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### ► To cite this version:

Juan Balinotti, Camila Mallie, Alberto Maffey, Alejandro Colom, Ralph Epaud, et al.. Inherited pulmonary surfactant metabolism disorders in Argentina: Differences between patients with SFTPC and ABCA3 variants. *Pediatric Pulmonology*, 2023, 58 (2), pp.540-549. 10.1002/ppul.26225 . hal-04200967

**HAL Id: hal-04200967**

**<https://hal.science/hal-04200967>**

Submitted on 8 Sep 2023

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**Inherited Pulmonary Surfactant Metabolism Disorders In Argentina:  
Differences Between Patients With SFTPC and ABCA3 Variants**

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**Key words:** *interstitial lung disease, pulmonary surfactant, ABCA3 deficiency, pulmonary surfactant protein C*

**Abbreviated title:** Differences between patients with SP-C and ABCA3 variants in Argentina

## **ABSTRACT**

**Background:** patients with inherited pulmonary surfactant metabolism disorders have a wide range of clinical outcomes and imaging findings. Response to current anti-inflammatory therapies has been variable and efficacy is unclear.

**Objective:** to describe and compare genetic, clinical, histological and computed tomography (CT) outcomes in a cohort of patients with variants in the genes encoding surfactant protein C (*SFTPC*) or adenosine triphosphate-binding cassette transporter A3 (*ABCA3*) in Argentina.

**Methods:** observational cohort retrospective study. Patients carrying variants in genes encoding SP-C and ABCA3 proteins were included.

**Results:** 14 patients met the inclusion criteria: *SFTPC* n=6, *ABCA3* n=8 (seven were heterozygous and one compound heterozygous). Neonatal respiratory distress was more frequent and severe in neonates with variants in the *ABCA3* gene. The onset of the disease occurred in infancy before the age of 20 months in all cases. Participants with pathogenic *ABCA3* variants had a severe clinical course, while long term outcomes were more favorable in patients with *SFTPC* mutations. Initial CT findings were ground glass opacities and intraparenchymal cysts in both groups. Over time, signs of lung fibrosis were present in 57% of patients with *ABCA3* variants and in 33% of the *SFTPC* group. The efficacy of anti-inflammatory interventions appears to be poor, especially for patients with *ABCA3* pathogenic variants.

**Conclusions:** clinical, histological, and CT features are similar in patients with *SFTPC* and *ABCA3* variants, however the latter have more severe clinical course. Current anti-inflammatory regimens do not appear to stop the progression of the disease.

## INTRODUCTION

Inherited pulmonary surfactant metabolism disorders are a type of children's interstitial and diffuse lung diseases caused by mutations in the genes encoding surfactant protein B (SP-B), surfactant protein C (SP-C), ATP-binding cassette transporter A3 (ABCA3) and thyroid transcription factor 1 (NKX2-1).<sup>1</sup> Mutations in genes encoding SP-C (*SFTPC*) or *ABCA3* are the most frequent and may cause sporadic or familial interstitial lung disease.<sup>2</sup>

Pulmonary surfactant is a mixture mainly of lipids and proteins that coat the distal air spaces and reduces surface tension at end expiration preventing atelectasis. Surfactant proteins are essential in the process of formation of pulmonary surfactant film, organizing and stabilizing the interconnected membranes that contact the air-liquid interface, ensuring correct recycling.<sup>3</sup>

The SP-C protein is a small protein encoded by a single gene on chromosome 8 and is synthesized from a precursor, pro SP-C, in the alveolar type II cells (AEC2s).<sup>4</sup> Although SP-C enhances adsorption and spreading of lipids at the air-liquid interface, the precise role of this peptide in the surfactant homeostasis and lung disease remains unclear.<sup>5</sup> Mutations in *SFTPC* gene, found in an autosomal dominant pattern, are responsible for the accumulation of pro SP-C in AEC2s, resulting in disruption of cellular homeostasis.<sup>3,6,7,8</sup> The classification of *SFTPC* mutations depends on the affected protein domain, which allows differentiation between BRICHOS and non-BRICHOS mutations, and physiopathological mechanisms are probably determined by the location of these mutations.<sup>9</sup>

