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# Case of familial interstitial lung disease attributed to ATP-binding cassette transporter 3 gene mutation in identical twins

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**Abstract.** Mutations in *ABCA3* can result in surfactant deficiency, leading to respiratory distress syndrome in term neonates, and interstitial lung disease (ILD) in children. Here, we report an extremely rare case of ILD in an identical twin with novel *ABCA3* germline mutations. Interestingly, they showed mostly similar, but slightly different, clinical features. Our cases suggest that, in addition to genetic factors, non-genetic factors are involved in the severity of the disease and its clinical course. Studies of gene-environment interactions, especially with twins, are needed, as they may contribute to the understanding of the clinical heterogeneity of ILD and its association with various underlying conditions as well as rare variant mutations.

**Key words:** *ABCA3* mutation, identical twins, interstitial lung disease

## Introduction

ATP-binding cassette transporter 3 (ABCA3) is predominantly expressed in lung tissue and is localized to the membranes of the lamellar bodies of alveolar type II cells, where its protein product is critical for pulmonary surfactant synthesis and processing (1). *ABCA3* mutations have been associated with lethal neonatal respiratory distress syndrome as well as pediatric and adult interstitial lung disease (ILD) (2-4).

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Here, we report a rare case of ILD in identical twins with novel compound heterozygous *ABCA3* mutations.

## Case report

An 18-year-old woman was referred to our hospital owing to the finding of an abnormal shadow on a chest radiograph. She had no smoking history herself, but her parents did (25 pack-years for her father, 5 pack-years for her mother). Physical examination revealed slight fine crackles upon lung auscultation on the upper back and no clubbed fingers. Chest radiograph showed bilateral reticular opacities in the lung fields, predominantly in the upper lobes of both lungs. A high-resolution computed tomography (CT) scan revealed upper lobe predominant fibrotic changes with architectural distortion and sparing of the bases (Figure 1A-1F).

