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CASE REPORT

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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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Key Clinical Message

We reported a case of a 7-year-old boy with pulmonary alveolar microlithiasis (PAM) and detected two novel compound heterozygous mutations of solute carrier family 34 member 2 (SLC34A2), EXON:2–6 duplication and c.1218 (EXON:11) C > A (p. Phe406Leu). His symptoms were nonspecific. Chest computed tomography (CCT) showed bronchiectasis, a mosaic feature, and extensive calcifications in both lungs. In addition, bronchoscopy showed bronchitis obliterans which has rarely been reported as a complication in the literature. This case aimed to explore the mechanism of PAM and emphasize the role of gene analysis in diagnosing rare pediatric diseases. Finally, we undertook a review of the current literature containing SLC34A2 gene mutations to update the gene mutation spectrum of PAM.

KEYWORDS

bronchiectasis, bronchitis obliterans, case report, mutation, pulmonary alveolar microlithiasis, SLC34A2

1 | INTRODUCTION

Pulmonary alveolar microlithiasis (PAM) is an uncommon, autosomal recessive lung disease with high penetrance (OMIM #265100) 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 by 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] Children are always

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detected in the early stage of PAM and usually remain asymptomatic when diagnosed, 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, tuberculosis, and subcutaneous emphysema.[11–14]

PAM is difficult to diagnose 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, there has been a tendency for gene analysis to play an increasingly important role in diagnostic procedures. 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.

2 | 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 (Figure 1A–D). The child continued to experience 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

