SLC34A2 Gene mutation of pulmonary alveolar microlithiasis: Report of four cases and review of literatures

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Summary
Pulmonary alveolar microlithiasis (PAM) is a rare autosomal recessive disorder characterized by the deposition of calcium phosphate microliths throughout the lungs. Currently the mutation of SLC34A2 gene was considered responsible for PAM. Here we reported the studies on mutation analysis of the SLC34A2 gene in three familial members and one unrelated subject of PAM by DNA direct sequencing. Meanwhile, we also reviewed and analyzed the published studies of the SLC34A2 gene mutation in PAM patients. The three familial patients were siblings of an inbred family whose parents were cousins. All four patients presented recurrent cough and exertional dyspnea. Diagnosis of PAM was made according to the typical manifestation of radiology. One homozygous mutation of the SLC34A2 gene, c.910A > T (p.K304X) was identified. The review of the SLC34A2 gene mutation showed multiple mutation symbols in PAM patients from China, Turkey, and Japan respectively. The present study supports that the clinical features, pathological and radiological characteristics of Chinese PAM patients are similar to those reported in other countries. Our investigation revealed that the c.910A > T mutation in the SLC34A2 gene was responsible for PAM patients in China. The review of literatures suggests that exon7 and exon8 seemed liable to be affected typical Mongoloid of PAM, and exon8 might be the screen target for Chinese patients.

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Introduction

Pulmonary alveolar microlithiasis (PAM [MIM 265099]) is a rare disease characterized by the deposition of calcium phosphate microliths throughout the lungs. It was first described by Puhr in 1933. Most patients are either asymptomatic for several years or even decades, or only complaining symptoms such as intermittent mild cough or slowly progressive dyspnea. The onset age of this potentially lethal disease varies from the neonatal period to 80 years. Following a chronic progressive course, PAM generally results in a slow deterioration of lung function. A sandstorm-like chest roentgenogram is a typical diagnostic finding. A variety of environmental factors have been suggested as the etiology, nevertheless, about one-third of the reported cases are familial. The most comprehensive report that described more than 500 cases worldwide in 2004 revealed that the prevalence of PAM was predominated in Europe (42.7%) and Asia (40.6%). And Japan, Turkey and India are the main source of PAM cases in Asia. However, very limited data concerning to Chinese PAM patients were reported. Recently, mutation in the SLC34A2 gene, which encodes the sodium dependent phosphate transporter, is considered to be responsible for PAM. However, its mutation symbols in different cases are not investigated yet for its limited data. Here we described a novel mutation of the SLC34A2 gene in an inbred family and a sporadic one, and reanalyzed the existing data of different mutations previously reported.

Materials and methods

Subjects and ethical considerations

A consanguineous family (see Fig. 1A) and one unrelated, sporadic PAM patient (see Fig. 1B) were recruited for this study. Patients were diagnosed based on characteristic chest X-ray and computer tomography (CT) findings. Written informed consent was obtained from either the patient or from an authorized family member. The study was approved by the Committee on Research with Human Participants at Zhejiang University.

Patients from the consanguineous family

Family member II:9, a non-smoking male worker, was diagnosed of PAM at the age of 25 years in a routine checkup. Eight years later, he gradually developed chronic cough and exercise-induced dyspnea. Follow-up chest CT scans revealed diffuse fine sand-like high density shadows in bilaterally fields, especially concentrated in the mid-lower zones. He was followed up once a year for more than thirty years and showed stable status. His parents were first cousins, the mother was healthy with normal chest X-ray manifestations, and the father deceased on his seventy year without case history. His two deceased sisters (II:3 and II:11) were both patients of PAM, the younger one (II:11) was the proband. Both of them were diagnosed in a high school regular checkup asymptomatically, developed respiratory failure 30 years later and finally succumbed to the disease five years ago. II:1, the elder brother of II:9, and III:6, the son of II:9, were unaffected with clear lung fields of chest CT scan.

The sporadic patient from a non-inbred family

This sporadic patient is a 42-year-old, non-smoking female, without any relationship to the foresaid family according to the detail survey of ancestry. She was also identified in a regular checkup at the age of 20. She kept asymptomatic until her 40s when she started experiencing exertional dyspnea. Physical examination revealed normal breath sound. Pulmonary function test demonstrated moderate impairment of ventilation function. A thorough survey of her family excluded the possibility of inbred marriage of her parents who were healthy. The Chest X-rays of all the family including her three brothers and a 20-year-old daughter were normal.

Genetic analysis

Genomic DNA was isolated from peripheral blood samples of the three men in the family (individuals II:1, II:9, III:6 in the pedigree shown in Fig. 1A), the sporadic PAM patient (individual II:7 in the pedigree shown in Fig. 1B) and 100 healthy volunteers using standard protocols. Applying online software of Primer3, 13 pairs of primers as showed in Table 1 were designed to amplify the coding exons of the SLC34A2 gene and the intronic flanking sequences. The amplifications of all exons were performed in thermocyclers (PerkinElmer, Inc, Foster City, Calif), starting with an initial denaturation of 4 min at 95 °C followed by 12 cycles of 35 s denaturation and 35 s annealing at 60 °C (0.5 °C decrease in each cycle, and 40 s extension at 72 °C). After amplification, PCR products were run on 6% PAGE gel to verify the specificities. Then, DNA sequencing was performed in an ABI 3100 Genetic analyzer (Applied Biosystems, Invitrogen Company, Shanghai). All identified disease-associated variants were examined for presence in the 100 controls (200 chromosomes) by direct DNA sequencing.

