe CF phenotypes.

**Keywords:** genetics, diagnosis, newborn screening, sweat chlorid.

### Resumo

**Objetivo:** este trabalho tem como objetivo descrever o diagnóstico e o acompanhamento de pacientes acometidos pelas manifestações da fibrose cística e grandes deleções do gene *CFTR*. Para isso, realizamos análise retrospectiva de prontuários, incluindo genotipagem e acompanhamento retrospectivo de dados clínicos e de função pulmonar.

**Descrição dos casos:** foram avaliados prontuários eletrônicos e impressos de pacientes acompanhados em ambulatório de referência em fibrose cística. Encontramos três pacientes com grandes deleções no gene *CFTR*, sendo dois deles heterozigotos (heterozigotos com deleção nos éxons de 2 a 3 e heterozigotos para deleções nos éxons de 25 a 27) e um deles homozigoto (homozigoto para as deleções nos éxons de 19 a 21.). Um paciente apresentou resultado falso negativo



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no sequenciamento genético completo. Todos os três receberam tratamento padrão para fibrose cística. Dois pacientes morreram de complicações pulmonares da fibrose cística. Portanto, achados falsos negativos no sequenciamento *CFTR* para o diagnóstico de fibrose cística são raros, mas podem ser mais frequentes em pacientes com grandes deleções.

**Conclusão:** grandes deleções de *CFTR* estão associadas a fenótipos graves de FC.

**Palavras-chave:** genética, diagnóstico, triagem neonatal, teste do cloro no suor.

## Introduction

Cystic fibrosis (CF) is an autosomal recessive, chronic and progressive genetic disease that affects several body systems. The cystic fibrosis gene is located on the long arm of chromosome 7, at locus q31, and is formed by 250 kilobases of DNA, with 27 exons, and has the property of coding an mRNA of 6.5 kilobases, which transcribes a protein transmembrane, ion transport regulator, composed of 1480 amino acids, known as Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*). Large deletions within *CFTR* have been estimated to constitute 1–2% pathogenic alleles, but the occurrence could be much higher in classical CF patients with one or none mutation detectable by the routine sequencing work-up.

In Brazil, the estimate incidence of CF is 1:7,576 live births, but it presents regional differences, with higher numbers in the South and Southeast due to the greater presence of immigrants of European origin, as well as differences in the genotypic profile, which show significant discrepancies according to the regions of the country (1). The disease has several possible presentations, with severe symptoms with rapid deterioration or mild symptoms with slow deterioration, and genetic sequencing has allowed the study of mutations and their relationship with disease manifestations. On the other hand, negative genetic sequencing in the presence of classic symptoms should be carefully interpreted. There are specific situations associated with the risk of false-negative findings in the routine "complete" genetic sequencing of coding regions. In these situations, intronic mutations or large deletions should be suspected, especially in families with a history of consangui-

nity and, therefore, a higher risk of homozygosity for infrequent mutations.

In the *CFTR* gene sequencing, the evaluation of the copy number variants (CNV) of the *CFTR* gene is frequently performed using the next-generation sequencing (NGS) technique. This test detects small deletions and duplications up to 17 bp, but large deletions and duplications may be not detected by this methodology. Other structural changes, such as inversions and translocations, may be also not detected. If some of these variations are suspected, methodologies such as array CGH, MLPA, qPCR or FISH can be used.

A retrospective analysis was performed on cases of patients with large deletions in the *CFTR* gene, who underwent standard treatment for CF at a referral outpatient clinic at Hospital São Lucas, Pontifical Catholic University of Rio Grande do Sul (PUCRS).

## Cases description

### Case 1

A 12-year-old female, consanguineous parents, with a family history of CF (cousin), was referred to the CF center in the first years of life reporting resistant stools, and persistent cough. Family came from a small town, rural area in Southern Brazil, and the family have Italian origin. In the month prior to the first visit, she had a positive oropharyngeal swab for *Pseudomonas aeruginosa*. At birth, she had an abnormal newborn screening test, with IRT of 405 ng/mL in the first days of life, associated with the manifestation of meconium ileus in the neonatal period. Sweat test showed chloride = 72 and 105 mmol/L in 2 different samples. Complete genetic sequencing (Sanger) was performed, which presented a negative result for *CFTR* mutations.

