Title: *ABCA3* gene and RDS in late preterm and term infants

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Neonatal respiratory distress syndrome (RDS) is caused by the deficiency or inactivation of pulmonary surfactant, and is commonly seen in early preterm infants due to their immature lung development. However, some maternal and neonatal characteristics in late preterm and term infants such as maternal diabetes, meconium aspirated pneumonia, neonatal sepsis, and severe intrapartum asphyxia, could also contribute to RDS. Existing evidence has demonstrated that RDS in late preterm and term infants is a somewhat distinct disease entity, with risk factors and clinical profiles that differ from those in early preterm infants [1-3]. In a neonatal unit, some late preterm or term infants experience RDS; however, the etiology has not been well defined after a routine workup. This unique RDS entity was referred to as unexplained respiratory distress syndrome (URDS) by numerous previous studies, as well as our study [4-7].

Given that a large body of literature has demonstrated the essential role genetic mechanism play in the pathogenesis of most URDS cases, numerous medical facilities have carried out clinical exome sequencing to identify the underlying genetic cause of URDS [8-10]. Among all of the genetic factors that contribute to the RDS, the most common is *ABCA3* gene mutation, which involves the assembly of pulmonary surfactant in the lamellar body of pneumocyte II [11]. Existing evidence indicated that patients with homozygous or compound heterozygous *ABCA3* gene mutations were commonly in critically ill conditions [12]. A single *ABCA3* mutation was also likely to increase the risk and severity of RDS [4, 13].

Unfortunately, in a considerable proportion of neonatal URDS patients, genetic testing fails to yield any abnormal findings, making these patients the “true URDS” patients. Currently, whether the *ABCA3*-mutated URDS patients have similar or more challenging clinical profiles to those without any genetic abnormalities continues to confound most neonatologists. An answer to this question would help to guide the management and predict

the clinical outcomes of neonatal URDS patients. The present study aimed to address this by comparing the clinical characteristics of late preterm and term infants with severe URDS with homozygous or compound heterozygous *ABCA3* mutations, a single *ABCA3* mutation, or no defined genetic abnormalities.

Patient selection

This single-center retrospective cohort study involved infants ⩾34 weeks' gestation with severe URDS who were admitted to Children's Hospital of Chongqing Medical University between January 2013 and December 2019. In this study, severe RDS was mainly defined according to the consensus of the Pediatric Acute Lung Injury Consensus Conference [14], and Montreux definition [15]: (1) manifestations and chest radiograph compatible with RDS; (2) on invasive mechanical ventilation with oxygenation index ⩾16, which was calculated based on the daily blood gas, or as an alternative measurement, on the subcutaneous oxygen tension [16, 17].

Almost all infants with severe RDS had undergone a comprehensive workup, including serial infection markers, chest radiograph, echocardiography, and blood and sputum pathogen testing. For all patients with severe RDS who responded inadequately to interventions and had unremarkable workup findings, trio exome sequencing on samples from patients and their parents were usually recommended. All URDS patients who underwent genetic testing were enrolled in this study. Those whose parents rejected genetic testing, or who had cardiopulmonary malformations, pulmonary hypoplasia, culture-positive sepsis, or known respiratory disease-associated gene mutations (such as *SFTPA1, SFTPA2, SFTPB, SFTPC, CHPT1, LPCAT1, PCYT1B, NKX2, CFTR,* and *FOXF1*)

were excluded. This study was approved by the Institutional Review Board of Children's Hospital of the Chongqing Medical University (No.2018-158) and was registered on clinicaltrials.gov (NCT04137783).

Exome sequencing and gene mutation detection

All infants in this study underwent trio exome sequencing after written consent had been obtained from their parents. A gene company (Chigene, Beijing) offered the sequencing as a clinical laboratory service. Sequence analysis of coding exons and flanking introns were performed as previously described [18, 19]. All samples were analyzed to detect frame-shift mutations, nonsense mutations, missense mutations, splicing site mutations, and in-frame indel mutations. Assessment of copy number variation was also performed from exome sequencing data using computational tools. A variant was strictly defined as a mutation if it had been previously described to cause disease with a presentation consistent with these patients, or resulted in an amino acid change or protein structure alteration to disrupt protein function that was predicted by both SIFT and PolyPhen for missense mutations [20, 21], and MaxEntScan and dbscSNV for splicing site mutations[22]. In the case of a novel mutation, phastCons and phyloP were used to determine the evolutionary conservation of the region where the mutation was located [23]. The American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG-AMP) criteria were applied to interpret the mutations [24]. The subjects in this study were categorized into three groups: homozygous or compound heterozygous *ABCA3* mutations, a single *ABCA3* mutation, and no defined genetic abnormalities.

Clinical profiles data

All relevant clinical data were extracted from a hospital information system.

All antepartum and postpartum data were collected and included: maternal age, parity, and pregnancy-related complications; mode of birth, amniotic fluid condition, and history of asphyxia and resuscitation; postnatal age of respiratory symptom onset, modalities of respiratory support, and the daily record of blood gas and subcutaneous oxygen tension; laboratory data including complete blood count, C-reactive protein, procalcitonin, blood and sputum culture, respiratory viral detection test, and genetic testing; radiographic examination including chest X-ray and echocardiogram; medications taken during the hospital stay.

Radiological scoring

All chest X-rays were reviewed on a hospital information system by one radiologist. The most severe images were scored according to the Fleischner Society criteria[25]. The chest X-ray was rated in three sections on both sides of the lung: apex to the carina, carina to the lower pulmonary vein, and lower pulmonary vein to the diaphragm. The incidence of radiological features, including ground-glass opacity, reticular pattern, air bronchogram, atelectasis, and air leak, were evaluated for each lung section. Each finding was scored as 0 = none, 1 = discrete,2=diffuse, or 3 = strong at each section. An overall cumulative score was calculated by adding the individual section scores together, with a maximum score of 18 for each patient.

Statistical Analysis

Analysis of variance (ANOVA) and the Chi-square or Fisher's exact tests were used to compare clinical characteristics and radiographic scores between different groups of patients. Tests for the differences in the age of symptom onset and the age of development of severe RDS between the groups were carried out using the log-rank test. A two-tailed *p*-value of

<0.05 was considered statistically significant.

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