Two novel gene mutations identified in a child with Pulmonary Alveolar Microlithiasis complicated with bronchitis obliterans: a case report and literature review

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Introduction

Pulmonary alveolar microlithiasis (PAM) is an uncommon, autosomal recessive lung disease with high penetrance (OMIM #265 100) and is considered to be a monogenic disorder. [1] The only known pathogenic gene is solute carrier family 34 member 2 (SLC34A2) (Entrez Gene ID 10568).[2-4] SLC34A2 mutations lead to the accumulation of calcium phosphate in the alveoli, restrict alveolar dilatation, and then progress to a restrictive lung function complicated with reduced dynamic and static volumes.[5, 6] Dyspnea is the most frequent symptom, followed by dry cough, chest pain, asthenia, pneumothorax, pulmonary fibrosis, and cor pulmonale.[7-10]While children are always in the onset at the early stage of PAM and usually remain asymptomatic when diagnosed; however, some can present with dry cough, exertional dyspnea, and chronic hypoxic signs, including clubbing.[3] Recently, some complications with PAM have been reported, such as asthma, pneumomediastinum, subcutaneous emphysema, and so on.[11-14]

PAM is difficult to be diagnosed because of nonspecific symptoms in children. The diagnosis of PAM is often based on radiographic studies at first, and an exact diagnosis requires at least one additional clinical feature including genetic testing demonstrating a mutation in SLC34A2, microlith analysis, or histopathology.[15] Gradually, it has been a tendency that gene analysis would play an increasingly important role in the diagnostic procedure. Bendstrup et al. summarized 30 genetic variants of SLC34A2 in 2020.[16]

In this case, we identified a PAM patient complicated with bronchitis obliterans by computerized tomography (CT), bronchoscopy and whole-exome-sequencing. Two novel compound heterozygous gene mutations, gain (EXON:2-6 duplication) and c.1218C>A (p. Phe406Leu) were identified to expand the spectrum of gene mutations.

Case history

A 2-year-old boy was admitted because of intermittent fever and cough in the past 15 days. At that time, chest computed tomography (CCT) showed bronchiectasis complicated with extensive pneumonia in both lungs (*Fig.1* A-D). The child remained paroxysmal irritating cough after a 10-day course of antibiotic treatment. His father had a chronic cough caused by smoking, his mother was in good health, and they were not consanguineous. His grandmother was diagnosed with bronchiectasis and he had no siblings. Genetic testing was recommended to his parents but was rejected. Thereafter, he was lost from follow-up until he was hospitalized at age 7 when he had been suffering a persistent cough and expectoration for the past 2 months. He had a dry cough occasionally and decreased exercise endurance over these past 5 years. He had not received systematic cardiopulmonary function assessments or effective treatments from

age 2 to 7. He denied any previous history of allergies, asthma, exposure to *Mycobacterium tuberculosis* or*adenovirus* infections. Physical examinations were unremarkable. Blood tests showed increased white blood cell count at 17.47*10ˆ9/L (NE%0.76, LY%0.17, MO%0.17). Pulmonary function tests (PFTs) showed a restrictive syndrome with a forced vital capacity (FVC) of 1.21L (60.5% of predicted), a mild obstructive ventilation dysfunction with a forced expiratory volume in 1 second/forced vital capacity ratio (FEV_1/FVC) of 69.8% and a positive bronchodilation test (BDT) with FEV_1 improvement ratio at 33.6%. CCT at age 7 (Fig.1 E-H) showed bronchiectasis, interlobular septal thickening and lung fibrosis. And some parts showed "mosaic" features. The mediastinal window showed nodular calcifications in the upper and lower lobes of both lungs (*Fig.1* I-P). Bronchoscopy showed bronchitis obliterans (*Fig.2*) and the Bronchoalveolar lavage fluid (BALF) was still turbid after repeating lavage. BALF metagenomic next-generation sequence (NGS) results suggested one positive pathogen (*Mycoplasma pneumoniae*). The Tuberculin skin test (TST) was medium positive (15mm). Two novel compound heterozygous mutations of the SLC34A2 gene (*Fig.3*) were identified by whole-exome sequencing, EXON:2-6 duplication (from the father) and c.1218 (EXON:11) C>A (from the mother). According to the American College of Medical Genetics (ACMG) guidelines in 2019, [17] the biological pathogenicity of the former was graded as "LIKELY" pathogenic, while the latter was graded as "UNCERTAIN" significance. Based on the evidence above, the patient was finally diagnosed with pulmonary alveolar microlithiasis (PAM). After intravenous injection of amoxicillin-clavulanate (600mg, three times a day), and oral azithromycin (250mg, every other day) for a week, his cough was partly relieved and was discharged.

