

A novel synonymous ABCA3 variant identified in a Chinese family with lethal neonatal respiratory failure

Weifeng Zhang

Quanzhou Women and Children's Hospital

Zhiyong Liu

Quanzhou Women and Children's Hospital

Yiming Lin

Quanzhou Women and Children's Hospital

Ruiquan Wang

Quanzhou Women and Children's Hospital

Jinglin Xu

Quanzhou Women and Children's Hospital

Ying He

Quanzhou Women and Children's Hospital

Fengfeng Zhang

Xiamen Genokon Medical Technology Co., Ltd.

Lianqiang Wu (✉ wulq205@sina.com)

Quanzhou Women and Children's Hospital

Dongmei Chen

Quanzhou Women and Children's Hospital

Research Article

Keywords: ABCA3 gene, synonymous variant, cryptic splice site, pulmonary surfactant, lethal neonatal respiratory failure

Posted Date: January 13th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-146531/v1>

License:  This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Abstract

Background: Lethal respiratory failure is mostly caused by pulmonary surfactant (PS) deficiency and is the main cause of neonatal death. PS metabolism dysfunction caused by mutations in the *ABCA3* gene is a rare disease with very poor prognosis. However, the underlying mechanism of genetic mutations causing PS metabolism dysfunction has not been fully elucidated yet. This study aimed to identify the genetic features in a family with lethal respiratory failure.

Methods: We studied members of a two-generation Chinese family including a female proband, her parents, her identical twin sister, and her older sister. Whole exome sequencing (WES), Sanger sequencing, and real-time quantitative polymerase chain reaction (qPCR) were used to identify and validate the *ABCA3* mutation. The potential pathogenicity of the identified synonymous variant was predicted using splice site algorithms (dbSNV11_AdaBoost, dbSNV11_RandomForest, and HSF).

Results: All patients showed severe respiratory distress, which could not be relieved by mechanical ventilation, supplementation of surfactants, or steroid therapy, and died at an early age. Molecular genetic analysis revealed that the patients had compound heterozygous *ABCA3* variants, including a novel synonymous variant c.G873A (p.Lys291Lys) in exon 8 derived from the mother and a heterozygous deletion of exons 4-7 derived from the father. The synonymous variant was consistently predicted to be a cryptic splice donor site that may lead to aberrant splicing of the pre-mRNA by three different splice site algorithms. The deletion of exons 4-7 of the *ABCA3* gene was determined to be a loss-of-function pathogenic variant.

Conclusions: We identified a novel synonymous variant and a deletion in the *ABCA3* gene that may be responsible for the pathogenesis in patients in this family. These results enrich the known mutational spectrum of the *ABCA3* gene. Moreover, the study of *ABCA3* mutations is helpful for the realization of patient-specific therapies.

Background

Neonatal hyaline membrane disease, neonatal transient tachypnoea, meconium aspiration syndrome, and infectious pneumonia are common causes of neonatal respiratory distress. Primary or secondary pulmonary surfactant (PS) deficiency causes fatal respiratory failure, the main cause of neonatal death. When a full-term newborn has persistent clinical and x-ray manifestations of respiratory distress syndrome (RDS), and the response to supplemental exogenous PS therapy is poor, the possibility of a rare inherited PS deficiency should be considered. The surfactant present in the lungs of all mammals is a complex compound of phospholipids and proteins. It maintains effective ventilation in the lungs by reducing the surface tension in the alveoli [1]. *ABCA3* (The adenosine triphosphate (ATP) binding cassette subfamily A, member 3) is a phospholipid transporter protein that regulates surfactant homeostasis and hydrolyses ATP [2, 3].

Surfactant metabolism disorders caused by genetic mutations are a group of diseases with a wide range of clinical manifestations, ranging from fatal respiratory distress in newborns to interstitial lung disease in children or adults [4, 5]. Mutations in the *ABCA3* (OMIM acc, No. 601615), *SFTPB* (OMIM acc, No. 178640), and *SFTPC* (OMIM acc, No. 178620) genes can cause qualitative and quantitative defects in surfactants [5]. However, surfactant deficiency is mainly caused by biallelic mutations in *ABCA3*, which is located on chromosome 16p13.3 [6]. *ABCA3* exon deletion mutation is rarely mentioned in previous reports, and a disease caused by synonymous mutations has only been reported last year [7]. In this study, we reported the clinical and genetic characteristics of a Chinese family, including three sisters who were born with fatal respiratory failure.

