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SLC34A2 gene compound heterozygous mutation identification in a patient with pulmonary alveolar microlithiasis and computational 3D protein structure prediction

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ABSTRACT

We recently diagnosed a patient with pulmonary alveolar microlithiasis (PAM). Because loss-of-function mutations of the *SLC34A2* gene are responsible for the development of PAM, we sought to sequence the *SLC34A2* gene of the patient and his direct relatives, with a purpose to identify mutations that caused the PAM of the patient as well as the carriers of his family. We found a novel compound heterozygous mutation of the *SLC34A2* gene in this patient, which were the mutations of c.1363T > C (p. Y455H) in exon 12 and c.910A > T (p. K304X) in exon 8. Computational prediction of three-dimensional (3D) structures of the mutants revealed that the Y455H mutation resulted in a formation of irregular coils in the trans-membrane domain and the K304X mutation resulted in protein truncation. Our study suggested that sequencing of the *SLC34A2* gene together with a computational prediction of the 3D structures of the mutated proteins may be useful in PAM diagnosis and prognosis.

Introduction

Pulmonary alveolar microlithiasis (OMIM [Online Mendelian Inheritance in Man] 265100) is a rare disease characterized by diffuse alveolar deposition of microliths (Ucan et al., 1993). PAM was first described by Malpighi in 1686 and named by Puhr in 1933 (Puhr, 1933). Since then, more than 500 cases

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have been reported all over the world (Mariotta et al., 2004). Among these patients, about one-third of them are familial. One remarkable feature of PAM is the late onset of symptoms despite the fact that it causes diffuse lung lesions. This is probably also the main reason why PAM is often incidental to other clinical diagnosis (Mariotta et al., 2004).

SLC34A2 is a type IIb sodium phosphate co-transporter which primarily expresses in alveolar type II cells. It is also the only known sodium-dependent phosphate transporter expressed in the lung. This protein plays a key role in clearing phospholipids of the alveolar space through transporting the phosphorus ion into the alveolar type II cells (Hashimoto et al., 2000). Mutations that cause SLC34A2 dysfunction or deficiency could therefore result in an accumulation of the local phosphate in lung parenchyma, which may represent the main mechanism associated with the microlith formation. In 2006, Corut et al. first identified a PAM locus on chromosome 4p15 by homozygosity mapping, and proposed that the mutation of the *SLC34A2* gene causes PAM (Corut et al., 2006). Huqun et al. employed a modified homozygosity mapping method and discovered homozygous exonic mutations on exon 8 of the *SLC34A2* gene in PAM patients they examined (Huqun et al., 2007). Other studies from Turkey, Japan and China have also described multiple mutations of this gene in PAM patients (Dogan et al., 2010; Ishihara et al., 2009; Ozbudak et al., 2012; Proesmans et al., 2012; Tachibana et al., 2009; Wang et al., 2010). However, the functions of these mutated proteins were not analyzed because of the poor understanding of their structures. Here we not only identified the mutations that caused PAM in this patient, but also computationally predicted 3D structures of these mutants. Our study may provide a novel strategy to help diagnose patients who may potentially develop PAM; it may also be used to estimate the prognosis.

Methods

Case report

A 43-year-old man from a non-inbred family was admitted to the hospital complaining of recurrent dyspnea for 1 year. The symptom started as mild chest tightness but deteriorated significantly within a year. He was healthy in the past and denied any history of medication. He had a history of smoking for 20 years with 20 cigarettes per day. His parents are not consanguineous, and he has a sister and a son. None of them complained of discomfort and their chest CT scans were normal.

Physical examination upon admission revealed apparent cyanosis of lips, venous varicose and slight bulb fingers. Crackle rales were heard in both lung fields. Laboratory tests revealed an increased level of hemoglobin (171 g/L) and a decreased level of PaO_2 (49.3 mm Hg); the serum calcium concentration and the tumor index panel were within the normal range. Ultrasound cardiogram showed enlargement of the right ventricle with severe pulmonary arterial hypertension. The dilation function of the left ventricle was found declined. Spirometry exam showed severe restrictive ventilatory disturbances and a decreased diffusing capacity (vital capacity, 44.2% of predicted, forced expiratory volume in 1 s, 84.4% of predicted, carbon monoxide transfer factor-single breath, 42.0% of predicted). Bronchoalveolar lavage fluid (BALF) was milky with negative Rivalta test. Smear of BALF showed fine sand inside. Chest computed tomography (CT) scan showed a diffused infiltration of fine sand-stones in both lungs mainly in the upper fields, and with interstitial changes in lower lung fields (Fig. 1A–D). Trans-bronchial lung biopsy showed lamellar microliths deposited in alveolar spaces and the pleura (Fig. 2A, B). The diagnosis of PAM was therefore established based on the symptoms, thorax imaging and the biopsy.

Genetic analysis

Genomic DNA was extracted from peripheral blood of the patient and his relatives as we previously described (Yin et al., 2013). The study was approved by the Ethics Committee of the second affiliated hospital, Zhejiang University School of Medicine (Ethic No. 2011-7), with a written consent form obtained from the subjects. Using the online software Primer3, 12 pairs of primers (Table 1) were designed to amplify the coding exons and the intronic flanking sequences of the *SLC34A2* gene. The amplifications were performed in thermocyclers (PerkinElmer, Inc, Foster City, CA, USA), starting with an