*ABCA3* is a transmembrane protein that uses energy from the hydrolysis of ATP to move substances across biological membranes and is encoded by the

large *ABCA3* gene on chromosome 16.<sup>10</sup> It is highly expressed in AEC2s where it is responsible for transportation of phospholipids into the lamellar bodies. *ABCA3* is critical for the assembly of pulmonary surfactant and the biogenesis and formation of lamellar bodies as well as for surfactant secretion and homeostasis.<sup>11,12</sup> *ABCA3* deficiency is an autosomal recessive disorder (OMIM 610921), and to date, more than 200 *ABCA3* mutations have been described.<sup>13</sup> Lung disease results from biallelic mutations, although there is evidence that monoallelic mutations may affect surfactant homeostasis.<sup>14,15,16,17</sup> Regarding missense variants, in vitro functional studies in biological models distinguished type I mutations with abnormal intracellular trafficking and abolished function and type II mutations with decreased ATP hydrolysis activity, therefore reduced *ABCA3* activity and a less severe phenotype in most cases.<sup>18</sup> Unfortunately, only a minority of *ABCA3* variants have been assessed by functional studies.<sup>19,20,21,22</sup>

The key pathological mechanisms why inherited pulmonary surfactant metabolism disorders cause lung disease are not fully understood.<sup>23</sup> Variants in the genes encoding the surfactant proteins can result in insufficient surfactant production, disrupted surfactant metabolism and secondary injury to alveolar type II cells leading to apoptosis.<sup>24,25</sup> This may trigger a wide range of clinical outcomes and computed tomography (CT) findings. Unfortunately, there is no improvement with exogenous surfactant administration (only transient)<sup>26,27</sup> citar Ciantelli and currently there are no randomized controlled trials that evaluate the effects of anti-inflammatory drugs.

In South America, there are few reports of surfactant disorders in children. This study aims to describe and compare genetic, clinical, histological and CT outcomes in a cohort of patients with *SFTPC* and *ABCA3* variants in Argentina.

## METHODS

**Design:** observational cohort retrospective study. Participants were recruited from a database created from a systematic record of data prospectively collected from all patients younger than 18 years old with interstitial lung disease who were followed at Ricardo Gutierrez Children's Hospital, Buenos Aires, Argentina.

**Study population:** patients with surfactant metabolism disorders related to SFTPC or *ABCA3* variants followed at the institution from July 2004 to July 2021. Individuals with heterozygous *SFTPC* variants and homozygous or compound heterozygous *ABCA3* pathogenic or likely pathogenic variants were included. Patients with clinical, CT and or histological findings consistent with a surfactant disorder with only one *ABCA3* variant already described according to ACMG-AMP recommendations<sup>21,28,29</sup> were also included after ruling out other chronic pulmonary diseases.

Molecular analysis of genes encoding the surfactant proteins *ABCA3*, SP-B and SP-C were performed by Sanger's technique or next generation sequencing (NGS) from EDTA blood samples obtained from the patients and parents for clinical purposes. NGS sequencing was performed in all the cases, including coding exons and their flanking regions, about 100 bp upstream and downstream were sequenced for each coding exon. Deletions/duplications were included in the genetic analysis. Deep intronic mutation at -98 of IVS25 was systematically investigated in *ABCA3* gene.<sup>30</sup>

Variant nomenclature was based on GenBank accession NM 001089 (*ABCA3*) and NM 003018 (*SFTPC*), with nucleotide one being the first nucleotide of the translation initiation codon ATG. We used the HGVS nomenclature followed by

their legacy name. Tests were performed at the genetic department of the Henri Mondor Hospital, Paris, France (n=11), John Hopkins Hospital, Baltimore, USA (n=1), Children's Hospital Colorado, USA (n=1) and at the Ricardo Gutierrez Children's Hospital, Buenos Aires, Argentina (n=1).

The following variables were recorded in the database: genetic results, demographics, perinatal and pertinent family history. Clinical features, pulmonary function tests (PFTs), lung biopsy reports, treatments regimens, oxygen requirements and duration of follow up. The Fan severity of illness score<sup>31</sup> was used to evaluate treatment response. High resolution computed tomography (HRCT) scans were reported by the investigators.

This study was approved by the Research Ethics Committee of the Ricardo Gutierrez Children's Hospital, and informed consent was waived.

**Statistical analysis:** the qualitative variables were reported in absolute values and percentages. The quantitative variables were expressed as mean and SD or median and interquartile range (IQR), as appropriate. Software STATA version 13 was used.

## **RESULTS**

From the 50 patients enrolled in the registry of interstitial lung diseases of infancy of our center, 14 met the inclusion criteria. Six patients had heterozygous *SFTPC* variants and 8 had *ABCA3* variants. In this group, only one had biallelic mutations and 7 patients were heterozygous. Demographic and clinical characteristics are listed in Table 1.