Methods

2.1 Clinical specimens

This study investigated a two-generation Chinese family, containing one female proband, her sisters, and her parents, from the Quanzhou Women and Children's Hospital. This study was approved by the ethics committee of Quanzhou Women and Children's Hospital and written consent was obtained from all participants involved.

2.2 DNA extraction and Sanger sequencing

Genomic DNA was extracted from peripheral whole blood or dried blood spots obtained from the proband, her parents, and her twin sister. We could not obtain the genomic DNA of the proband's older sister because she died early. All DNA was extracted from peripheral blood white blood cells. The coding region and flanking intron sequences of *ABCA3* (NM_001089) genes were amplified using standard polymerase chain reaction (PCR) conditions and bi-directional DNA sequencing. The DNA from the proband, her parents, and her twin sister was analysed using Sanger sequencing for the *ABCA3* variant c.G873A (p.Lys291Lys). DNA sequences, including the candidate mutation, were amplified using the forward primer *ABCA3-F*:5'-AAGTCACTCTGTTGCCCAA-3' and the reverse primer *ABCA3-R*:5' -CACCTATAGTCCAACTACTC-3'. SuperReal PreMix Plus (SYBR Green) (FP205, Tiangen Biochemical Technology Co., Ltd., Beijing, China) was used. The PCR cycle included the following steps: 2 min at 95 °C, followed by 36 cycles of 30 s at 95 °C, 1 min at 60 °C, 1 min at 72 °C, and a final step at 72 °C for 2 min.

2.3. Whole exome sequencing (WES)

WES was performed to identify any underlying pathogenic variants in the proband. First, the Blood Genome Extraction Kit (Tiangen Biochemical Technology Co., Ltd.) was used to extract the genomic DNA from the leucocytes in the blood sample. DNA was sheared with the Bioruptor Pico Sonication System. The NEBNext Ultra II DNA Library Prep Kit (New England Biolabs, Ipswich, MA, USA) was used for library preparation. The samples were submitted for 150bp pair-end sequencing, performed on NextSeq 500 Sequencing System (Illumina, San Diego, USA) by the Genonok Medical Laboratory, Xiamen, China. Trimmomatic (Usadel Lab, Aachen, Germany) was used to perform quality control and remove data of low quality. Burrows-Wheeler Alignment tool (BWA) was used to align reads to the reference (GRCh37/hg19). Variant discovery and genotyping were performed with GATK (<https://software.broadinstitute.org/gatk/>) and annotated with ANNOVAR. Common variants, such as intergenic, upstream, downstream, intronic, and synonymous variants, and variants with minor allele frequency (MAF) > 1% in the 1,000 genome, ExAC, and gnomAD databases, were then filtered out. Moreover, in silico programs were used to predict the deleterious effect of each variant on the function of proteins, including: REVEL, ClinPred, SIFT, Polyphen2, LRT, MutationAssessor, PROVEAN, CADD, MutationTaster, dbScn11_AdaBoost, dbScn11_RandomForest, and Human Splicing Finder (HSF). Genotype-phenotype analyses were performed using the Exomiser [8] and Phenolyzer [9] software programs. Finally, we read the results according to the standards and guidelines of American College of Medical Genetics and Genomics (ACMG).

2.4 Real-time quantitative PCR

The deletions from exon 4 to 7 of *ABCA3* gene were validated with real-time quantitative PCR. The method was described above. All reactions were performed in triplicate. Primer sequences are presented in Table 1.

