CASE REPORT

A Frame-Shift Mutation in the SLC34A2 Gene in Three Patients with Pulmonary Alveolar Microlithiasis in an Inbred Family

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Abstract

Pulmonary alveolar microlithiasis (PAM) is a rare disease characterized by the presence of small calculi in the alveolar space. The SLC34A2 is thought to be responsible for the disease. We encountered three siblings of an inbred family who have PAM. We examined the family of the proband who was admitted with dyspnea on exertion and cough, and eventually was diagnosed with PAM. Genetic analysis revealed that both parents (a consanguineous marriage) of the proband were carriers with heterozygous mutation of SLC34A2 gene, and three of their children were diagnosed with PAM with homozygous mutation in the SLC34A2 gene. These findings suggest that impaired activity of the SLC34A2 gene may be responsible for familial PAM.

Key words: familial pulmonary alveolar microlithiasis, SLC34A2 gene, inbred family

(Int Med 49: 45-49, 2010)
(DOI: 10.2169/internalmedicine.49.2702)

Introduction

Pulmonary alveolar microlithiasis (PAM) is a rare disease characterized by the presence of small calculi (calcium phosphate) in the alveolar space (1-3). PAM is more prevalent in some countries such as Italy, USA, and Turkey, as well as Japan. Turkey represents 30% of the worldwide literature on PAM (1, 4-6). The disease is predominant in men, especially among sporadic cases in Turkey. About one-third of the reported cases are familial and the man to woman ratio is equal in familial cases (1-3). Hypothetical mechanisms for the etiology and pathogenesis of the disease are an inborn error of metabolism, an unusual response to an unspecified pulmonary insult, an immune reaction to various irritants, and acquired abnormality of calcium and phosphorus metabolism (7). In contrast, as horizontal transmission among siblings born to unaffected patients has been shown and a high rate of consanguinity was present among the parents of the affected individuals, PAM has been considered to be an autosomal recessive disorder (3, 8). Recently, a few reports have described the role of mutation in the SCL34A2 gene (the type IIb sodium-phosphate cotransporter gene) (8, 9). Here, we describe mutations in SLC34A2 gene in an inbred Turkish family with three siblings diagnosed as PAM.

Subjects

Mutation screening of the SLC34A2 gene in six members of the family of the index case was performed. Written informed consent was obtained from all subjects or their parents.

Sibling 1. (proband)

A 28-year-old man presented with dyspnea on exertion and cough. Bibasilar fine crackles were heard on chest auscultation. Hematology, biochemistry and urinalysis were within normal limits. His chest radiograph showed the presence of bilateral micronodular calcified densities, involving mid- and lower zones of lungs, obliterating cardiac, mediastinal, and diaphragmatic borders. Computed tomography (CT) of the chest revealed diffuse calcified opacities with

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Received for publication July 22, 2009; Accepted for publication August 26, 2009
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extensive conglomeration (Fig. 1a, b). Pulmonary function tests showed a mild restrictive pattern; forced vital capacity (FVC) of 3.39 L (69% predicted); a forced expiratory volume in 1 s (FEV1) of 2.89 L (75% predicted) and an FEV1/FVC ratio of 0.87. Single breath carbon monoxide diffusion capacity (DLCO) showed a DLCO of 6.3 mmol/kPa/min (75% predicted), and adjusted DLCO/VA of 1.64 mmol/kPa/min (128% of predicted). Room air arterial blood gas analysis demonstrated mild hypercapnia (PaCO2 47 Torr, PO2 77 Torr, O2 saturation 96%, and pH 7.44). Bone scintigraphy revealed bilateral intense 99mTc uptake in the lung. There was no history of exposure to dust or fumes, and sputum smear and culture for acid-fast bacilli were negative. The histological examination of transbronchial biopsy confirmed the diagnosis of PAM. He said that his brother had similar symptoms; therefore, we invited the family members for examination.