Discussion

Pulmonary alveolar microlithiasis (PAM) is a rare genetic disease characterized by the accumulation of microliths in the pulmonary alveolar space.[1, 3, 10, 18] These microliths induce a chronic inflammation of the alveolar septa, responsible for a chronic interstitial lung disease leading to respiratory failure and lung fibrosis. Common symptoms are dyspnea, dry cough, chest pain, hemoptysis, asthenia, and possible occurrence of pneumothorax. Children are always in the onset at the early stage of PAM and are often misdiagnosed because of nonspecific symptoms such as dry cough, acute respiratory failure and asthenia.[19] As early symptoms are imperceptible, PAM has a low diagnostic rate in children aged [?] 5 years, accounting for only 2%-3% of all cases (28 cases to 1022 cases in the most recent all-age cohort).[19] In our study, we reported a case of PAM in a 7-year-old boy diagnosed by genetic testing and CT findings.

As reported in the literature, symptoms, signs, serological tests or imaging features of PAM are not typical at the early stage, especially in children. The diagnosis of PAM is often based on radiographic images at first, and a definitive diagnosis requires at least one additional clinical feature including genetic testing demonstrating a mutation in SLC34A2, microlith analysis or histopathology.[15]Genetic testing demonstrates pathogenic mutations in SLC34A2 are highly specific for PAM,[15] and because of less invasive than lung biopsy or transbronchial biopsy, it is more frequently used to confirm PAM diagnosis in children [?]5 years of age than in the all-age cohort.[1] Especially in families with unknown genetic backgrounds, genetic investigations are highly recommended to identify possible variants of SLC34A2. In the cases of suspected PAM with no prior family history, it is also preferred to perform genetic analysis. In our case, we tried to persuade the parents to perform a lung biopsy/transbronchial biopsy or whole-exome sequencing on the patient at age 2, but they rejected which caused delayed diagnosis, while finally diagnosed by gene analysis.

Up to date, there have been approximately 40 pathogenic variants in SLC34A2 reported. Based on the summary of SLC34A2 gene mutations by Bendstrup et al,[16] we searched PubMed and Web of Science in these 3 years until Feb 1, 2023, and updated 7 novel pathogenic variants including this case in*Table 1* , namely c.286 C>T ,[20] c.448 G>A ,[21] c.524-1 G>C ,[22]EXON 2-6 duplication, c.1218 C>A, c.1493 G>T ,[23] c.1653_1660del.[24] Types of DNA variants include 4 substitutions, 1 deletion, 1 splicing site and 1 duplication. According to the literature, there is no clear correlation between genotype/phenotype. Jönsson et al. demonstrated that the disease severity was associated with the pathogenicity of the variants,[6] but this needs to be investigated in a larger patient population. In our case, we identified two heterozygous mutations in SLC34A2, EXON:2-6 duplication and c.1218C>A in EXON 11. The EXON:2-6 duplication

was predicted to disrupt the reading frame and leaded to the transcription factor degradation. Compared to mutations of a single exon, 5 consecutive exons duplication in the coding region tended to cause loss of function. In monogenic autosomal recessive disease, duplications within one pathogenic gene could cause dysfunctions or correspond different phenotypes.[25] The missense variant, c.1218C>A in EXON 11 was also predicted to be pathogenic by forecasting tools, like PROVEAN, SIFT, Polyphen2, Mutation Taster and Revel analyzing conservatism. And gene frequency in normal general was below 0.0005. The compound heterozygous mutations eventually leaded to dysfunction in SLC34A2.