Table 1. Primer sequences used in exon 4 to 7 of *ABCA3* gene amplification

Exons	forward	Reverse
<i>ABCA3</i> -e4	5'-CCCACTCTGCGTGTTC-3'	5'-TGCCTGAGCACAGCCATC-3'
<i>ABCA3</i> -e5	5'-AGTCGGAAAATGTGCCAAC-3'	5'-AAGGGATGTAGGCAAGCTCC-3'
<i>ABCA3</i> -e6	5'-TCTGCCAGTGACCTGAACC-3'	5'-ACGCTGGACGAGCAGTTGT-3'
<i>ABCA3</i> -e7	5'-TATCACCTACGGTTCAGTTACAC-3'	5'-CCTTGGTCTGGGTTGG-3'
<i>ALB</i>	5'-TCTGCTCTCCTGCCTGTTCT-3'	5'-TCGCCTGTTACCAAGGAT-3'

Results

3.1 Clinical information

The patients were from a family in Quanzhou, Fujian province, China (Fig. 1a). The proband and her identical twin sister were born without complications by caesarean delivery at 38 weeks and three days, with Apgar scores of 10 at one, five, and ten min. Birth weight of the proband and her sister was 2,650 g and 2,300 g, respectively. Their mother had no fever, no infection, and no intrauterine hypoxia in late pregnancy. After birth, the proband suffered from shortness of breath, groaning, and cyanosis, and was transferred to the neonatal intensive care unit (NICU). The proband was treated with non-invasive positive pressure ventilation, invasive mechanical ventilation, and high frequency oscillation ventilation. They were also treated with a combination of PS supplementation, antibiotics, inhalation of nitric oxide (NO) to reduce persistent pulmonary hypertension, and the anti-inflammatory methylprednisolone. The echocardiogram did not reveal any structural abnormalities of the heart. The chest radiograph of the proband at birth showed reticular granular blur of both lungs (Fig 1b), and then progressed to bilateral "white lungs" (Fig 1c) at 6 days of admission. Despite the above treatments, the proband's condition worsened rapidly, presenting with refractory dyspnoea, hypoxemia, persistent pulmonary hypertension, eventually leading to death at the age of 23 days. Her identical twin sister presented a similar profile and died of the same cause 23 days after birth.

Inquiring about family history revealed that the proband had an older sister born from her mother's first pregnancy more than a year earlier. The older sister was delivered by vaginal delivery at 38 weeks and six days after an uncomplicated pregnancy with Apgar score of 10 at one, five and ten min and birth weight of 3,200 g. Her condition was similar to that of the proband. However, she had a relatively long clinical treatment process. Fifteen days after the birth, the patient's breathing improved after supplementation with PS, and she was out of oxygen therapy for eight days. However, she still showed symptoms of respiratory failure and she was unable to leave the oxygen support until her death. From 35 to 42 days after birth, she was continuously treated with methylprednisolone and azithromycin, but no significant improvement was observed. Airway CT reconstruction at 56 days showed right side tracheal bronchus and bronchopulmonary dysplasia (BPD) (Fig. 1d,e). She died 109 days after birth due to refractory dyspnoea and hypoxemia.

The clinical presentations and suspicious family history led us to hypothesise that there was a genetic cause. Peripheral blood samples were obtained from the infants and parents for WES and further genetic analysis. However, we were unable to obtain pathological specimens because the parents refused fiberoptic bronchoscopy, lung biopsy, and autopsy.

3.2 Sequencing and qPCR results: *ABCA3* mutations identified by WES

Two *ABCA3* variants were identified by WES of the proband's DNA sample, namely a heterozygous deletion of exons 4-7 (Fig. 2a) and a novel heterozygous synonymous variant c.G873A (p.Lys291Lys) in exon 8 (Fig. 2d). The *ABCA3* gene variants were subsequently determined to be *in trans* in the proband, with the unaffected father found to be carrying the heterozygous deletion of exon 4-7 (Fig. 2b) and the unaffected mother carrying the heterozygous synonymous variant c.G873A (p.Lys291Lys) (Fig. 2e).