Mother

A 54-year-old obese woman complained of dyspnea on exertion. She was a self-reported never smoker and was using antihypertensive medication. Her physical examination was normal. Routine laboratory examination including complete blood count, biochemistry and urinalysis were normal, as well as chest radiography.

Father

A symptom-free 51-year-old farmer, self-reported current smoker (40 pack/years). His physical examination was normal. Routine laboratory examination including complete blood count, biochemistry and urinalysis were normal, as well as chest radiography.

Sibling 3.

Brother

One year older than proband (29 years old). He suffered from cough, dyspnea on exertion, nausea, and headache. Breath sounds were diminished on chest auscultation, particularly at the lower lung zones. Bibasilar fine crackles were heard. Erythrocyte sedimentation rate was 52 mm/h. His blood urea nitrogen and creatinine levels were 62 mg/dL and 5.7 mg/dL, respectively. Bilateral diffuse micronodules were observed on chest radiography. Calcified micronodules with rare conglomeration were seen on HRCT. Pulmonary function tests showed a FVC of 3.80 L (75% predicted); a FEV1 of 3.03 L (71% predicted) and an FEV1/FVC ratio of 0.80. Diffusion capacity studies showed a DLCO of 7.1 mmol/kPa/min (60% predicted), and DLCO/VA of 1.38 mmol/kPa/min (64% predicted). Room air arterial blood gas analysis demonstrated mild hypoxemia (PaO2 58 Torr, PaCO2 35 Torr, O2 saturation 90%, and pH 7.46). Scintigraphy revealed bilateral intense 99mTc uptake in the lungs. He was diagnosed with renal failure because of hypertension, and began to receive dialysis three times a week.

Sibling 7.

The 11-year-old sister of the index case complained of loss of appetite and dyspnea on exertion. Routine laboratory examination including complete blood count, biochemistry and urinalysis were normal. Chest X-ray revealed, although not intense, diffuse micronodular infiltration (Fig. 2).
Pedigree and mutations of the SLC34A2 in all available members of family including three siblings (1, 3 and 7) with pulmonary alveolar microlithiasis. Arrows indicate the frameshift mutations.

Sibling 8.

A 21-year-old man, self-reported never-smoker and symptom-free. Chest radiography was normal, as well as physical examination and routine laboratory tests.

Testicular ultrasound was negative for microlithiasis in both man patients (index case and sibling 3) with PAM.

We could not evaluate siblings 2, 4, 5, and 6.

Pedigree and mutations of the SLC34A2 in all available members of the family including the three siblings (1, 3 and 7) with pulmonary alveolar microlithiasis are shown in Fig. 3.

Genotyping

Total genomic DNA was extracted from blood samples of all available family members by the invisorb kit extraction system (Invitex, Germany). Exons 1 and 2 of SLC34A2 gene were simultaneously amplified and directly sequenced.

The primer details were:

SLC-exon1-2 F: 5´-CCACCCAGTTGATGCTTTGC-3´
SLC-exon1-2 R: 5´-GGGACAGGAGGATGATGACA-3´

PCR products were performed in a thermal cycler Applied Biosystem 2720. The profile consisted of an initial step of 5 minutes at 95°C; followed by 35 cycles of 30 seconds at 94°C, 30 seconds at 58°C, and 45 seconds at 72°C; and a final extension step of 10 minutes at 72°C. Computer analyses of the PCR products (519 bps) were performed using custom programs and the nucleotide sequencing of products were made (AB, 310) for each person.

We found a T base insertion that causes frameshift mutations in homozygous (siblings 1, 3 and 7) and heterozygous members (both parents and sibling 8) of the current family.

