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Long-term Follow-up in Adult Patients with Cystic Fibrosis and Deep Intronic Splicing Variants

Seguimiento a largo plazo en pacientes adultos con fibrosis quística y variantes de splicing en regiones profundas de los intrones

Dear Editor,

Bi-allelic pathogenic variants in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene are the cause of cystic fibrosis (CF).[1] More than 2000 specific variants have been reported to date. Most of these alterations are detected in exons and exon-intron boundaries (splicing variants). Changes in the promoter region, full or partial gene deletions, and more recently, deep intronic splicing variants (DISV) account for the remaining cases. DISV are alterations in the DNA sequence of intronic regions that generate cryptic splicing sites that favour the transcription of intronic sequences (pseudoexons) in mRNA molecules. These pseudoexons serve as templates for the synthesis of dysfunctional proteins, such as the CFTR protein in the case of CF, or create premature stop codons in the pre-mRNA molecules that result in nonsense mediated decay. These pathogenic variants could account for a majority of the 2–5% of remaining unknown variants in both cystic fibrosis (CF) and CFTR-related disorders (CFTR-RD) patients. The study of intronic sequences requires complex and expensive technology.[2] We implemented next-generation sequencing (NGS) to study CF patients in our Genetic Laboratory in 2014 and were able to identify DISV in the second allele of four historical clinical CF cases that have been followed-up in our CF Unit during the last decades.

In order to assess the role of DISV on CF diagnosis, we present 4 cases of patients with clinical suspicion of CF but with incomplete genotype, in whom we detected DISV by NGS technologies.[3] We used the *CFTR* MASTR™ Dx kit (Multiplicom, Niel, Belgium) directed to the 27 *CFTR* coding exons, selected intronic regions or variants and part of the *CFTR* promoter region.[4] Cases 1–3 were diagnosed as CF during childhood by clinical manifestations and positive sweat test. However, only one mutated allele was detected in each patient during infancy. Case 4 was followed–up in our Unit since the age of 19.

The clinical and demographic characteristics of the patients are shown in Table 1.

The deep intronic variant c.1680-883A>G (rs1554388867, Legacy name c.1811+1637A>G, intron 12) was detected in cases 1–3, and the variant c.870-1113_870-1110del (rs397508809, Legacy name c.1002-1110_1113del, intron 7) in case 4. In the first three cases, CF was diagnosed during childhood owing to clinically compatible symptoms and positive sweat tests. The fourth case was initially classified as CFTR-RD at 19 years of age because of clinically compatible symptoms, an inconclusive sweat test (<60 mmol/L Cl^-), and the detection of a single variant in the genetic analysis. CF was confirmed genetically 20 years later following the detection of a DISV in the second allele. This patient presented a severe phenotype with altered lung function, low body mass index (BMI), great extent of bronchiectasis, and a significant delay in diagnosis that could have negatively affected disease progression.

The deep intronic variant c.870-1113_870-1110del, which was found in case 4, is located in intron 7 of the *CFTR* gene and alters the mRNA splicing process creating a pseudoexon with a 101 nucleotide sequence between exons 6b and 7. This variant was initially identified in one patient with a clinically compatible phenotype and a negative sweat test, and in three Italian patients with a classic CF phenotype.[5,6] Moreover, 17 patients with CF from other studies have also been diagnosed with this pathogenic variant.[7–9] The patients have been reported as manifesting a wide spectrum of phenotypes including severe disease, delayed diagnoses, higher frequencies of diffuse bronchiectasis, with or without colonisation by *Pseudomonas aeruginosa*, pancreatic sufficiency (PS) that may progress to pancreatic insufficiency (PI) (as in our case 4 patient at 32 years old), and positive or inconclusive sweat tests. The variability of the phenotype has been related to levels of aberrant mRNA, which is affected by the expression of splicing factor SRp75. Decreasing levels of this factor have been shown to correct abnormal splicing of the variant.[7]