The deletion of exon 4-7 of the *ABCA3* gene was identified by bioinformatic analysis of single-gene copy number variants using NGS data. This loss-of-function variant was absent from the gnomAD and ExAC population database. It was interpreted as pathogenic according to the ACMG guidelines [10]. The c.G873A (p.Lys291Lys) novel variant was synonymous, however, it was also absent from gnomAD, HGMD, 1,000 Genomes and EXAC gene databases. Furthermore, it was consistently predicted to be a novel cryptic splice donor site by three different splice site algorithms (dbSNV11_AdaBoost, dbSNV11_RandomForest, and HSF) within exon 8 of the *ABCA3* gene, which may lead to aberrant splicing of the pre-mRNA. Given the consistent *in silico* prediction of a splicing site, the absence in control populations, and presence of *in trans* with the pathogenic variant (deletion of exon 4-7) in two patients, both the proband and her identical twin sister, this variant was also interpreted as likely pathogenic according to the ACMG guidelines (PM2_supporting, PP3, PM3_strong). We further confirmed the *ABCA3* mutations in DNA sample of the proband's identical twin sister. The result of real-time quantitative PCR experiment revealed a heterozygous deletion mutation of exon 4-7 (Fig. 3a). The heterozygous c.G873A (p.Lys291Lys) mutation of the *ABCA3* gene was identified by Sanger sequencing (Fig. 2g). These results were consistent with the proband. Therefore, the evidence above supported the diagnosis of autosomal recessive PS metabolism dysfunction caused by deficiency of *ABCA3*.

Table 2. Identified *ABCA3* variants identified

Gene	Chromosome	Nucleotide Change	Protein Change	Variant type	In silico prediction	Genotype	Parent of origin
<i>ABCA3</i>	Chr16:2390573-2326806	NM_001089: (Chr16:2390573-2326806)X1	Exon 4-7 deletion	Deletion	LOF	Heterozygous	Paternal
<i>ABCA3</i>	Chr16:2369582	NM_001089: exon8:c.873G>A	p.Lys291Lys	Synonymous	Cryptic splice site activation	Heterozygous	Maternal

Table 3. *In silico* prediction of the *ABCA3* synonymous variant c.873G>A

Software	Score	Interpretation
AdaBoost ^a	1	splice-altering
Random Forest ^a	0.976	splice-altering
HSF ^b	-10.08 (87>76.92=>-11.59%)	Alteration of the WT donor site, most probably affecting splicing.

^aAdaBoost and Random Forest: A score of more than 0.6 is predicted as splice-altering. [11]

^bHSF: A score of less than 0 is determined as splice site broken. (<https://hsf.genomni.com/>)

Discussion

Human respiration depends on the extensive gas exchange surface area provided by the expanded alveoli. Lipid-rich lung surfactants keep the alveoli unobstructed throughout the air-liquid interface. The appropriate synthesis, transport, secretion, absorption, and recycling of alveolar epithelial type II cells (AEC2) ensure the intact function of surfactants, and alveolar macrophages also participate in the absorption and recycling of the surfactants [12]. *ABCA3*, a transmembrane phospholipid glycoprotein, is a member of the ABC ATP binding cassette family that is essential for the formation of lamellar bodies (LBs) and phospholipid transport, as well as assembly and generation of surfactant [13, 14]. *ABCA3* mutations may affect a range of physiological processes. These have been confirmed in studies with samples from patients with *ABCA3* mutations, as well as in ACE2 model A549 and HEK293 cells after wild-type *ABCA3* expression [15].

Fatal RDS caused by biallelic mutation in newborns with congenital surfactant deficiency was first reported in 2004 [16]. So far, more than 200 *ABCA3* mutations have been found, and about three-quarters of patients present with complex heterozygotes [8]. At present, the incidence of *ABCA3* mutations in newborns is not clear. Wambach et al. predicted that the disease incidence of the *ABCA3* mutation ranged from 1:4,000 to 1:17,000 in

individuals of European and African descent [17]. A study in Nanning, China, showed that *ABCA3* is the most frequent gene mutation affecting the function of surfactants, at 2.7% [18].

Although uniparental disomy has been reported, the most common pattern of *ABCA3* mutations is autosomal recessive, requiring mutations in both alleles [19]. Kröner et al. studied 242 patients with interstitial lung disease (ILD) with *ABCA3* gene sequencing and found that 69 patients had at least one mutation, of which 40 had two different pathogenic mutations [20].