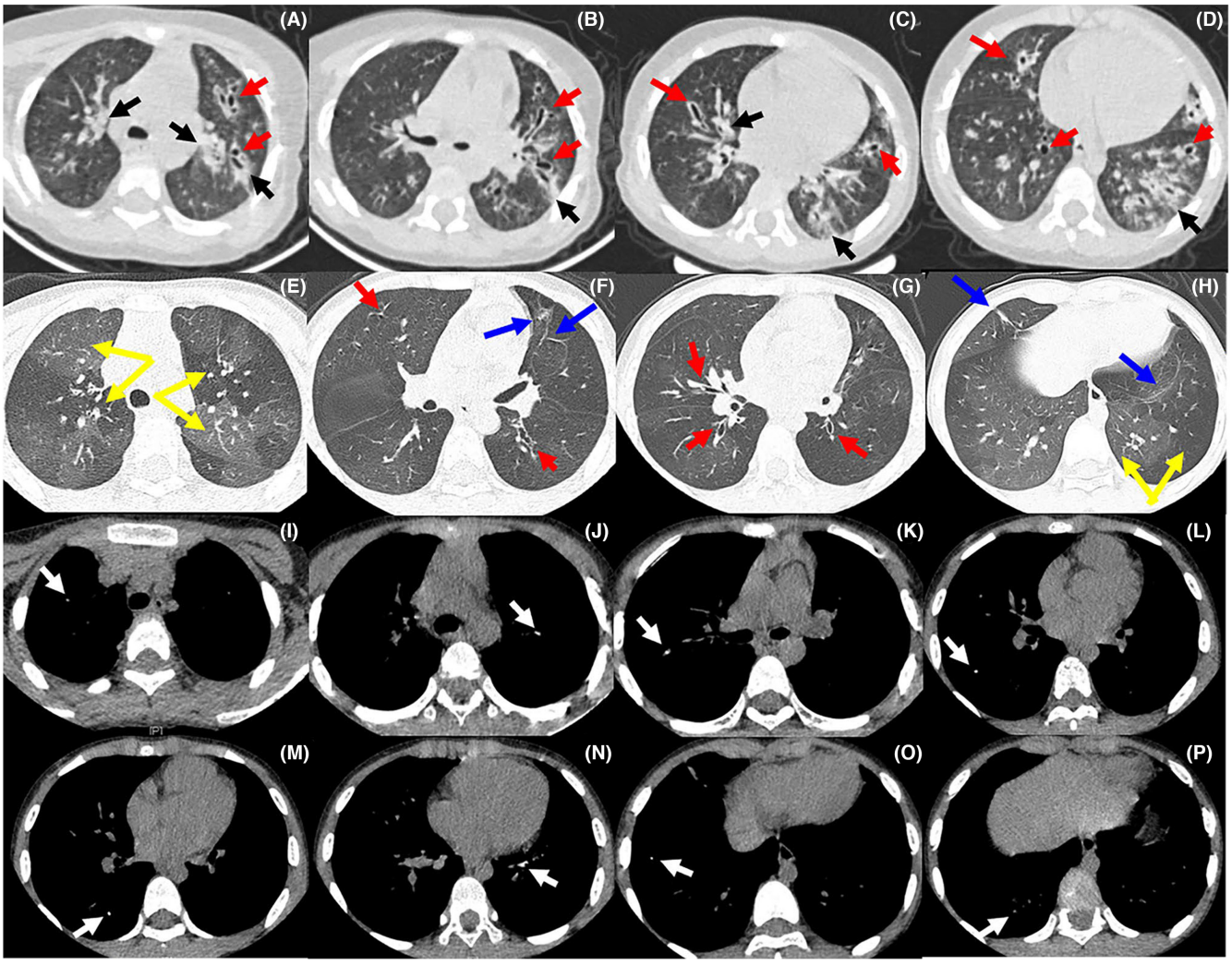

FIGURE 1 Chest computed tomography of the patient. (A–D) Chest computed tomography of the 2-year-old boy. Chest CT showed bronchiectasis (red arrow) complicated by inflammation (black arrow) in both lungs. (E–P) Images of profound diagnosis with PAM at age 7. Lung windows showed mosaic features (yellow arrow), bronchiectasis (red arrow), and interstitial changes (blue arrow). Mediastinal windows showed extensive calcifications (white arrow) in both lungs.

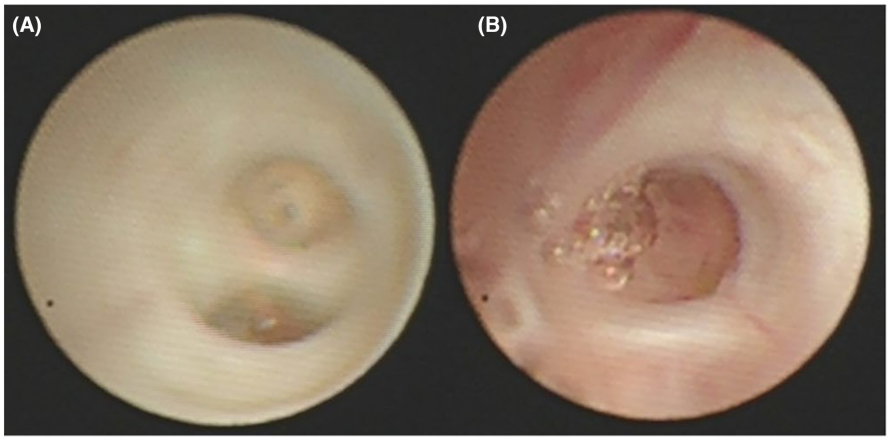

FIGURE 2 Features of bronchitis obliterans under bronchoscopy. (A, B) Bronchoscopy showed that bronchitis obliterans was covered by a smooth-surfaced membrane involving many of the subsegmental airways of both lungs.