The deep intronic variant c.1680-883A>G is a more recently described variant located in intron 12[10] that results in aberrantly spliced transcripts due to the inclusion of a pseudo-exon. Only three cases with this splicing intronic variant have been referenced in the literature and included in the CFTR-France Database.[11] These three reported patients were diagnosed during childhood (2 months, 5 months, and 3 years) and were compound heterozygous with a positive sweat test, with or without PI, with respiratory symptoms at diagnosis, and without further follow-up data.[11] Our three patients with this intronic variant (cases 1–3) now aged 49, 19 and 22 years respectively, are the only ones with available long-term follow up and progression data. They were diagnosed during childhood with positive sweat tests, are compound heterozygous for severe pathogenic variants and have PI, bilateral bronchiectasis, bronchial colonisation by *Staphylococcus aureus*, *Achromobacter* sp.

Table 1
Clinical characteristics.

	Case 1	Case 2	Case 3	Case 4
Deep intronic splicing variant	c.1680-883A>G (Intron 12)	c.1680-883A>G (Intron 12)	c.1680-883A>G (Intron 12)	c.870-1113_870-1110del (Intron 7)
Variant second allele[a] (Legacy name)	c.254G>T (G85V)	c.3909C>G (N1303K)	c.3909C>G (N1303K)	c.1521_1523delCTT (delF508)
Gender	Female	Male	Female	Female
Age at diagnosis of CF, years	5	3	6	39
Age at deep intronic splicing variant detection, years	41	18	21	39
Current age, years	49	19	22	43
Symptoms at diagnosis	Repeated episodes of bronchitis	Repeated episodes of pneumonia	Repeated episodes of pneumonia	Repeated episodes of bronchitis
Sweat test, mmol/L [Cl^-]	83	101	116	50
NPD	ND	ND	ND	Positive
Bronchial colonisation	*Staphylococcus aureus*	*Achromobacter* sp.	*Achromobacter* sp., *Pseudomonas aeruginosa*	*Staphylococcus aureus*, *Haemophilus influenzae*, *Achromobacter* sp.
Bronchiectasis	Upper lobes	Diffuse	Diffuse	Diffuse
Sinusitis and/or nasal polyposis	Yes	Yes	Yes	Yes
FVC, predicted percentage	81	93	41	56
FEV1, predicted percentage	87	83	20	44
O_2 saturation, %	98	98	94	98
BMI	37.2	20.8	17	19.6
Pancreatic insufficiency	Yes	Yes	Yes	No → Yes[b]
Pancreatitis	No	No	No	Yes
Haemoptysis	No	Yes	Yes	Yes

Abbreviations. CF: cystic fibrosis; NPD: nasal potential difference; ND: not done; FVC: forced vital capacity; FEV1: forced expiratory volume in the first second; BMI: body mass index.

[a] Variant cDNA name.

[b] At 32 years old.

or *Pseudomonas aeruginosa*, sinusitis and/or nasal polyposis, and preserved lung function, except for case 3, who had very impaired lung function and required a lung transplant at the age of 21. Our data support the involvement of this variant with a severe CF phenotype, in similar manner to c.1680-877G>T and c.1680-886A>G variants,[12] and shows the long-term evolution of these patients when they are subjected to careful follow-up measures (case 3 was referred to our Cystic Fibrosis Unit just for lung transplant). Bonini et al.[10] developed oligonucleotides that can correct aberrant splicing with the use of target site blocker treatment (TSB), opening the alternative of a tailored therapy for the causative defect. The use of other techniques capable of correcting aberrant splicing caused by other deep intronic variants have also been described recently.[13–16]

In conclusion, in CF cases with only one pathogenic variant detected, it is important to sequence the entire *CFTR* gene regions to confirm genetic diagnosis for management of the patient and to provide adequate genetic counselling. The increasing number of DISV reported[10,17,18] will provide a better understanding of their pathogenic role in altering mRNA transcription using current technologies, such as NGS and new genome editing tools. This is essential in the present era for designing specific therapeutic approaches to correct the altered CFTR and move towards personalised medicine[19] or 4P medicine ('personalised', 'predictive', 'preventive' and 'participatory').