### **Genetics features:**

Half of the participants with *SFTPC* mutations (3/6) were heterozygous for the p.Ile73Thr mutation. One patient with p.Ile73Thr (patient 6) had an additional

*ABCA3* variant which could have an additional pathogenic effect. The *SFTPC* mutation was *de novo* on half of the cases (3/6) and was found once in an asymptomatic mother, with normal CT and PFT at 26 years of age.

Three novel pathogenic variants were described in the *ABCA3* group. Patients suspected of *ABCA3* deficiency presented in only one case with biallelic variants (patient 7, compound heterozygous). The other 7 cases presented monoallelic variants (heterozygous). Patients “12,13,14” were heterozygous carriers of a likely pathogenic single variant (p.Arg288Lys, R288K) already described as a type II mutation with normal trafficking and protein processing, but decreased ATPase activity.<sup>20</sup> In the other 4 cases, apart from for the likely pathogenic p.Arg1561Gln variant found in a *CFTR* mutation carrier (patient 11), the pathogenic effect of the *ABCA3* variants found in these monoallelic cases was clear (novel frameshift and nonsense mutations, large deletion or previously published mutation). The mother of patient 9, who died at 25 months, had the same single variant as her son. She is asymptomatic with normal PFTs. Detailed genetic information is presented in Table 2.

#### **Neonatal and clinical features:**

Most of the patients were born full term. Severe neonatal respiratory distress with need for mechanical ventilation was present in 88% (7/8) of cases with *ABCA3* variants, meanwhile only 16% (1/6) of neonates with *SFTPC* deficiency. Clinical manifestations began before 20 months of age in all cases. Although, neonatal features were more severe in patients with *ABCA3* variants, clinical manifestations after the neonatal period were similar in participants with *SFTPC* and *ABCA3* variants: tachypnea, hypoxemia, chronic cough and failure to thrive. Clinical course was more severe in patients with *ABCA3* variants (especially in



the subject with biallelic variants and in those with pathogenic monoallelic variants), persisting symptomatic with home oxygen requirements. Two patients died, at 1 month (patient 8, compound heterozygous) and at 25 months (patient 9). Two are listed for lung transplant (11 and 14 years old, respectively). Patients (12,13,14) who were carriers of the single likely pathogenic variant (R288K) had neonatal respiratory distress but their clinical and functional outcomes were different from the rest of the ABCA3 patients (table 3).

Long term outcomes within the *SFTPC* group were more favorable. 5/6 patients (83%) were weaned off oxygen, median duration of home oxygen was 41 months (min and max values: 7 – 84), although 3 of these 5 patients persist with oxygen saturation (SpO<sub>2</sub>) below 88% assessed by the 6-minute walk distance test. Detailed information is listed in Tables 1 and 3.

#### **High resolution computed tomography features:**

Initial HRCT findings were mainly ground glass opacities (GGO) and intraparenchymal cysts in participants with *ABCA3* and *SFTPC* mutations. Over time, GGO tend to decrease and septal thickening as well as intraparenchymal cysts tend to progress. In older patients, signs of lung fibrosis such as honeycombing or architectural distortion were present in 57 % of patients with *ABCA3* variants and in 33% of the SP-C subjects. One individual with a *SFTPC* mutation improved his CT, while none in the *ABCA3* group. HRCT features are presented in Table 4. Tomographic changes over time of patient 6 are shown in Figure 1.

#### **Pulmonary function tests: definer aca comillas o parentesis**

Infant PFTs were measured by the rapid thoracoabdominal compression technique at partial volumes.<sup>32</sup> Two patients “10,12” with *ABCA3* variants had an

obstructive pattern ( $V_{\max}FRC$  less than - 2 SD). The rest of the participants “3, 5” with *SFTPC* mutations and “13” with *ABCA3* deficiency presented normal infant PFTs. Spirometry, plethysmography and carbon monoxide diffusion capacity (DLCO) were performed in only 5 patients (3 *ABCA3* and 2 *SFTPC*) who were able to perform reproducible and acceptable maneuvers according to international guidelines.<sup>33</sup> In this limited sample, all of the patients had abnormal PFTs. One individual with a *SFTPC* mutation (5) presented a restrictive pattern and the other (6) presented a mixed pattern. One patient (8) with an *ABCA3* variant showed a severe obstructive pattern with extremely high residual volume due to decreased alveolar elastic recoil and airway closure at high lung volumes secondary to extensive areas of emphysema and parenchymal destruction seen in his chest CT scans. The other two patients “14” and “15” with monoallelic variant R288K, are siblings and had moderate - severe obstructive spirometric pattern.