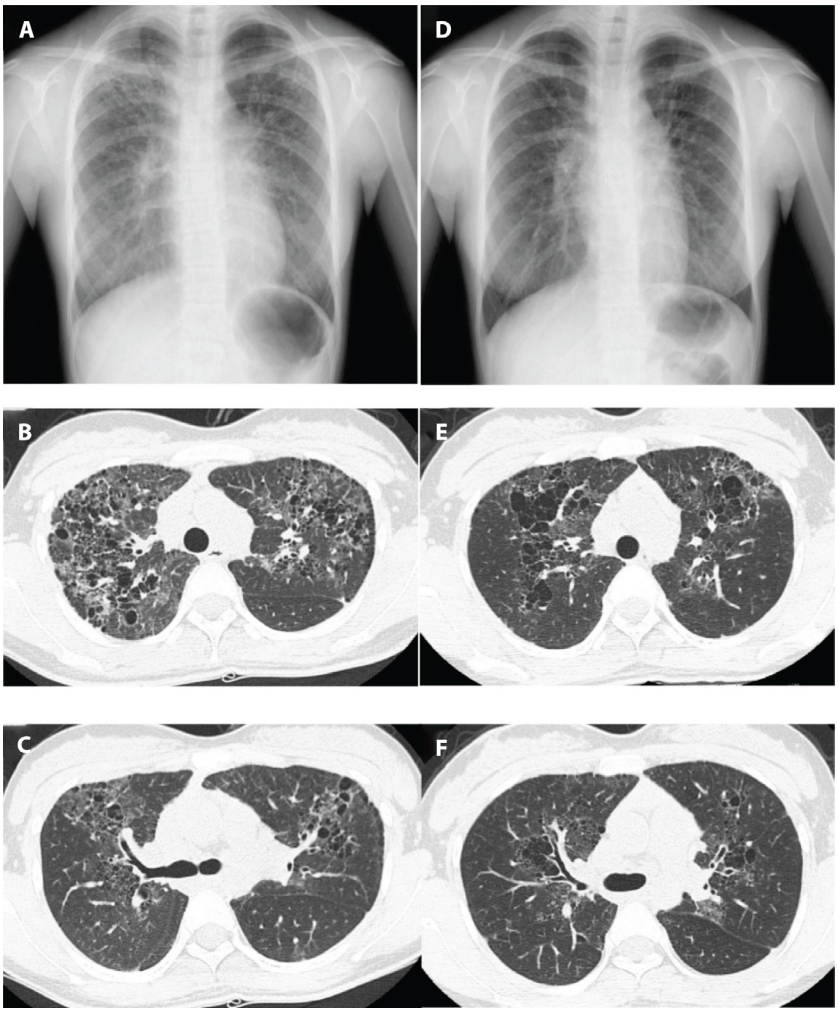


**Figure 1.** In both patients, chest radiography showed bilateral reticular lung field opacities, predominantly in the upper lobes of both lungs (A; proband, D; her sister). A high-resolution CT scan demonstrated upper lobe predominant fibrotic changes with cystic formation, sparing the bases (B, C; proband, E, F; her sister). The findings of her sister were milder than those of the proband.

Blood tests revealed elevated levels of serum Krebs von den Lungen-6 antigen, surfactant protein D and lactate dehydrogenase. Pulmonary function tests yielded results suggestive of restrictive ventilatory impairment. Bronchoalveolar lavage fluid did not show any dominant cell types apart from a slight increase in eosinophils (Table 1). The patient had an identical twin sister. They lived with their parents and went to the same school until they were 15 years of age. Her twin sister had similar findings, although they were relatively mild (Figure 1, Table 1). Surgical lung biopsy specimens showed mild-to-moderate alveolar thickening with cellular infiltration and some degree of fibrosis, resembling cellular and fibrotic NSIP patterns. Additionally, in some parts, accumulation of alveolar macrophages in the alveolar spaces was seen as a desquamative interstitial pneumonia (DIP)-like reaction; in other regions, deposition of focal dense fibrosis was also observed (Figure 2A and 2B).

Whole exome sequencing (WES) of both patients' genomes revealed a missense mutation of *ABCA3*: C to T substitution at c.596 in exon 7 (c.596C>T) and C to T substitution at c.737 in

**Table 1.** Comparison of laboratory data on admission between two patients

| | Proband | Sister |
|---|---|---|
| WBC (/μL) | 9250 | 9410 |
| LDH (IU/L) | 329 | 191 |
| KL-6 (U/mL) | 2242 | 645 |
| SP-D (ng/mL) | 300 | 139 |
| pH | 7.37 | 7.38 |
| $PaO_2$ (Torr) | 81.0 | 85.0 |
| $PaCO_2$ (Torr) | 43.7 | 43.4 |
| VC, L (predicted %) | 1.50 (49.4) | 1.98 (61.3) |
| FVC, L (predicted %) | 1.23 (45.3) | 1.70 (54.1) |
| $FEV_1$, L (%) | 1.08 (87.8) | 1.44 (84.7) |
| DLco, ml/min/mmHg (%) | 10.82 (57.3) | 13.56 (72.9) |
| 6MWT, m, Lowest $SpO_2$ | 485, 88% | 530, 95% |
| BALF recovery rate, % | 72 | 75 |
| Cell count | $1.71\times10^5$/ml | $0.84\times10^5$/ml |
| Macrophage, % | 88.4 | 92.0 |
| Neutorophil, % | 1.0 | 1.0 |
| Lymphocyte, % | 4.2 | 4.8 |
| Eosinophil, % | 6.4 | 2.2 |
| CD4/CD8 ratio | 1.74 | 1.28 |

Abbreviations: WBC: white blood cell, LDH: lactate dehydrogenase, KL-6: Krebs von den Lungen-6 antigen, SP-D: surfactant protein D, VC: vital capacity, FVC: forced vital capacity, FEV1: forced expiratory volume in one second, DLco: diffusing capacity for carbon monoxide, 6MWT: six-minute walk test, BALF: bronchoalveolar lavage fluid.

exon 8 (c.737 C> T). WES also revealed that the c.737 C> T and c.596C>T variants were inherited from their father and mother, respectively (Figure 3A). MUC5B and other genetic polymorphisms known to cause ILD have not been detected. Both patients were educated to avoid secondhand smoking and activities that increase the risk of pulmonary infection.