In this study, we identified heterozygous deletion mutations in exons 4-7 of *ABCA3* gene in both proband and paternal samples through WES. Furthermore, according to the qPCR results, a heterozygous deletion mutation of exons 4-7 of *ABCA3* gene was present in the twin sister of the proband. There have been few reports on exon deletions in *ABCA3*. The large homozygous deletion mutation in exon 2-5, the heterozygous deletion mutation in exon 13-18, and the heterozygous deletion mutation in exon 12 have been reported to cause neonatal respiratory distress [19, 21, 22]. In animal experiments, a mouse model with the deletion of *ABCA3* exons 4-7 showed the mechanism of *ABCA3* deletion leading to respiratory failure in mice [16]. Through WES, the *ABCA3* gene C.G873A: p.yS291Lys heterozygous mutation was identified in the samples of the proband and their mother, and the same results were found in the twin sister by Sanger sequencing. The heterozygous mutation of *ABCA3* gene C.G873A is a synonym mutation, predicted to be a novel cryptic splice donor site by three different splice site algorithms, which is absent from gnomAD, HGMD, 1,000 Genomes and EXAC gene databases. In 2014, Wambach et al. reported that some synonymous mutations were found in newborn RDS patients. However, these mutations could not be predicted to lead to abnormal RNA splicing by algorithms, and no clear RDS mechanism has been determined [23]. Oltvai et al. first reported a patient with an *ABCA3* synonymous mutation. The clinical manifestations and weak *ABCA3* immunostaining provided evidence that the c.2883C > T p.Gly961Gly mutation behaved like a "mosaic null" allele. Moreover, it also showed that the novel RNA splicing site caused by the *ABCA3* synonymous mutation may have a new pathogenesis [7]. This is the second time that this synonymous mutation has been reported as a cryptic exonic splicing variant in *ABCA3*. At the same time, NGS sequencing did not find other mutations affecting surfactant. The combination of clinical phenotypes, algorithm predictions, and genetic findings supports our theory that these mutations are pathogenic.

There is a recognized correlation between genotype and phenotype in *ABCA3* deficiency. Patients with two null mutations, defined by premature stop codon or frameshifts, have earlier symptoms and greater mortality in the first year of life [24]. It is generally believed that besides lung transplantation, patients with *ABCA3* gene mutations have no effective treatments although the combination of corticosteroids, macrolides, and hydroxychloroquine has been used in clinical empiric therapy. However, studies have shown that exogenous surfactants, whole-lung lavage, hydroxychloroquine, and corticosteroids have significant but multiple transient effects on individuals [20, 24]. Moreover, blinded controlled treatment evaluations for rare diseases have not yet been proven feasible [25]. Our patients had a fatal early-onset disease with serious progression, needing long-term respiratory support and oxygen supplementation. Even the repeated use of surfactants, macrolides, and corticosteroids did not lead to significant clinical improvement. All three patients eventually died at an early stage in life.

However, this study had some limitations. This was a single-centre retrospective study of only one pedigree. Future studies will need to use multicentre, standardized clinical, genetic, pathological, and imaging data. Our research will next verify the impact of this mutation on the splicing of *ABCA3* at the RNA level.

Conclusions

In conclusion, we reported the clinical and genetic features of a Chinese family with compound heterozygous mutation in the *ABCA3* gene: a novel variant c.G873A:p.Lys291Lys and the deletion of exons 4-7. Reports on novel *ABCA3* gene mutations, clinical course, and treatment response, especially *ABCA3* gene synonymous variants that cause the disease, will help further understand the diagnosis and treatment strategies for *ABCA3* deficiency.

List Of Abbreviations

PS: pulmonary surfactant; WES: whole exome sequencing; PCR: polymerase chain reaction; RDS: respiratory distress syndrome; ATP: adenosine triphosphate; CT: computerized tomography

Declarations

Ethics approval and consent to participate

The parents of the patients signed written informed consent and agree themselves and their children to take part in this study and using the relevant data and information for scientific research.

Consent for publication

We confirm that the parents of the patients signed written informed consent for publication of their own and children's genetic data, clinical details and/or any accompanying images.

Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

No funding to declare

Authors' contributions

WZ and ZL conceived the study, carried out the first draft of the manuscript. DC and LW helped critically revise the manuscript for important intellectual content, were mentors who designed and guided the research study. YL and FZ carried out the mutation analysis. RW, YH and JX cared for patients and collected the clinical data.

Acknowledgements

We would like to thank Editage (www.editage.cn) for English language editing.