#### **Lung biopsies and histopathological findings:**

Initially, access to genetic analysis was difficult, and for that reason lung biopsies were performed in 6 cases. All the participants with *SFTPC* and *ABCA3* variants had similar histological findings, except patient 12 (single variant R288K) who had mild airway fibrosis (constriction of lumen), mucus stasis and distension of airspaces. Alveolar wall thickening, interstitial smooth muscle hyperplasia, alveolar type II cells hyperplasia, foamy macrophages and mild lymphocytic inflammation were characteristic (Figure 2, A and B). In 4 patients (6,8,11 and 13) alveoli were filled with lipoproteinaceous material (PAS +). Histological patterns are detailed in Table 3. We were not able to perform electron microscopy

to describe abnormalities in lamellar body ultrastructure or immunohistochemical staining of ABCA3 protein.

### **Treatment Response:**

Detailed information about treatment regimen and response evaluated by the Fan severity of illness score are listed in Table 3. All the cases with *SFTPC* mutations received intravenous methylprednisolone pulses at 10 mg/kg for 3 consecutive days plus hydroxychloroquine and 4/6 also received azithromycin. Patients with ABCA3 variants received pulses of methylprednisolone (50%), hydroxychloroquine (25%) and azithromycin (62%). Individuals with *SFTPC* mutations had a better response to anti-inflammatory drugs in terms of clinical score than subjects with ABCA3 variants. Over time, 2 of 6 patients with *SFTPC* mutations and most of the patients with ABCA3 variants (except the 3 patients with the monoallelic variant R288K) tended to progress to lung fibrosis, even when anti-inflammatory treatment was initiated at the early onset of symptoms.

### **DISCUSSION**

This article describes for the first time in Argentina a cohort of patients with inherited pulmonary surfactant metabolism disorders. Clinical, histological, and CT features are similar in children with *SFTPC* and ABCA3 genomic variants. Identification of the gene is necessary for an accurate diagnosis. Clinical course is usually severe, especially in cases with biallelic ABCA3 mutations.<sup>27</sup> Course can be variable for infants and children with missense, splicing and in-frame insertions/deletions variants.<sup>34</sup> Patients with *SFTPC* mutations show an unpredictable broad clinical spectrum, ranging from asymptomatic to lung fibrosis. Individuals with the same mutation may present very different outcomes.<sup>35,36</sup> PFTs and CT changes occur over time, reflecting interstitial

inflammation and progressive lung fibrosis. Unfortunately, current anti-inflammatory treatments do not seem to stop the progression of the disease although there are some reports of response to steroids and hydroxychloroquine.<sup>9,27,37</sup>

In terms of genetic findings, the SP-C mutation p.Ile73Thr appears to be the most frequent mutation described for this gene also for Hispanic population.<sup>37,37</sup> Kröner and colleagues have shown that genotype for *SFTPC* mutations alone does not predict the clinical course.<sup>24</sup> Our patients with *SFTPC* mutations, varied from asymptomatic to hypoxemia on exertion or pulmonary fibrosis and these outcomes were not associated with the type of mutation. Participants 1 and 3 presented mutations in the BRICHOS domain, which is associated to neonatal respiratory distress.<sup>6</sup> Furthermore, some members of the same family with the same mutation were completely asymptomatic.<sup>35</sup> Wambach and colleagues,<sup>36</sup> found genotype-phenotype correlation for infants with biallelic loss of function variants in *ABCA3*, but not a clear association for missense, splicing and in-frame insertions/deletions. In our study, only one patient showed biallelic mutations so we cannot suggest a genotype-phenotype correlation, although this patient died during the neonatal period. Clinical course was severe in most of our patients with monoallelic pathogenic variants. One died at 25 months and two are on the lung transplant list. Patients with a single likely pathogenic variant R288K described as type II mutation with normal trafficking and protein processing, but decreased ATPase activity<sup>20</sup> presented neonatal respiratory distress, and their clinical, tomographic and histological outcomes were different from the rest of the *ABCA3* patients.