## Discussion

This is the first adolescent twin case harboring a rare variant, *ABCA3*, associated with ILD. Recently, Wang et al. reported that late preterm or term infants with unexplained respiratory distress syndrome due to homozygous or compound heterozygous *ABCA3* mutations exhibit more challenging clinical profiles (5). Zhang also reported that an *ABCA3* variant was identified in a family with lethal neonatal respiratory failure (6). Both studies included twins, but all patients died at an early age; no precise descriptions were provided. Our patients presented a relatively milder chronic condition, allowing us to observe differences in clinical features between the twins (Figure 3B).

Previous studies have reported cases of neonatal respiratory distress syndrome with the compound mutation involving the c.737 C>T variant (7,8). However, there have been no reports of ILD associated with the c.596 C>T variant. In silico analysis shows that the c.737 C>T variant is deleterious in several algorithms, while the c.596C>T variant has conflicting results (Table 2). According to the American College of Medical Genetics and Genomics guidelines (9), the c.737 C>T variant is predicted to be classified as 'likely pathogenic' (PM1+PM2+PP3+PP4). The c.596 C>T variant is also predicted to be classified as 'likely pathogenic' (PM1+PM2+PM3+PP4). Experimental evidence has shown that missense mutations in the extracellular domain (ECD)1 region of *ABCA3* affect protein trafficking, leading to the accumulation of ABCA3 protein in the endoplasmic reticulum (10). In this case, both mutations are located in the region encoding the ECD1 protein. Therefore, the compound mutation of c.737 C>T and c.596 C>T is the most likely cause of ILD.

Studies on identical twins are important to exemplify the clinical heterogeneity of the disease, showing that even genetically identical individuals can present with different clinical manifestations. These differences emphasize the importance of environmental factors and their influence on phenotypes. The identical twin ILD patients with *ABCA3* mutations in our study showed mostly similar but slightly different clinical features, including clinical/radiological findings and clinical course, suggesting that non-genetic factors may be involved in the onset of the disease. A previous study reported that environmental factors such as smoking and/or infection can affect the clinical course of the disease (11). *ABCA3* mutations cause severe surfactant metabolic dysfunction and accumulation of abnormal surfactant proteins in the endoplasmic reticulum (ER), leading to ER stress (12). Persistent ER stress induces apoptosis and results in alveolar epithelial injury. Additionally, viral infection and smoking exposure are