References

1. Nogee LM: Interstitial lung disease in newborns. *Semin Fetal Neonatal Med* 2017, 22(4):227-233.
2. Bullard JE, Wert SE, Whitsett JA, Dean M, Nogee LM: ABCA3 mutations associated with pediatric interstitial lung disease. *Am J Respir Crit Care Med* 2005, 172(8):1026-1031.
3. Zhou W, Zhuang Y, Sun J, Wang X, Zhao Q, Xu L, Wang Y: Variants of the ABCA3 gene might contribute to susceptibility to interstitial lung diseases in the Chinese population. *Sci Rep* 2017, 7(1):4097.
4. Griesse M, Haug M, Brasch F, Freihorst A, Lohse P von Kries R, Zimmermann T, Hartl D: Incidence and classification of pediatric diffuse parenchymal lung diseases in Germany. *Orphanet J Rare Dis* 2009, 4:26.
5. Malý J, Navrátilová M, Hornychová H, Loosman AC: Respiratory failure in a term newborn due to compound heterozygous ABCA3 mutation: the case report of another lethal variant. *J Perinatol* 2014, 34(12):951-953.
6. Gonçalves JP, Pinheiro L, Costa M, Silva A, Gonçalves A, Pereira A: Novel ABCA3 mutations as a cause of respiratory distress in a term newborn. *Gene* 2014, 534(2):417-420.
7. Oltvai ZN, Smith EA, Wiens K, Nogee LM, Luquette M, Nelson AC, Wikenheiser-Brokamp KA: Neonatal respiratory failure due to novel compound heterozygous mutations in the ABCA3 lipid transporter. *Cold Spring Harb Mol Case Stud* 2020, 6(3).
8. Robinson PN, Köhler S, Oellrich A, Wang K, Mungall CJ, Lewis SE, Washington N, Bauer S, Seelow D, Krawitz P *et al*: Improved exome prioritization of disease genes through cross-species phenotype comparison. *Genome Res* 2014, 24(2):340-348.
9. Yang H, Robinson PN, Wang K: Phenolyzer: phenotype-based prioritization of candidate genes for human diseases. *Nat Methods* 2015, 12(9):841-843.
10. Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E *et al*: Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015, 17(5):405-424.
11. Jian X, Boerwinkle E, Liu X: In silico prediction of splice-altering single nucleotide variants in the human genome. *Nucleic Acids Res* 2014, 42(22):13534-13544.
12. Perez-Gil J, Weaver TE: Pulmonary surfactant pathophysiology: current models and open questions. *Physiology (Bethesda)* 2010, 25(3):132-141.
13. Besnard V, Matsuzaki Y, Clark J, Xu Y, Wert SE, Ikegami M, Stahlman MT, Weaver TE, Hunt AN, Postle AD *et al*: Conditional deletion of Abca3 in alveolar type II cells alters surfactant homeostasis in newborn and adult mice. *Am J Physiol Lung Cell Mol Physiol* 2010, 298(5):L646-659.
14. AlAnazi A, Epaud R, Heena H, de Becdelievre A, Miqdad AM, Fanen P: The most frequent ABCA3 nonsense mutation -p.Tyr1515* (Y1515X) causing lethal neonatal respiratory failure in a term neonate. *Ann Thorac Med* 2017, 12(3):213-215.
15. Flamein F, Riffault L, Muselet-Charlier C, Pernelle J, Feldmann D, Jonard L, Durand-Schneider AM, Coulomb A, Maurice M, Nogee LM *et al*: Molecular and cellular characteristics of ABCA3 mutations associated with diffuse parenchymal lung diseases in children. *Hum Mol Genet* 2012, 21(4):765-775.
16. Shulnenin S, Nogee LM, Annilo T, Wert SE, Whitsett JA, Dean M: ABCA3 gene mutations in newborns with fatal surfactant deficiency. *N Engl J Med* 2004, 350(13):1296-1303.