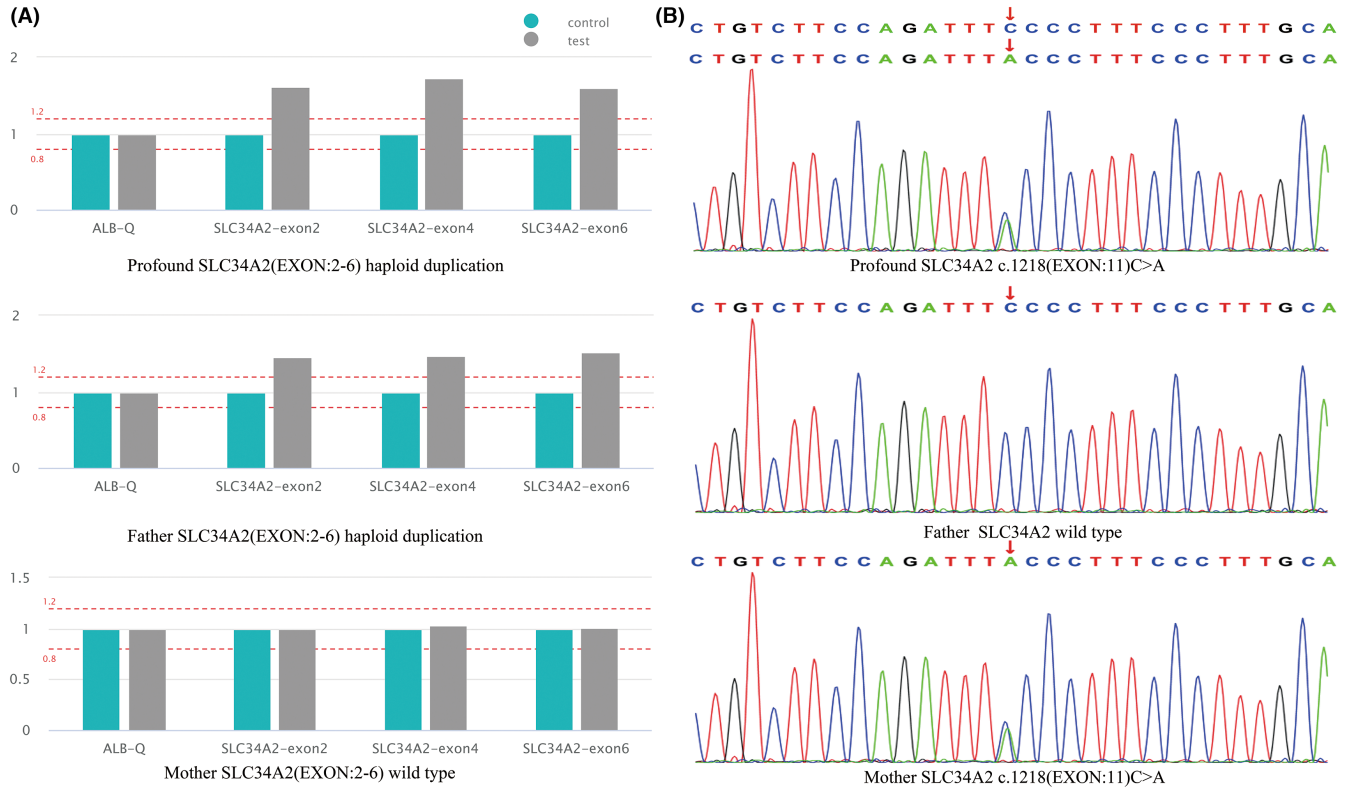

FIGURE 3 Results of whole-exome-sequencing. (A) The mutation of gain (EXON:2–6 duplication) from his father. The variant was predicted to disrupt the reading frame and lead to transcription factor degradation. (B) The c.1218 (exon 11) C > A mutation from his mother. The missense variant was suspected to be a pathogenic gene mutation.

suspicious 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 seven when he had been suffering a persistent cough and expectoration for the past 2 months. He occasionally had a cough and decreased exercise endurance over the 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, or exposure to *Mycobacterium tuberculosis* or *adenovirus* infections. Physical examinations were unremarkable. Blood tests showed increased white blood cell count of 17.47*10^9/L (NE% 0.76, LY% 0.17, MO% 0.17). Pulmonary function tests (PFTs) showed restrictive syndrome with forced vital capacity (FVC) of 1.21 L (60.5% of predicted), mild obstructive ventilation dysfunction with forced expiratory volume in 1 second/forced vital capacity ratio (FEV_1/FVC) of 69.8% and positive bronchodilation test (BDT) with FEV_1 improvement ratio of 33.6%. CCT at age seven (Figure 1E–H) showed bronchiectasis, interlobular septal thickening and lung fibrosis. Some parts showed mosaic features. The mediastinal

window showed nodular calcifications in the upper and lower lobes of both lungs (Figure 1I–P). Bronchoscopy showed bronchitis obliterans (Figure 2), and the bronchoalveolar lavage fluid (BALF) was still turbid after repeated lavage. BALF metagenomic next-generation sequencing (NGS) results suggested one positive pathogen (*Mycoplasma pneumonia*). The tuberculin skin test (TST) was medium positive (15 mm). Two novel compound heterozygous mutations of the SLC34A2 gene (Figure 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 PAM. After intravenous injection of amoxicillin-clavulanate (600 mg, three times a day) and oral azithromycin (250 mg, every other day) for a week, his cough was partly relieved, and he was discharged.