The manifestations of PAM are not classical at the early stage, since the microliths have not caused obvious respiratory dysfunction. The present patient became symptomatic at age 2 and was diagnosed at age 7. His main symptoms were intermittent fever, cough, and expectoration which were consistent with the characteristics of children suffering from PAM. Intermittent fever and cough appear as first symptoms in children cohort and CCT is performed for other reasons such as a viral or bacterial lung infection. Combining with respiratory infections tends to be the first reason for children's admissions, and it is also an opportunity to find abnormal chest images. Further genetic testing or biopsy confirm the diagnosis of PAM. We hypothesized that PAM combined with recurrent respiratory infections, which could be rational to explain why dry cough, fever, acute respiratory failure are frequent symptoms in PAM. Furthermore, recurrent infections are one of the factors resulting in bronchiectasis.

Notably, our patient showed unusual bronchiectasis at age 2. And CCT at age 7 showed that central bronchiectasis had contracted. However, Deniz and his partners found a different observation that peripheral bronchiectasis was seen at a high incidence rate of 60% (6/10) and none of the group (mean age: 22±3.2) had central bronchiectasis.[26] Pathophysiological mechanisms of bronchiectasis include persistent bacterial infections, dysregulated immune responses, airway obstruction and impaired mucociliary clearance.[27] Most common pathogenic causes associated with development of bronchiectasis in children are idiopathic factors, post-infection, congenital immunodeficiency or associated with dysplastic syndromes. [28] For our patient, the hereditary factor could be the primary reason when bronchiectasis was noticed in early childhood since his grandmother and his father had respiratory diseases. Following hereditary factor, airway epithelium was destroyed due to respiratory infections. Especially complicated with persistent infections, central bronchiectasis could be more severe at young age.

In addition, our patient's PFTs showed a restrictive syndrome with FVC of 1.21L (60.5% of predicted), a mild obstructive ventilation dysfunction with FEV_1/FVC of 69.8%, and a positive BDT with FEV_1 improvement ratio at 33.6%. PAM caused by SLC34A2 mutations lead to the accumulation of calcium phosphate in the alveoli, restrict alveolar dilatation, and then progress to a restrictive lung function impairment. Our case also showed a mild obstructive ventilation dysfunction possibly because of excessive sputum. Furthermore, his positive BDT suggested airway spasm, possibly associated with asthma or*Mycoplasma pneumoniae* . The patient denied history of allergies or asthma and lung auscultation had no sonorous rhonchi or sibilant wheezes. There was no insufficient evidence to diagnose asthma. *Mycoplasma pneumoniae*is considered as a factor resulting in a trigger in recurrent wheezing and exacerbations of asthma in children.[29] Besides, there may be bias in this data of BDT, especially under the condition of a reduced vital capacity, which needs multiple measurements.

As PFTs showed a restrictive ventilation dysfunction and CCT showed mosaic sign, bronchitis obliterans was diagnosed by bronchoscopy.[30, 31]Histologically, bronchiolitis obliterans is defined by obliteration of the lumen of bronchioles owning to inflammation, granulation tissue or scarring.[32] Bronchial obliteration presents as a complete obliteration of the bronchus by a smooth-surfaced membrane.[33] Typically, such changes are associated with chronic inflammation of the bronchial walls and cartilage destructions, resulting in structural shifts such as thickening, bronchiectasis, and fibrosis.[34-36] Then the bronchial or bronchiolar lumina may be contracted or dilated and filled with mucopurulent debris[34], with partial or complete luminal obliterans.[35-37] In addition, infections also play an important role in the development of bronchitis obliterans and the most common post-infection pathogens occurrence of bronchiolitis obliterans in children are *Mycoplasma pneumoniae* , *adenovirus* ,*respiratory syncytial virus* , *influenza* , *measles* and*tuberculosis.* [38] *Mycoplasma pneu-*

*monia*adheres to the ciliated columnar epithelium of the respiratory tract and produces local cytotoxic.[39] And P1-adhesin, a transmembrane protein, helps *Mycoplasma pneumonia* in cell-to-cell transfer which eventually increases the infective surface area and results in extensive airway epithelium damage.[40] *Mycoplasma pneumoniae* was detected in our case through BALF-NGS, therefore, bronchitis obliterans in our case was considered to be the results of bronchiectasis and *Mycoplasma pneumoniae* infection. Currently, there has been no evidence to contact PAM with infections, but it has been predicted that environmental factors such as exposure to passive smoking and infections may accelerate the process of PAM.