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Conflicts of interest

The authors have no conflict of interest to disclose.

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Membrana de oxigenación extracorpórea (ECMO) como terapia puente a la cirugía en paciente con síndrome de distrés respiratorio agudo (SDRA) debido a la rotura de un quiste hidatídico pulmonar

Pulmonar Extracorporeal Oxygenation Membrane (ECMO) as Bridge Therapy to Surgery in a Patient with Acute Respiratory Dystres Syndrome (ARDS) due to the Rupture of a Pulmonary Hydathid Cyst

Estimado Director,

Las indicaciones de la membrana de oxigenación extracorpórea (ECMO), como soporte vital transitorio en pacientes con insuficiencia respiratoria o cardiaca graves, refractarias a tratamiento convencional, se han ampliado considerablemente en los últimos 20 años[1]. La ECMO puede utilizarse en el síndrome de distrés respiratorio agudo (SDRA) y en otras formas de insuficiencia respiratoria potencialmente reversible como puente a la recuperación, a una intervención quirúrgica definitiva[2] o al trasplante[3]. Igualmente, se ha descrito la utilización de la ECMO en contextos de infecciones respiratorias graves como las causadas por virus influenza A H1N1[4] y, más recientemente, por coronavirus tipo 2 del síndrome respiratorio agudo grave[5]. Sin embargo, no existen muchos datos sobre su potencial uso en complicaciones debidas a infecciones parasitarias[6,7]. Presentamos el caso de un paciente con diagnóstico de quistes hidatídicos pulmonares bilaterales en tratamiento antiparisatorio previo a la intervención quirúrgica que sufrió la rotura de uno de los quistes y que precisó de soporte vital con ECMO como puente a la cirugía definitiva.

Se trata de un paciente de 21 años de origen peruano, alérgico a metamizol y diazepam, sin hábitos tóxicos ni antecedentes médicos de interés, que acudió al Servicio de Urgencias por tos y expectoración hemoptoica de 3 días de evolución, sin otra sintomatología acompañante. En la radiografía de tórax se objetivaron 2 masas de bordes bien definidos en ambos lóbulos inferiores y signos de hiperinsuflación pulmonar (fig. 1 A). La tomografía computarizada torácica evidenció 2 lesiones quísticas pulmonares: una de 10,5 × 9,8 cm en lóbulo inferior derecho y otra de 6,2 × 6,1 cm en lóbulo inferior izquierdo. Los hallazgos radiológicos eran sugerentes de quistes hidatídicos pulmonares sin signos de complicación. Tras obtenerse el título positivo de anticuerpos frente a *Echinococcus granulosus*, se decidió iniciar tratamiento antiparasitario con albendazol (400 mg/12 h) y praziquantel (1.200 mg/12 h) previo a la intervención quirúrgica. Las pruebas de función respiratoria del paciente reflejaron una alteración ventilatoria restrictiva.

Durante el período de espera para la intervención quirúrgica el paciente presentó de forma espontánea dolor en el hemitórax izquierdo y tos, seguidos de vómica escasa y expectoración hemoptoica. En la exploración física del paciente destacaba taquipnea (> 25 rpm), taquicardia (> 130 lpm), hipotensión (103/57 mm Hg) y $SatO_2$: 92% con aporte de oxígeno en gafas nasales a 2 l por minuto. La analítica destacaba leucocitosis de 11.420/μl, con 6,2% de eosinófilos y PCR de 4,36 mg/dl. La radiografía de tórax mostró un nivel hidroaéreo en la región basal del hemitórax izquierdo compatible con quiste hidatídico complicado con comunicación con la vía aérea (fig. 1 B). En pocas horas el paciente sufrió deterioro generalizado e insuficiencia respiratoria aguda y precisó ingreso en la unidad de cuidados intensivos (UCI) e intubación orotraqueal urgente. A pesar de llevar a cabo una ventilación pulmonar protectora, el paciente presentó empeoramiento