Literature review

We searched the articles in the PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and China National Knowledge Infrastructure (CNKI) databases (http://www.cnki.net) using the keyword "pulmonary alveolar microlithiasis, SLC34A2". All the papers were reviewed directly by two investigators of our team. The mutation information of the SLC34A2 gene from these articles was collected and reanalyzed together with our result for more comprehensive knowledge about its role in this disease.

Results

Genotyping results

Member II:9 in the consanguineous family and the sporadic case (member II:7 from the non-inbred family) harbored a same homozygous mutation c.910A > T (p.K304X) in the SLC34A2 exon8 (Fig. 2), which was reported previously.
Family 1 member III:6 who was clinically unaffected showed the same variant c.910A > T (p.K304X) in a single chromosome (Fig. 2). Family 1 member II:1 and 100 healthy volunteers didn’t have this variant. No other variants in exons 1–7 and 9–13 were detected. Immediate truncation from Lys led by this mutation would result in premature termination, which was predicted to cause loss of function of the protein.

Review of SLC34A2 mutation in PAM

Eight articles involving 25 cases studied the SLC34A2 gene mutations in PAM cases. Eight cases from an inbred Chinese family and one case from Sweden showed negative results. Other 6 articles with 21 cases demonstrated multiple mutation symbols of the SLC34A2 gene in PAM patients from China, Turkey, and Japan respectively. Among these reports, three point deletions, one deletion plus insertion mutation, five point mutations, one small fragment deletion and one large fragment deletion were identified (Table 2).

Discussion

In present study, we described the clinical features and SLC34A2 gene mutations in an inbred PAM family and an unrelated, sporadic patient and reviewed SLC34A2 gene mutations in PAM patients. Our diagnosis of PAM was established based on the characteristic chest X-ray and CT scan findings with the diffuse intra-alveolar lamellar...
microliths in the lung tissue without obvious clinical manifestation.\textsuperscript{3,7,22} Identification of a homozygous mutation in the \textit{SLC34A2} gene further clinched the diagnosis.\textsuperscript{8,16,17}

The clinical features of our patients were unremarkable compared with previous reported cases.\textsuperscript{1−8,16−19,23} Both cases showed early onset, slow developing course, no obvious difference could be noted between the familial cases and sporadic case, either. Though extrapulmonary calcium deposits in testis, kidney, urethra, gallbladder and aortic valve has been reported,\textsuperscript{5,6,23,24} our patients didn’t present those complications. In spite of its slow development, the prognosis of this disease is not as good as expected. Two (family 1 member II:3 and II:11) of three familial cases died of respiratory failure. Lung transplantation is suggested the only promising treatment, though long-term result is not proved.

\textbf{Figure 2} Sequence analysis of the c.910A > T mutation in the available members of family 1 and the sporadic patient of family 2. The affected nucleotide was indicated with an arrowhead. An A to T homozygous mutation at nucleotide 910 (arrow) in the coding region of exon8 of the \textit{SLC34A2} gene was observed in family 1 member II:9 (B) and the sporadic patient (D). Family 1 member III:6(A) was detected a heterozygous mutation at the same site. The point mutation was not seen in family 1 member II:1(C) and healthy control (E).
The SLC34A2 gene mutation in PAM

Table 2 SLC34A2 Sequence variants identified in patients with PAM.