As for PAM, we should distinguish between miliary tuberculosis (PAM occurs frequently in countries where *Mycobacterium tuberculosis*is common), hemosiderosis, silicosis, carcinomatosis, or sarcoidosis.[19] When there are extensive calcifications in both lungs on mediastinal windows, we should especially distinguish them from miliary tuberculosis (TB).[41] Miliary tuberculosis is a potentially fatal form of disseminated disease due to the hematogenous spread of tubercle bacilli to the lungs, and other organs. It results in the formation of millet seed-sized (1 to 2 mm) tuberculous foci.[42]However, the size (3 to 6 mm) of calcifications in our child's imaging was bigger than that of miliary TB and calcifications were distributed in the middle and lower lobes. While in miliary TB, innumerable micronodular (1 mm) infiltrates, diffusely scattered in both lungs, especially the lung apices.[43] Besides, there was no obvious lymphadenopathy and no associated cavities to spread satellite lesions. No evidence of tuberculosis was found in BALF-NGS. Though the patient's TST was positive, probably because of vaccination. To summarize, TB infection is not considered at this time and calcifications in PAM are quite different from TB in location and size.

Conclusion

PAM is rarely diagnosed in children, especially under 5 years old and this may be related to the lack of obvious clinical symptoms and imaging features. Our patient was diagnosed by identifying two novel gene mutations which expanded the spectrum of genetic mutations in PAM, however, no specific genotype-phenotype could be concluded and a larger population review or further investigations are needed. This unique case could help us to further explore the mechanism of PAM and emphasize the role of gene analysis in diagnosing rare pediatric diseases.

Author contributions

Meiyu Zhang: Writing-original draft. **Man Gao:** Writing-review and editing.**Yuhuan Liu** : Validation. **Wang Kun:** Validation.**Siyan zhou:** Data curation. **Haoran Jing:** Investigation.**Yin Guo:** Supervision. **Fanzheng Meng:** Supervision.

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Conflicts of interest

The authors have no funding and conflicts of interest to disclose.

Data availability statement

All data generated or analyzed during this study are included in this published article.

Consent statement

The patient's parent has provided informed consent for publication of the case.

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Figure legends

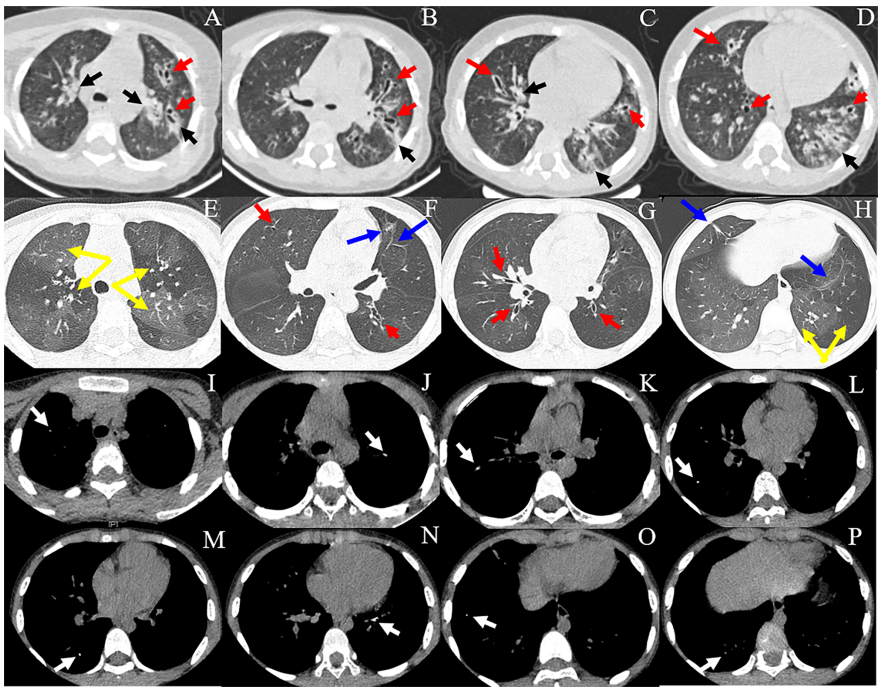

Figure 1: The chest computed tomography of the patient. (A-D) The chest computed tomography of the 2-year-old boy. Chest CT showed bronchiectasis (red arrow) complicated with inflammation (black arrow) in