Discussion

SLC34A2 (the type IIb sodium-phosphate cotransporter
Table 1. Symptoms of Available Family Members (n=6) and Mutations in the SLC34A2 Gene

<table>
<thead>
<tr>
<th>Family Members</th>
<th>Age and Gender</th>
<th>Symptoms</th>
<th>Mutation in the SLC34A2 gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>Father</td>
<td>51-yr-old male</td>
<td>Asymptomatic</td>
<td>Heterozygous frameshift</td>
</tr>
<tr>
<td>Mother</td>
<td>54-yr-old female</td>
<td>Dyspnea on exertion, obesity</td>
<td>Heterozygous frameshift</td>
</tr>
<tr>
<td>Sibling 1 proband</td>
<td>28-yr-old male</td>
<td>Dyspnea on exertion, cough</td>
<td>Homozygous frameshift</td>
</tr>
<tr>
<td>Sibling 2</td>
<td>male</td>
<td>-</td>
<td>Not examined</td>
</tr>
<tr>
<td>Sibling 3</td>
<td>29-yr-old male</td>
<td>Dyspnea on exertion, cough, headache, and nausea</td>
<td>Homozygous frameshift</td>
</tr>
<tr>
<td>Sibling 5</td>
<td>male</td>
<td>-</td>
<td>Not examined</td>
</tr>
<tr>
<td>Sibling 6</td>
<td>female</td>
<td>-</td>
<td>Not examined</td>
</tr>
<tr>
<td>Sibling 7</td>
<td>female</td>
<td>-</td>
<td>Not examined</td>
</tr>
<tr>
<td>Sibling 7</td>
<td>11-yr-old female</td>
<td>Dyspnea on exertion, and loss of appetite</td>
<td>Homozygous frameshift</td>
</tr>
<tr>
<td>Sibling 8</td>
<td>male</td>
<td>Asymptomatic</td>
<td>Heterozygous frameshift</td>
</tr>
</tbody>
</table>

gene) is highly expressed in the lung (specifically in type II alveolar cells), is directly related to calcium or phosphate metabolism, and the mutations abolish the normal gene function. Dysfunction of \( SLC34A2 \) may reduce the clearance of phosphate and may lead to the formation of microliths (9). Corut et al identified homozygous mutations in \( SLC34A2 \) in a large family with PAM; however, the family had mild clinical phenotypes, with the exception of the smokers (8). On the other hand, all three of the present cases with homozygous mutations were symptomatic and their chest radiographs revealed radiologically significant PAM with calcified microliths in both lungs.

Asymptomatic patients with PAM have frequently been reported (2, 3), all three of our cases had complained of dyspnea on exertion but the heterozygous carriers (parents and one boy) were asymptomatic. These findings suggest that all siblings of any age of the index case should be examined for the presence of PAM.

As PAM has been reported in twins and siblings rather frequently (10-13), it has been suggested that genetic factors may be responsible for the disease. We identified homozygous (n=3 siblings) and heterozygous (n=3, both parents and one sibling) frame-shift mutations in the \( SLC34A2 \) gene in a ten-member family. All siblings with the homozygous mutation in the \( SLC34A2 \) (n=3) were diagnosed with PAM. Homozygous frame-shift mutation in the \( SLC34A2 \) gene was reported in seven unrelated patients with PAM by Corut et al (8). A mutation in the \( SLC34A2 \) gene was presented in all six Japanese patients reported by Huqun and colleagues (9). More recently, by a genome-wide SNP study, Ishihara et al (14) detected an intragenetic deletion in \( SLC34A2 \) of a case with pulmonary alveolar microlithiasis. We identified frame-shift mutations in \( SLC34A2 \) gene by directly sequencing technique in affected patients. Our results support the role of mutations of the \( SLC34A2 \) gene in the diagnosis of PAM. The truncated and/or absence of functional protein that encode by \( SLC34A2 \) gene is compatible with calcium phosphate deposition in alveolar cells in the current three first degree probands and causes full penetrance of PAM.

**Conclusion**

As consanguineous marriages are relatively frequent in some regions of the world, siblings of a patient with PAM should be carefully examined for PAM and for mutations in \( SLC34A2 \) gene in these regions. All families with PAM or a mutation in \( SLC34A2 \) gene should be informed about the risks of consanguineous marriage.

**References**

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