Three novel mutations in the CFTR gene identified in Galician patients

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Abstract

We report three novel CFTR missense mutations detected in Spanish patients from Galicia (North West of Spain). In the first case, a patient homozygous for a novel S1045Y mutation died due to pulmonary problems. In the other two cases, both heterozygous for novel mutations combined with the F508del mutation, clinical symptoms were different depending on the mutation, detected as M595I and A107V.
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1. Introduction

More than 1400 different mutations within the CFTR gene have been described to date [1]. It is known that there are mutations associated with severe cystic fibrosis (CF) phenotype and mutations associated with milder or atypical CF disease. The potential of a mutation to contribute to the phenotype depends on its type, localization in the gene and interactions with secondary modifying factors [2,3]. It is assumed that nonsense, frameshift and splice site mutations result in the loss of functional CFTR. Missense mutations are more problematical in that they may only partially affect CFTR function. As the amount of functioning CFTR appears to be related to clinical status, some mutations with more modest effect may present congenital absence of the vas deferens with or without mild respiratory disease.

From 2004, when we started sequencing the exonic regions of the CFTR gene in our laboratory, we have sequenced nearly 150 samples of patients from Galicia (North West of Spain) with possible or independently diagnosed cystic fibrosis. From these analyses we discovered three mutations in the CFTR gene not previously described in the literature: one homozygous S1045Y and two compound heterozygous, A107V and M595I, both combined with the F508del mutation. Each of these is a new missense mutation located in exons 17A, 4 and 13, respectively (Table 1). No other accompanying mutations were identified after complete sequencing of the CFTR gene in each case.

2. Materials and methods

Genomic DNA was extracted from anticoagulated blood using the Wizard Genomic DNA purification kit (Promega).

The sequencing reaction was performed using the Big Dye terminator v3.1 cycle sequencing kit (Applied Biosystems) and analyzed on an ABI 3730XL Genetic Analyzer (Applied Biosystems).

3. Case reports

3.1. Case 1: patient homozygous for mutation S1045Y

A female born in 1972, nulliparous, diagnosed with allergic bronchial asthma at her local primary healthcare centre with allergic sensibility to dust mites, gramineous pollen and

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Table 1
New CFTR mutations found in Galician patients and bioinformatic predictions

AA change	Exon	Polyphen [a]	PSIC [b]	PMUT [c]	Reliability [d]
S1045Y	17A	Possibly damaging	1.864	Pathological	7
M595I	13	Benign	1.310	Neutral	3
A107V	4	Possibly damaging	1.602	Pathological	2

[a] Polyphen prediction about the impact of the amino acid substitution on the function of the protein.
[b] Polyphen value which correlates with the probability of one missense mutation to be pathogenic. Large differences in PSIC score (above 1.5) may indicate that the substitution of interest is rarely or never observed in the protein family.
[c] PMUT prediction about the pathological character of the mutation.
[d] PMUT index which values vary between 0 and 9. Low values correspond to poorer predictions and higher values to better predictions.

Aspergillus. In 1988 she suffered slight acute pancreatitis. In 2001 the patient was diagnosed with bronchopulmonar allergenic aspergillosis.

In 2005 she was hospitalized due to a deterioration of her respiratory symptoms and suggestive data of cystic fibrosis: dyspnoea, productive cough, nasal congestion, diffuse bronchiectasis with central predominance and elevated sweat tests (>90 mEq/L). In the familial study it was found that her only siblings (two brothers) had both died, one of them with biliary pathology and the other with cardiac pathology. In the blood analysis there was leucocytosis (11,300 cel/mm^3), eosinophilia (8.3%) and a total IgE of 1508 ku/L to *Aspergillus.* In the sputum culture *Candida albicans* was isolated while spirometric tests showed levels: FVC (Forced Vital Capacity) 67%, FEV$_1$ (Forced Expiratory Volume) 53%, FEV$_1$/FVC 78%.

From that year the patient suffered many hospitalizations due to respiratory infections with *Pseudomona aeruginosa*, receiving several cycles of antibiotic treatments and tobramicin inhalation over extended periods. At that moment the possibility of a lung transplant was suggested because of the deteriorating respiratory condition.

In April of 2007 and after many urgent hospitalizations because of worsening breathing symptoms (oxygen saturation 88% with a Ventimask at 100%) the patient was admitted to intensive care, was intubated and connected to a mechanical ventilatory system receiving treatment with antibiotics. After a brief initial improvement the patient suffered a fresh deterioration with leucocytosis and raised fever. *P. aeruginosa* and *Morganella morganii* were isolated from cultures.

After the patient stabilized, she was moved to a transplantation unit where copious and thick mucous secretions were found in bronchoscopy throughout the bronchial tree, especially in the upper lobe. At the same time the secretions were cleaned and cultured, showing the presence of *P. aeruginosa* with mucoid morphotype. Twelve days later the patient presented an oliguric renal failure and began a progressive respiratory and haemodynamic deterioration resulting in refractory shock and death three days after.

In this patient we found amino acid change S1045Y: serine (TCT) to tyrosine (TAT) at codon 1045 in exon 17A of CFTR. Interestingly, there are no mutations described in this codon to date.

Further familial studies showed that both parents had the same mutation in heterozygous form and that there was parental consanguinity (first cousins).

3.2. Case 2: patient M595I/F508del

A male born in 1991, the first live birth (two previous stillbirths) with no neonatal pathology. From the first year of life he suffered frequent catarrhs with dry cough at night and distress. He was diagnosed with allergenic rhinitis and bronchial asthma with sensitivity to domestic dust mites in 1999. In a routine consultation the patient showed a sweat test with elevated chloride levels, so was referred to a cystic fibrosis unit.