both lungs. (E-P) The imagings of profound diagnosed with PAM at age 7. Lung windows showed "mosaic" feature (yellow arrow), bronchiectasis (red arrow), and interstitial changes (blue arrow). Mediastinal windows showed extensive calcifications (white arrow) in both lungs.

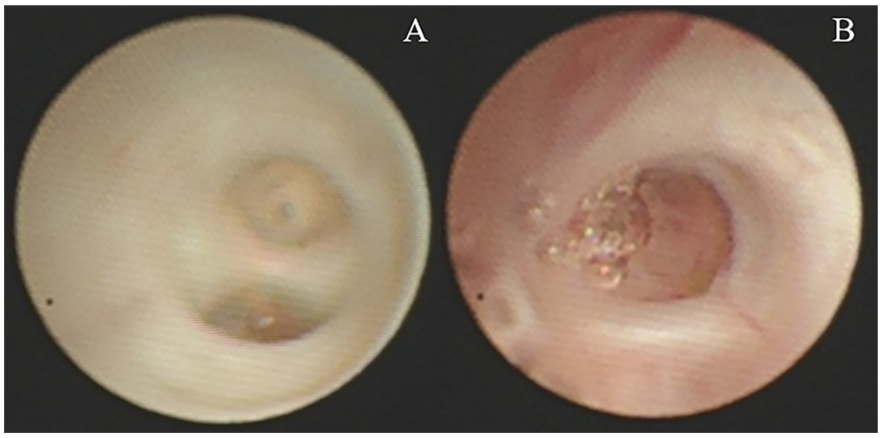

Figure 2: Features of bronchitis obliterans under bronchoscopy. (A-B) Bronchoscopy showed bronchitis obliterans was covered by a smooth-surfaced membrane in involving many of the subsegmental airways of both lungs.

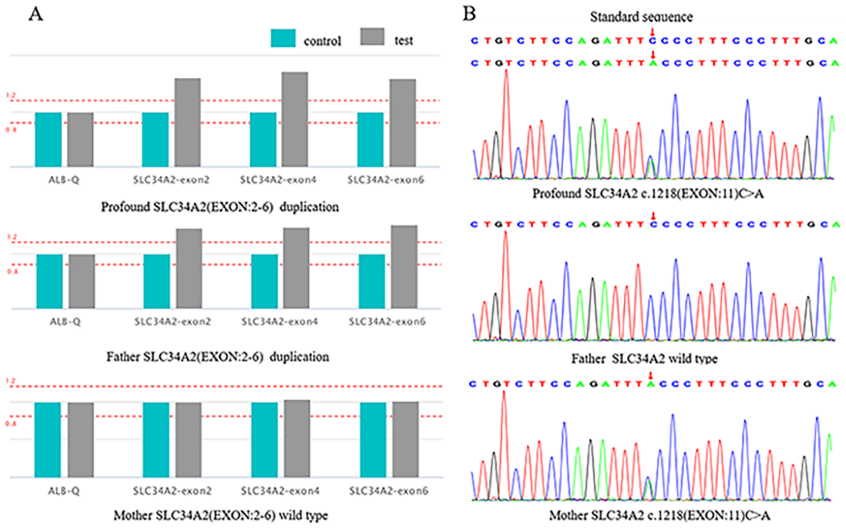

Figure 3: The results of whole-exome-sequencing. (A) The mutation of gain (EXON:2-6 duplication) from his father. The variant was predicted to disrupt the reading frame and leaded to transcription factor degradation. (B) The mutation of c.1218 (exon11) C>A from his mother. The missense variant was suspected as a pathogenic gene mutation.

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