Although negative *CFTR* sequencing, the patient maintained the follow-up in CF specialized center, with appropriate therapy for cystic fibrosis and routine check-up tests. During follow-up, she presented respiratory infections, in addition to respiratory colonization by bacteria associated of cystic fibrosis. Considering the clinical mani-

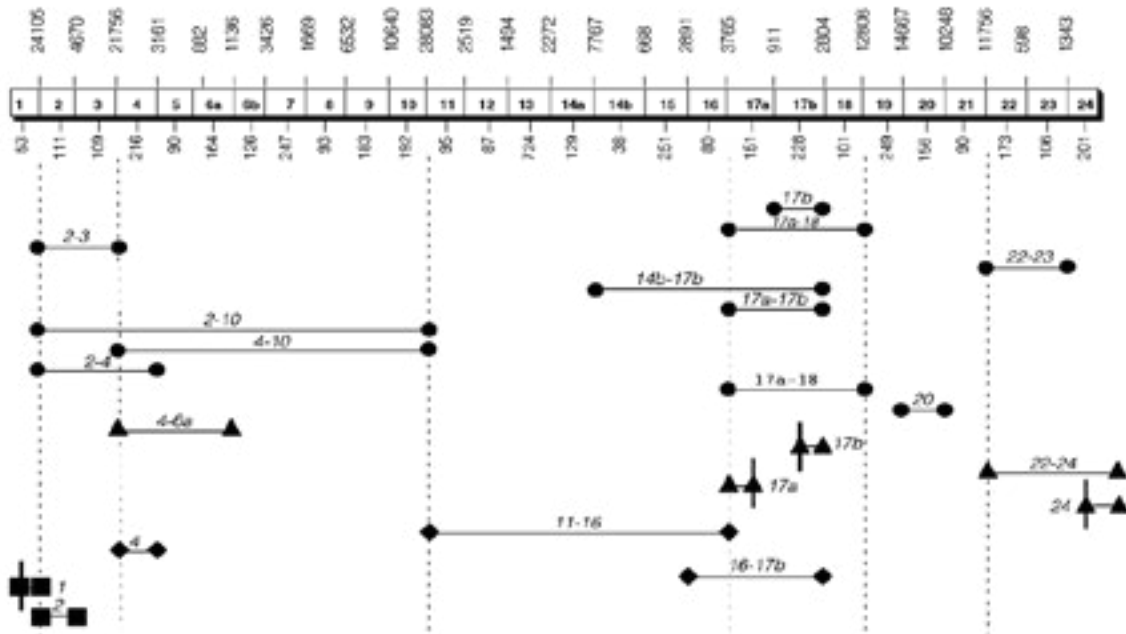
festations with classic CF symptoms, the consanguineous parents and the presence of a cousin with CF, a new complete genetic sequencing, now analyzed through the NGS sequencing (Illumina HiSeq). In a second test, the genetic sequencing showed a homozygosity in the *CFTR* gene, with a deletion in exons from 19 to 21, encompassing approximately the Chr7:117250573-117245767 region, in homozygosity. Genetic counseling was carried out. Finally, the patient continued standard treatment for cystic fibrosis, with no change in CF management. However, it was important to understand genetics of the case reported and how "complete" *CFTR* gene sequencing has also limitations to define CF diagnosis.

### Case 2

Male patient, with a history of frequent hospitalizations for treatment of exacerbations. He presents at the consultation with a complaint of dry cough with clear secretion. At birth, he did not perform a newborn screening test for IRT. In a sweat test performed at 2 years of age, he showed Cl = 79 and 80mmol/L, in two different samples. Upon request for a genetic test, one mutation was identified with amplification of exon 10 of the *CFTR* gene, by polymerase chain reaction (PCR), which showed heterozygosity for the G542X mutation. Detection for F508del mutation and simultaneous analysis of further gene variations were negative. 10 years later, he performed NGS sequencing (Illumina HiSeq), for registration purposes in the Brazilian CF Registry. On examination, in addition to confirming the heterozygous G542X mutation, a large deletion of exons 25-27 was identified, encompassing approximately the Chr:7:117,304,713 – 17,307,162 region. During follow-up, the patient continued to use appropriate therapy for CF, with pancreatic enzymes, vitamins, inhaled alfadornase, hypertonic saline, inhaled and systemic antibiotics as needed. He had episodes of hospitalization and frequent pulmonary exacerbation. CT scans have shown progression of lung disease with diffuse bronchiectasis. He died with 24 years old after massive hemoptysis.