Our CT findings as well as histological features are consistent with those already published.<sup>37,38,39</sup> Bilateral GGO and lung cysts in an infant born full term with rapid breathing suggests an interstitial lung disease and the possibility of a genetic surfactant disorder. Most of our patients presented the typical histopathology of a surfactant dysfunction disorder: thickened alveolar septa with alveolar epithelial hyperplasia, foamy macrophages and lipoproteinaceous material filling alveoli.<sup>40</sup> As electron microscopy was not performed, we were not able to assess abnormalities in lamellar bodies seen in individuals with *SFTPB* and *ABCA3* mutations. There is no specific CT feature or histopathological pattern that distinguishes the type of surfactant disorder. Access to genetic testing provides precise diagnosis and can permit avoidance of a lung biopsy.

This article provides detailed genetic, clinical, functional, histological and CT information of a cohort of patients with genetic surfactant disorders for the first time in our country. Because it is a retrospective design, missing data elements may account for information bias. In addition, the small number of participants does not allow us to make statistical comparisons between patients with *ABCA3* and *SFTPC* mutations. Aside from this weakness, our results are consistent with other publications.<sup>27,28,</sup>

Interestingly, only 1/8 cases with *ABCA3* deficiency had biallelic mutations. The other 7 cases presented monoallelic *ABCA3* variants. From this subgroup, 4 of them presented pathogenic variants, and one died and two are listed for lung transplant, suggesting that even single alleles may contribute to severe disease.<sup>41,42</sup> In certain cases when only one *ABCA3* pathogenic variant is identified, and typical clinical symptoms persist over time plus characteristic tomographic and histological features are consistent with a surfactant disorder,

the diagnosis of a *ABCA3* deficiency should be considered after ruling out other pathologies. As *ABCA3* deficiency is an autosomal recessive disorder, we cannot rule out the possibility that we missed a second mutation with our genetic tests (in gene promoter, non coding exons and deep introns). Moreover, this could be a frequent mutation in our population, which remains to be identified. Unfortunately, electron microscopy and mRNA analysis on lung tissue or in bronchoalveolar lavage fluid could not be performed. It is also possible the phenotype could be caused by an additional mutation in another gene that impairs surfactant homeostasis.

*SFTPC* and *ABCA3* variants may produce surfactant deficiency and or an abnormal surfactant metabolism that may result in chronic injury to AEC2s.<sup>24,25</sup> Different phenotypes and outcomes cannot be explained only by genotype. Possible explanations for this discordance may be the presence of modifier genes, differences in penetrance, exposure to environmental stresses and maybe to the singular response to treatments.<sup>32</sup> These observations raise the question of which are the main mechanisms involved in the natural history of the disease.

Most of our patients with pathogenic *ABCA3* variants (biallelic or monoallelic) presented severe clinical outcomes. The benefits of therapeutic interventions with steroids, hydroxychloroquine and azithromycin seems poor in these cases.<sup>32</sup> Unfortunately, to date there is no specific treatment that benefits these patients. Given that *ABCA3* and *CFTR* (Cystic fibrosis transmembrane regulator) are both ATP binding cassette transporters, channel potentiators such as Genistein or Ivacaftor, the latter successfully used for Cystic Fibrosis patients with missense mutations may be a therapeutic option.<sup>43</sup> Another option for

individuals with *ABCA3* nonsense mutations maybe targeted therapeutic medications such as Ataluren used in CF or Duchenne Muscular dystrophy.<sup>44</sup> Patients with *SFTPC* mutations usually show better outcomes<sup>6,24,45</sup> and the efficacy of anti-inflammatory drugs assessed by clinical scores or in terms of weaning of oxygen appear to be better but unfortunately not in terms of progression of lung fibrosis. We cannot determine if the clinical course was defined by early and aggressive treatment or because of spontaneous improvement. It is possible that antifibrotic agents, such as tyrosine kinase inhibitors (Nintedanib),<sup>46</sup> may be useful in the future to prevent progressive lung fibrosis in pediatric patients. Finally, promising genetic therapy focused on the specific type of mutation and underlying mechanisms may be the solution for subjects with *SFTPC* and *ABCA3* mutations.

## **CONCLUSION**

Clinical, histological, and CT features are similar in patients with *SP-C* and *ABCA3* mutations, however the latter present with more severe neonatal and clinical outcomes. Routine PFTs and CT scans should be performed to detect early fibrotic changes even in asymptomatic patients. Current anti-inflammatory treatments do not seem to stop the progression of the disease. Improving access to genetic testing in Latin America would allow for earlier diagnosis and would give an opportunity for genetic counselling while novel therapies continue to evolve.

## **Acknowledgements:**

The authors want to thank Dr. Shai Manzuri, Dr. María Esnaola, JP Piñero and Mariana Mallie for help in revising and improving the manuscript.



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