3 | DISCUSSION

PAM is a rare genetic disease characterized by the accumulation of microliths in the pulmonary alveolar space.[1,3,10,18] These microliths induce chronic inflammation of the alveolar septa, which is responsible for 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 detected in 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–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 that pathogenic mutations in SLC34A2 are highly specific for PAM,[15] and because it is 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 cases of suspected PAM with no prior family history, genetic analysis is also preferred. 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 the idea, which caused delayed diagnosis. However, the patient was finally diagnosed by gene analysis.

To date, approximately 40 pathogenic variants in SLC34A2 have been reported. Based on the summary of SLC34A2 gene mutations by Bendstrup et al.,[16] we searched PubMed and Web of Science for the recent 3 years until Feb 1, 2023, and updated seven novel pathogenic variants 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] The types of DNA variants included four substitutions, one deletion, one splicing site and one duplication. According to the literature, there is no clear correlation between genotype/phenotype. Jönsson et al. demonstrated that 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 as 'likely pathogenic' and c.1218C > A in EXON 11 as "uncertain significance". The EXON:2–6 duplication was predicted to disrupt the reading frame and lead to transcription factor degradation (PVS1). Compared to mutations of a single exon, five consecutive exons duplicated 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 to different phenotypes.[25] The missense variant c.1218C > A in EXON 11 was also predicted to be pathogenic by forecasting tools, such as PROVEAN, SIFT, Polyphen2, Mutation Taster and so on (PP3). Moreover, the latter variant was considered for pathogenicity in trans (PM3) and could not be upgraded to strong due to a lack of multiple observations. Both gene frequencies in large general population were below 0.0005 (PM2). The compound heterozygous mutations eventually led 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 patient in our case became symptomatic at age two and was diagnosed at age seven. His main symptoms were intermittent fever, cough, and expectoration which were consistent with the characteristics of children suffering from PAM. Intermittent fever and cough appeared as the first symptoms in the pediatric cohort, and CCT was performed for other reasons, such as viral or bacterial lung infection. Respiratory infections tend to be the first reason for children's admissions, and it is also an opportunity to find abnormal chest images.

TABLE 1 Characteristics and gene mutations of the updated published cases of pulmonary alveolar microlithiasis.

Author, Year	Age, sex, geographic origin	Initial symptoms	Lung imaging findings	Gene mutations in SLC34A2	Complications
Yazdani, 2020	45, Female, UK	Chronic dry cough	CXR: Innumerable small pulmonary nodules producing a 'sandstorm' appearance; HRCT: Widespread interlobular septal thickening and a dense micronodular pattern.	Homozygous c.1653_1660del	Not detailed
Cheng, 2022	36, Male, China	Progressive dyspnea and aphasia for 2 days	CCT: Diffused calcified micronodules and pleural calcification.	Homozygous c.524-1G > C[a]	Ellis–van Creveld syndrome; Hypertension; Edentulous; Chronic kidney dysfunction
Panjwani, 2022	23, Male, Ireland	Fever (38°C), productive cough of 2 days	CXR: Bilateral nodular opacities; HRCT: Diffuse bilateral sand-like calcifications with superimposed regions of ground-glass opacity. The pleural surfaces were seen as a black line and diffuse pleural calcifications.	Homozygous c.1493G > T	*Influenza B*, Past infection with *Mycoplasma pneumonia*
Liu, 2022	11, Male, China	Asymptomatic	CXR: Pulmonary interstitial lesions; Enhanced CT: Diffuse interstitial changes in both lungs with pleural calcification on both sides.	Heterozygous c.286C > T c.910A > T[b]	*Aspergillus fumigatus* infection
Our case, 2023	7, Male, China	Cough and expectoration for 2 months	CCT: Bronchiectasis, subpleural interstitial thickening, interlobular septal thickening and a "mosaic" feature. The mediastinal window showed calcifications in both lungs.	Heterozygous EXON:2–6 duplication c.1218 C > A	Bronchiectasis; Bronchitis obliterans; *Mycoplasma pneumonia*

Abbreviations: BDT, Bronchial dilation test; CCT, Chest computed tomography; CXR, Chest X-ray; FEV_1/FVC, Forced expiratory volume in 1 second/forced vital capacity ratio; FVC, Forced vital capacity; HRCT, High-resolution computed tomography; PFTs, Pulmonary function tests; SLC34A2, Solute carrier family 34 member 2.