### Case 3

An 8-year-old male patient was referred to the CF center for his first consultation, presenting digital clubbing and complaining of occasional abdominal pain and cough. He brings tests, with sputum showing *Pseudomonas aeruginosa*. At birth, he did not perform a newborn screening test for IRT. In a sweat test performed at 7 years of age, he showed Na of 63mmol/L and Cl of 68mmol/L. In the same year, he performed genetic testing that showed heterozygosity of the *CFTR* gene, with deletion of exons 2,3 and 7T polymorphism (c.743 + 1 G> A). During follow-up, he was hospitalized for pulmonary exacerbation and underwent usual drug treatment, with alfa-dornase, hypertonic solution, azithromycin, vitamins, and respiratory physiotherapy. He died with 21 years after a severe pulmonary exacerbation with mechanical ventilation.



**Figure 1.** Schematic diagram of fully characterized *CFTR* genomic rearrangements involving deletions. Upper panel genomic structure of the *CFTR*. Spanning 189 kb on chromosome 7q31 the gene comprises 27 exons, and encodes a 6.5 kb transcript. Numbers above and below denote the sizes (bp) of the introns and exons respectively. Lower panel characterized large genomic rearrangements involving deletions of the *CFTR* gene. simple deletions with short direct repeats. complex deletions with short insertions of 3–6bp. complex deletions with small insertions. Complex deletions with large insertions of 4100bp. Adapted from: Férec C et al. (2).

## Conclusions

There are more than 2000 mutations over all 27 exons of *CFTR* coding sequence described, in which those variants considered pathogenic are divided into six classes according to their mechanisms and clinical significance (3). Cystic fibrosis genetic testing detects common *CFTR* gene mutations screening to diagnose or identify carriers of the disease by NGS to evaluate *CFTR* copy number variants. Although traditional techniques (PCR, Sanger) and even NGS includes most of the described coding *CFTR* gene mutations, there are limitations regarding its accuracy, and they should be carefully analyzed. Sporadic mutations might occur due to panel or database coverage, as well as the difficulty in distinguishing pathogenic variants from polymorphisms that are not related to disease manifestation. Otherwise, false-negative screening, in which are related to 1-3% of the *CFTR* mutations, may result due to inability to detect large deletions and dupli-

cations (4). Mutations in the CF gene that remain unidentified might be located within introns or in regulatory areas that are not typically examined and could also involve gene rearrangements like significant deletions in one copy of the gene, which may not be detectable using existing PCR-based methods (5). In this sense, negative results of genetic sequencing regarding the presence of classic symptoms must be interpreted carefully. According to these considerations, there are reports reinforcing the hypothesis that patients carrying a homozygous mutation for large *CFTR* deletions could result in the false-negative misdiagnosis of a rare and potentially severe disease presentation. In addition, these situations may occur due to high heterogeneity of the population, and it is particularly important in families with a history of consanguinity and therefore increased risk of homozygosity for infrequent mutations. To evaluate and confirm the presence of such

deletions, there are strategies to explore, such as fluorescent-based real-time polymerase chain reaction (PCR), multiplex ligation-dependent probe amplification (MLPA) or array comparative genomic hybridization (CGH) (6). In the case one, we observed that the second genetic test denoted a homozygous deletion of exon 19 to 21 at the chr:7:117:250:573-117:254:767 position, corroborating with clinical screening and confirming the presence of CF. Similarly, Essawi et al reported a 19 to 21 exon-deletion in *CFTR* gene undetected by NGS that was only confirmed by subsequent MLPA analysis (7). Also, in the CFTR2 database (8), there is only five patients with the *CFTR*dele19-21 described. Moreover, there is no data described in the CFTR2 about the mutation of case two – which led us to think about severity of the mutation, since the patient died at age of 25 with classic CF treatment. To close our series of cases, the case three is not that rare, with several mutations described in CFTR2 database.

In conclusion, these case series reports reinforce the importance of complete gene sequencing in the confirmation of CF diagnosis, since large deletions may be not that rare. Also, negative results of genetic sequencing must be interpreted carefully, since the misleading of diagnosis confirmation can happen, especially when it comes to rare deletions. Furthermore, large deletions are associated with severe phenotypes and there is still not treatment available with CFTR modulators. Further studies focusing on these gene variations may be important to understand better the prognosis and the potential therapeutics.

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### Conflicts of interest disclosure

The authors declare no competing interests relevant to the content of this study.

### Authors' contributions

All the authors declare to have made substantial contributions to the conception, or design, or acquisition, or analysis, or interpretation of data; and drafting the work or revising it critically for important intellectual content; and to approve the version to be published.

### Availability of data and responsibility for the results

All the authors declare to have had full access to the available data and they assume full responsibility for the integrity of these results.

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