In 2007 radiology failed to show any pulmonary lesions or gastroenterologic pathology, but chloride levels were still very elevated (>100 mEq/L) while spirometry was also normal. The clinical evolution was practically asymptomatic, showing only bronchial asthma episodes. The age of the patient precluded the ascertainment of the sterility status.

In this patient we found amino acid change M595I: methionine (ATG) to isoleucine (ATA) at codon 595 in exon 13 of CFTR. A previously reported mutation [4] described at the same codon resulted in a change from methionine to threonine. The patient we describe was found to have a F508del mutation in combination with M595I.

3.3. Case 3: patient A107V/F508del

A male born in 1981, diagnosed with clinical symptoms compatible with cystic fibrosis at 8 years old, with a F508del mutation detected at that stage. The patient showed a serious respiratory condition with the following spirometric data: FVC: 40%, FEV$_1$: 27%, FEV$_1$/FVC: 55%.

In 1993 he required hospitalization for a thoracic drainage due to a pneumothorax. In 2005 he presented a haemoptysis with embolyzation where *Staphylococcus maltophilia* and *Staphylococcus aureus* were cultured. In 2007 he was hospitalized again with an important haemoptysis (350 cc approx.) without any other symptoms. Blood analysis showed leucocytosis, microcitic hypochromic anaemia and acute respiratory insufficiency. Thorax radiography gave bilateral brochiectasis with central and left predominance. In the sputum culture *C. albicans* and *S. aureus* were isolated. The haemoptysis was self limited and probably related to a bronchial sepsis. The sweat test showed chloride levels of 96 mEq/L. The patients required substitutive enzymatic treatment with pancreatic enzymes.

In this patient we found an amino acid change A107V from alanine (GCT) to valine (GTT) at codon 107 in exon 4 of the CFTR. A mutation recently described in the same codon resulted in a change from alanine to glycine in a patient with asthenospermia [5].

4. Discussion

We identified three novel missense mutations that led to different amino acid changes in the CFTR protein and to different clinical symptoms in the patients. To predict the possible impact

of the amino acid substitutions in the structure and function of the protein, we used the bioinformatic tools PolyPhen [6] and Pmut [7] which take into account structural and evolutionary properties to characterize a substitution [8,9].

The patient homozygous for mutation S1045Y resulted in an amino acid change from serine to tyrosine that appeared to be the cause of a serious cystic fibrosis condition with multiorganic repercussions and fatal consequences for the patient. PolyPhen and Pmut predicted a possibly damaging or pathological repercussion, respectively, for this mutation. The vast majority of mutations are at frequencies under 0.1% or represent private mutations, normally with a background of the F508del major mutation. So, in the simple cystic fibrosis heterozygotes, any effect of the rare point mutations on the products of the normal CFTR gene cannot be estimated directly due to the co-function of the *trans* normal CFTR gene. Therefore rare cases of homozygosity, related in the described case to consanguinity, can help to understand the effect of the respective mutations on CFTR gene function.

The patient with genotype M595I/F508del did not show severe respiratory symptoms. In fact, with appropriate follow up, the patient seemed to be practically asymptomatic. The substitution of methionine for isoleucine in amino acid 595 appeared to show mild clinical effects. In this case the prediction of PolyPhen and Pmut programs gave a perfect correlation with the clinical status of the patient, predicting the mutation to be benign and neutral, respectively.

In the last case, a patient with the A107V/F508del genotype showed important pulmonary problems. The alanine to valine substitution of amino acid 107 appeared to give more serious repercussions in the clinical evolution of the patient. Predictions of the programs were once again in accordance with the symptoms: PolyPhen predicted possibly damaging effects and Pmut pathological effects.

These three cases clearly reflect a common problem in the genetic counselling of cystic fibrosis, specifically that the spectrum of mutations in the CFTR gene gives rise to a highly variable clinical phenotype that may not be predictable from the genotype. In these cases bioinformatics tools can help guide advice given to the patient, but even with such predictions it is impossible to have certainty of the clinical evolution of the condition. In our study we detected three patients each with novel mutations and indicating different clinical evolution, ranging from a patient that was nearly asymptomatic to a patient who died because of the illness.

We also note that these patients would not have been genetically identified using the current recommended panel for CFTR genetic analysis, particularly the panel based on the American College of Medical Genetics guidelines [10]. This panel is used as a standard in many countries and used as the core mutation set to develop different commercial kits. After several years of experience in our laboratory, we have realized the importance of sequencing the whole CFTR gene, especially having identified an allele with a mutation (generally included in commercial kits). It is not unusual to encounter mutations that are not included in commercial kits and we have experienced this situation on several other occasions in addition to the cases described here.

Finally, case 1 illustrates the importance of an early genetic analysis for patients with suspicion of cystic fibrosis. The patient, homozygous for mutation S1045Y, probably would have been benefit from an early antibiotic treatment avoiding the severe lung problems that finally developed. An early genetic analysis can be useful even for patients showing no clear symptoms of cystic fibrosis in order to avoid this events. Furthermore, the case illustrates the limitations inherent in the use of mutation panels for CF neonatal screening and the necessity of complete CFTR gene analysis or more complete mutation panels. In Galicia we are developing a neonatal screening program based in a panel with all CFTR mutations previously detected in our population, assisted by complete CFTR gene analysis when clinical data indicate suspicion of CF.

Acknowledgments

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