17. Wambach JA, Wegner DJ, Depass K, Heins H, Druley TE, Mitra RD, An P, Zhang Q, Nogee LM, Cole FS *et al*. Single ABCA3 mutations increase risk for neonatal respiratory distress syndrome. *Pediatrics* 2012; 130(6):e1575-1582.
18. Chen YJ, Chen SK, DePass K, Wegner DJ, Hamvas A, Nong GM, Wang YZ, Fan X, Luo JS: [Pulmonary surfactant associated gene variants in mixed ethnic population of Han and Zhuang]. *Zhonghua Er Ke Za Zhi* 2012; 50(11):843-846.
19. Henderson LB, Melton K, Wert S, Couriel J, Bush A, Ashworth M, Nogee LM: Large ABCA3 and SFTPC deletions resulting in lung disease. *Ann Am Thorac Soc* 2013; 10(6):602-607.
20. Kröner C, Wittmann T, Reu S, Teusch V, Klemme M, Rauch D, Hengst M, Kappler M, Cobanoglu N, Sismanlar T *et al*. Lung disease caused by ABCA3 mutations. *Thorax* 2017; 72(3):213-220.
21. Carrera P, Ferrari M, Presi S, Ventura L, Vergani B, Lucchini V, Cogo PE, Carnielli VP, Somaschini M, Tagliabue P: Null ABCA3 in humans: large homozygous ABCA3 deletion, correlation to clinical-pathological findings. *Pediatr Pulmonol* 2014; 49(3):E116-120.
22. Chen J, Nong G, Liu X, Ji W, Zhao D, Ma H, Wang H, Zheng Y, Shen K: Genetic basis of surfactant dysfunction in Chinese children: A retrospective study. *Pediatr Pulmonol* 2019; 54(8):1173-1181.
23. Wambach JA, Wegner DJ, Heins HB, Druley TE, Mitra RD, Hamvas A, Cole FS: Synonymous ABCA3 variants do not increase risk for neonatal respiratory distress syndrome. *J Pediatr* 2014; 164(6):1316-1321 e1313.
24. Wambach JA, Casey AM, Fishman MP, Wegner DJ, Wert SE, Cole FS, Hamvas A, Nogee LM: Genotype-phenotype correlations for infants and children with ABCA3 deficiency. *Am J Respir Crit Care Med* 2014; 189(12):1538-1543.
25. Beers MF, Mulugeta S: The biology of the ABCA3 lipid transporter in lung health and disease. *Cell Tissue Res* 2017; 367(3):481-493.