[a]c.524-1G > C in SLC34A2 was also identified in a 3-year-old girl with Bartter syndrome and Sheng et al identified another variant, c.448G > A, by a Sanger sequencing analysis of the SLC34A2 gene of all members of this family.

[b]c.910A > T in SLC34A2 has been summarized by Bendstrup et al in 2020.

Further genetic testing or biopsy confirmed the diagnosis of PAM. We hypothesized that PAM combined with recurrent respiratory infections could explain why dry cough, fever, and 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 two. CCT at age seven showed that the severity of central bronchiectasis had been reduced. However, Deniz and his partners found a different observation; peripheral bronchiectasis had a high incidence rate of 60% (6/10), and no one in 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] The most common pathogenic causes associated with the development of bronchiectasis in children are idiopathic factors, postinfection, congenital immunodeficiency or 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. Regarding hereditary factors, the airway epithelium was destroyed due to respiratory infections. Especially when complicated with persistent infections, central bronchiectasis could be more severe at a young age.

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

As PFTs showed restrictive ventilation dysfunction and CCT showed mosaic signs, bronchitis obliterans was diagnosed by bronchoscopy.[30,31] Histologically, bronchiolitis obliterans is defined by obliteration of the lumen of bronchioles owing 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 destruction, 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 postinfection pathogens causing bronchiolitis obliterans in children are *M. pneumonia*, *adenovirus*, *respiratory syncytial virus*, *influenza*, *measles,* and *tuberculosis*.[38] *M. pneumonia* adheres to the ciliated columnar epithelium of the respiratory tract and induces local cytotoxicity.[39] P1-adhesin, a transmembrane protein, helps *M. pneumonia* in cell-to-cell, transfer which eventually increases the infective surface area and results in extensive airway epithelium damage.[40] *M. pneumonia* was detected in our case through BALF-NGS; therefore, bronchitis obliterans in our case was considered to be the result of bronchiectasis and *M. pneumonia* infection. Currently, there is no evidence of 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.

For PAM, we should distinguish from miliary tuberculosis (PAM occurs frequently in countries where *M. tuberculosis* is common), hemosiderosis, silicosis, carcinomatosis, and sarcoidosis.[19] The child did not have a fungal infection, non-necrotizing granulomatous inflammation, siderophores in broncho-alveolar lavage fluid, dust inhalation history or abnormal protein deposition. Other diseases have been excluded except for TB. When there are extensive calcifications in both lungs on mediastinal windows, we should especially distinguish them from miliary tuberculosis (TB).[41] PAM has been wrongly diagnosed as miliary tuberculosis in more than 72 cases.[1] Miliary tuberculosis is a potentially fatal form of disseminated disease due to the hematogenous spread of tubercle bacilli to the lungs, and other organs. This results in the formation of millet seed-sized (1 to 2 mm) tuberculous foci.[42] However, the size (3–6 mm) of calcifications in our patient's imaging was larger than that of miliary TB, and calcifications were distributed in the middle and lower lobes. In miliary TB, innumerable micronodular (1 mm) infiltrates are diffusely scattered in both lungs especially the lung apices.[43] In addition, there was no obvious lymphadenopathy and no associated cavities to spread satellite lesions. No evidence of tuberculosis was found in BALF-NGS. 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.

4 | CONCLUSION

PAM is rarely diagnosed in children, especially those under 5 years old, which may be related to the lack of obvious clinical symptoms and imaging features. Our patient was diagnosed by identifying two novel gene mutations that 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. **Kun Wang:** Validation. **Siyan Zhou:** Data curation. **Haoran Jing:** Investigation. **Guo Yin:** Supervision. **Fanzheng Meng:** Supervision.

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CONFLICT OF INTEREST STATEMENT

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

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONSENT

Written informed consent was obtained from the patient's parent to publish this report in accordance with the journal's patient consent policy.

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