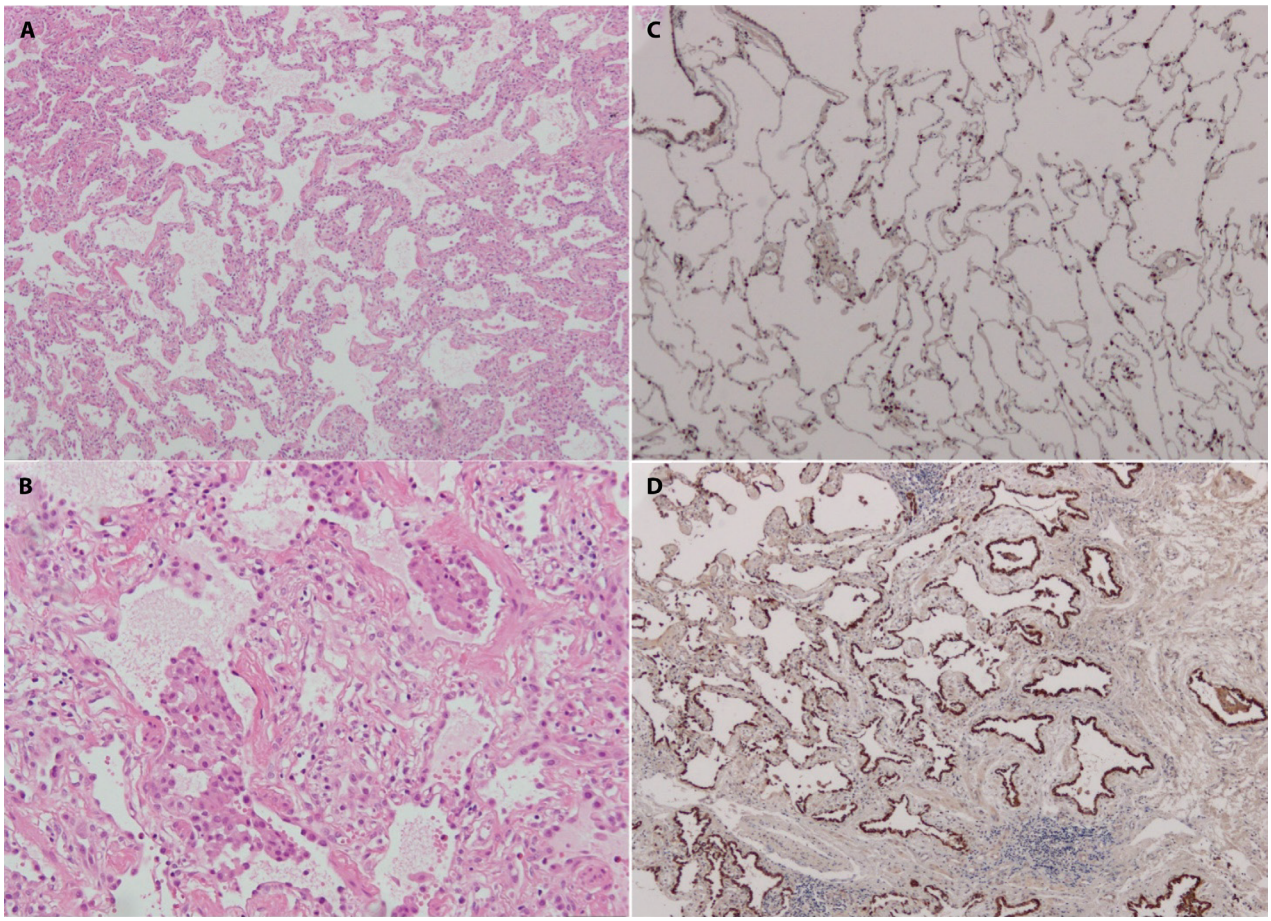


**Figure 2.** Surgical lung biopsy specimens of the proband show mild to moderate alveolar thickening with cellular infiltration and some degree of fibrosis, resembling cellular and fibrotic NSIP patterns; in some parts, deposition of focal dense fibrosis is also seen (A, Hematoxylin and eosin stain, ×40). Accumulation of alveolar macrophages in the alveolar spaces is seen namely desquamative interstitial pneumonia (DIP)-like reaction (B, Hematoxylin and eosin stain, ×100). Immunohistochemical analysis of lung tissue showed that the type 2 alveolar epithelium in this case (D, ×40) was strongly stained for proSPC compared to control alveoli (C, ×40).

known to increase ER stress (13). In this case, it was observed that the sisters had different frequencies of respiratory symptoms, which suggested exposure to viral infections based on their medical histories, as shown in Figure 3B. Therefore, the role of these environmental factors on ER stress could explain the differences in disease development between identical twins. The limitation of this report is that epigenetic analysis was not examined in these cases.

Regarding the clinical relevance of a slight increase in eosinophils in BAL, a few eosinophils were also observed in part of the biopsy. However, the blood tests of the both twins did not indicate any elevation in eosinophils, and there was no history of allergies. It is necessary to collect a greater number of cases to clarify the potential role of intrapulmonary eosinophils in the pathogenesis of the disease.

Attention should be paid to patients with chronic stable adolescent or adult ILD, as they may have *ABCA3* mutations. It might be important to avoid environmental factors such as cigarette smoke and infection. Despite the difficulty of identifying and establishing a relationship between specific environmental factors, such as smoking or infection, and the development of clinical manifestations, the investigation of these components, especially with twin studies, may contribute to improving our understanding of the factors influencing the progression of ILD.