Figures

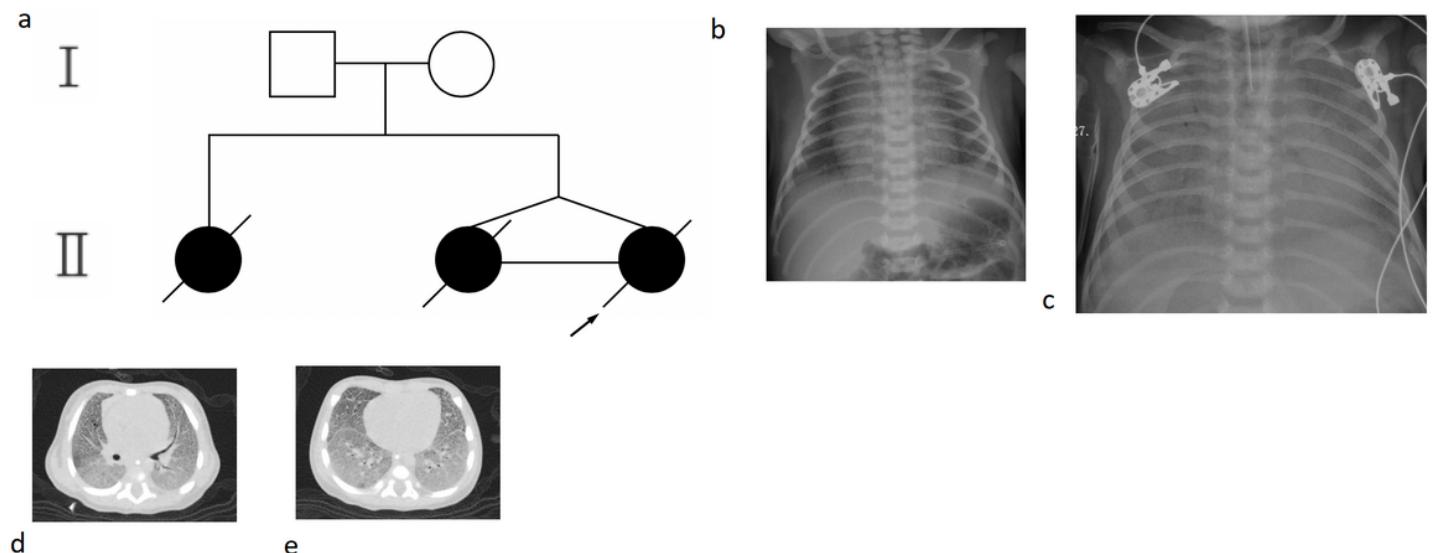


Figure 1

Clinical information of the family. (a) Pedigree of the family. The arrow denotes the proband and the filled black symbols represent the affected members. (b,c) X-rays of the proband revealed the pulmonary interlobular inflammation effusion and white lungs. (d,e) The computed tomography airway reconstruction of the older sister showed bronchopulmonary dysplasia.

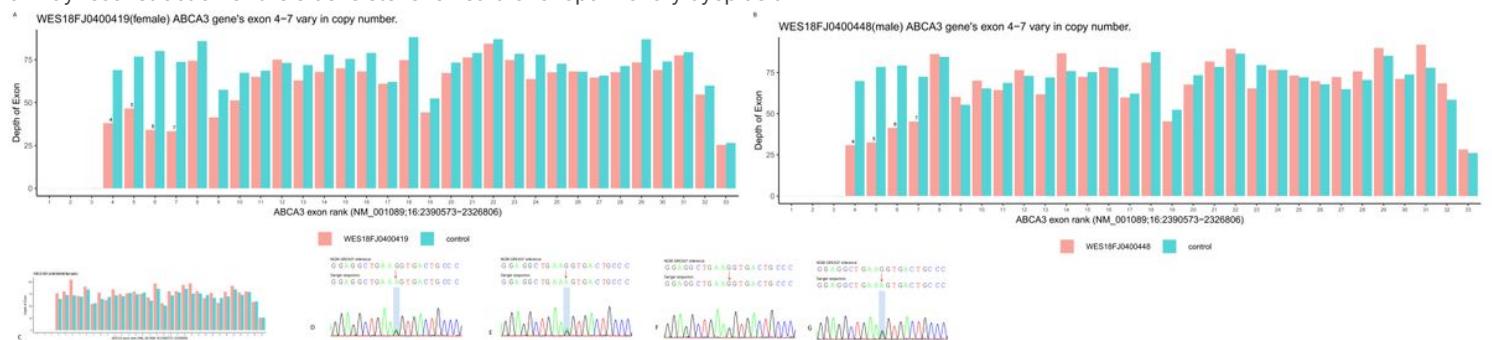


Figure 2

ABCA3 mutations in this family. (a,b) Results of whole exome sequencing (WES) showed deletion mutation of exon 4-7 of ABCA3 gene in the samples of the proband and their father. (c) Deletion mutation of exon 4-7 of ABCA3 gene not found in the samples of the proband's mother. (d,e) WES showed heterozygous mutation (c.873G>A) of ABCA3 gene in the samples of the proband and their mother. (f) Heterozygous mutation (c.873G>A) of ABCA3 gene in the samples of the proband's father. (g) Sanger sequencing of ABCA3 gene identified the heterozygous mutation (c.873G>A) in the identical twin sister of the proband.

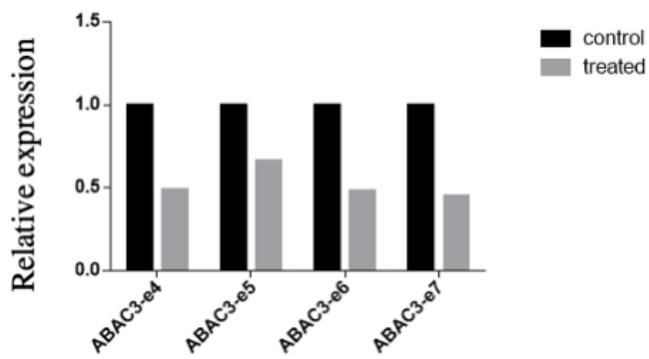


Figure 3

ABCA3 mutation in the identical twin sister of proband. (a) The expression of exon 4-7 of ABCA3 gene were confirmed by RT-PCR. All DNA extracted from peripheral blood white blood cells.