<table>
<thead>
<tr>
<th>Origin of patient</th>
<th>Cases</th>
<th>Mutation (Location)</th>
<th>Effect on translation</th>
<th>Predicted consequence on protein</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turkish 1</td>
<td>1</td>
<td>c.[-6773_-6588del] + [-6773_-6588del]</td>
<td>Promoter ... exon1</td>
<td>Not synthesized</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>Turkish 3</td>
<td>3</td>
<td>c.[114delA] + [114delA]</td>
<td>Exon3 Frameshift</td>
<td>Truncation</td>
<td>Ref. 17, Ref. 20</td>
</tr>
<tr>
<td>Turkish 1</td>
<td>1</td>
<td>c.[226C &gt; T] + [226C &gt; T]</td>
<td>Exon3 p.Q76X</td>
<td>Truncation</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>Turkish 2</td>
<td>2</td>
<td>c.[316G &gt; C] + [316G &gt; C]</td>
<td>Exon4 p.G106R</td>
<td>Substitution</td>
<td>Ref. 17, Ref. 20</td>
</tr>
<tr>
<td>Japanese 2</td>
<td>2</td>
<td>c.[857-871delinsAAGTTATCGCTTTTTCATC] + [857-871delinsAAGTTATCGCTTTTTCATC]</td>
<td>Exon7 Frameshift</td>
<td>Truncation</td>
<td>Ref. 16</td>
</tr>
<tr>
<td>Chinese 4</td>
<td>4</td>
<td>c.[910A &gt; T] + [910A &gt; T]*</td>
<td>Exon8 p.K304X</td>
<td>Truncation</td>
<td>Ref. 8</td>
</tr>
<tr>
<td>Japanese 4</td>
<td>4</td>
<td>c.[IVS5-1G &gt; A] + [IVS5-1G &gt; A]</td>
<td>Exon8</td>
<td>Aberrant splicing</td>
<td>Ref. 16</td>
</tr>
<tr>
<td>Turkish 1</td>
<td>1</td>
<td>c.[1328delT] + [1328delT]</td>
<td>Exon11 Frameshift</td>
<td>Truncation</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>Turkish 2</td>
<td>1</td>
<td>c.[1342delG] + [1342delG]</td>
<td>Exon12 p.V448X</td>
<td>Truncation</td>
<td>Ref. 17</td>
</tr>
<tr>
<td>Turkish 1</td>
<td>1</td>
<td>c.[1456C &gt; T] + [1456C &gt; T]</td>
<td>Exon13 p.Q486X</td>
<td>Truncation</td>
<td>Ref. 19</td>
</tr>
<tr>
<td>Japanese 1</td>
<td>1</td>
<td>a 5.5 kb long homozygous deletion</td>
<td>Exon2–6 ...</td>
<td>...</td>
<td>Ref. 18</td>
</tr>
</tbody>
</table>

* denotes the results of this paper.

Though one third of cases are familial suggested genetic basis might be the etiology of PAM, it is until 2006 the SLC34A2 gene has been identified to be responsible for PAM. However, currently we only collected 21 cases about SLC34A2 gene mutation in PAM. In those reports, eleven different mutations of SLC34A2 gene were detected without obvious hotspot region among those patients. Exon1, 2, 3, 4, 6, 7, 8, 11, 12 and 13 were involved mainly. The mutation site in Turkish is versatile except exon7 and exon8. Chinese patients only have mutation in exon8. Japanese patients demonstrated a 5.5 kb long homozygous deletion. All of those mutations were homozygous. Most of these mutations happened on the exons, truncation of protein was the predominant result except exon7 and exon8. Chinese patients only have mutation in exon8. Japanese patients demonstrated a 5.5 kb long homozygous deletion. All of those mutations were homozygous. Most of these mutations happened on the exons, truncation of protein was the predominant result of these mutations. All the patients originated from different regions or countries. Thus, it is more likely that the mutation is recurrent rather than identical by descent. As for the different races, Turkish obviously showed more involved exons, and the mutation in Japanese and Chinese was relatively simple, mainly in exon7 and exon8, which suggests exon7 and exon8 might be liable to be affected in. However, the mutation symbols of their patients are quite different as we noted in Table 1, in spite of the fact that Japanese and Chinese belongs to the same race, Mongoloid, and are close in area. This phenomenon might be explained by the influence of environment and life style on genetics.

Our investigation demonstrated a reported mutation of homozygous mutation (c.910A > T) in exon8 of the SLC34A2 gene, which caused premature termination. The truncated protein lacking five functional domains is about half the size of the full-length protein, and has definitely lost its normal function. Although segregation of the mutation could not be confirmed by studying all affected family members due to the death of the two elder sisters, the asymptomatic heterozygous carriers (family 1 member II:9) with normal chest X-ray finding provided the strong evidence. In Chinese patients, both the two unrelated affected familial patients and the sporadic patient had the same nonsense mutation in the exon8 of SLC34A2 gene. The high consistency of the mutation site releases a strong signal that exon8 might be a good target for future gene screening in Chinese PAM cases. However, more cases should be collected to confirm it.

No disease-causing mutations or single nucleotide polymorphisms in the SLC34A2 gene were identified in an inbred, Chinese PAM family reported by Yang Y et al. Recently, the same result has been reported in a PAM patient from Sweden. It suggests that PAM may be a genetically heterogeneous entity that arises from mutations in genes other than SLC34A2 although the mutation could be in the 5’ and 3’ untranslated regions, intron and copy number problems.

In sum, our investigation revealed a homozygous mutation of the SLC34A2 gene in PAM patients. The review of literatures suggests that exon7 and exon8 seemed liable to be affected typical Mongoloid of PAM, and exon8 might be the screen target for Chinese patients.

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Conflict of interest statement

Xinzhen Yin contributed to the design of the study, acquisition of patient, analysis and interpretation of results, and drafting the article. Huiying Wang contributed to the design of the study, patient collection, literature review and revision of the manuscript critically for important intellectual content. Dingwen Wu contributed to the design of the study, genetic analysis and writing the manuscript. Guohua Zhao contributed to the design of the study, literature review, genetic analysis and revision of the manuscript critically for important intellectual content. Jingxin Shao contributed to the design of the study, patient collection, literature review, results interpretation and writing the manuscript. Yu Dai contributed to the design of the study, patient collection, literature review, genetic analysis and writing the manuscript.
the study, literature review, data interpretation and writing the manuscript.

The authors have no conflict of interest to disclose.

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