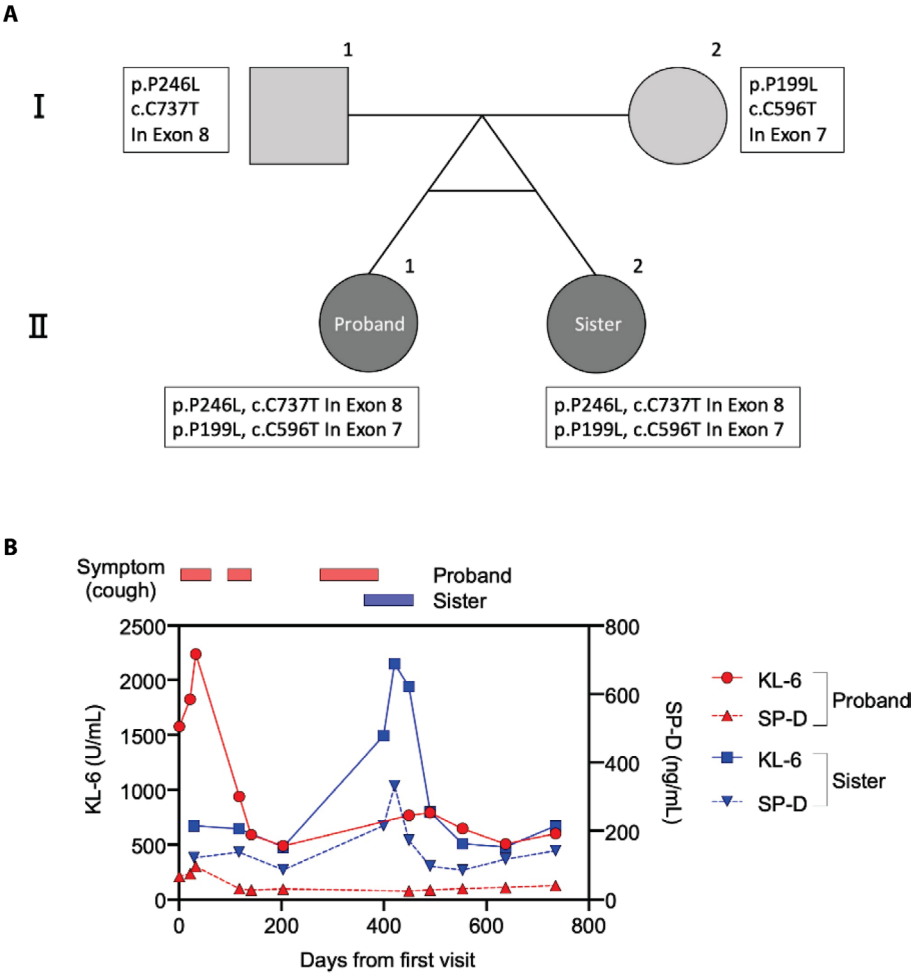


**Figure 3.** Genetic results of the family (A). Whole exome sequencing (WES) analysis in twins reveals missense mutation of ABCA3; C to T substitution at c.596 in exon 7 (c.596C>T) and C to T substitution at c.737 in Exon 8 (c. 737 C> T). WES identifies c. 737 C> T and c.596C>T variants inherited from his father and mother, respectively. The clinical course of twins after proband's initial visit (B). The transitions of symptoms and biomarkers (KL-6; serum Krebs von den Lungen-6 antigen, SP-D; surfactant protein D) are different between twins.

**Table 2.** Summary of the features of the identified ABCA3 variants

| | **ABC3A variants** | |
|---|---|---|
| | **c.737 C>T (p.P199L)** | **c.596 C>T (p.P246L)** |
| Allele frequency (gnomAD) | 0.000007526 | 0.00001163 |
| Mutation Taster | disease causing | polymorphism |
| PolyPhen-2 | 0.999 (probably damaging) | 0.014 (benign) |
| CADD | 25.1 (likely deleterious) | 15.99 (likely deleterious) |
| M-CAP | 0.07 (possibly pathogenic) | 0.683 (possibly pathogenic) |

Abbreviations: CADD: the Combined Annotation Dependent Depletion; M-CAP: Mendelian Clinically Applicable Pathogenicity.

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**Conflict of Interest:** we declare that we have no commercial associations (e.g. consultancies, stock ownership, equity interest, patent/licensing arrangement etc.) that might pose a conflict of interest in connection with the submitted article.

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