Alpha-1-antitrypsin Deficiency in Fraternal Twins Born with Familial Spontaneous Pneumothorax

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Abstract

Background: Spontaneous pneumothorax is a rare condition that occurs when air accumulates in the pleural space without preceding trauma or obvious underlying lung disease. A positive family history is found in about 11.5% of cases and is referred to as familial spontaneous pneumothorax (FSP). In adults and adolescents, FSP has been mechanistically and genetically linked to Birt-Hogg-Dube syndrome, Marfan syndrome, alpha-1-antitrypsin (AAT) deficiency, hemochromatosis, and Ehlers-Danlos syndrome. In neonates, the mechanism of FSP is poorly understood and is likely multifactorial. FSP is uncommon in neonates with four cases reported in the literature.

Case: The twins' family history is remarkable for reactive airway disease and a female sibling born with spontaneous pneumothorax. The family had no history of connective tissue disorders, renal cancer, or dermatologic diseases. AAT phenotype analysis revealed a normal phenotype with no abnormalities noted.

Results: Total serum AAT concentrations were 117 and 132 mg/dL for twin A and B, respectively, (reference interval 100-200 mg/dL). Analysis of their AAT genotype revealed that they were both compound heterozygous for rare SERPINA1 alleles. Additional serum and whole blood specimens were collected from the twins and their parents. Total AAT concentrations of the mother and father were 153 and 107 mg/dL, respectively (reference interval 100-200 mg/dL).

Conclusions: These findings suggest that the combination of A60T;K129E and S330F AAT alleles are likely deleterious and may play a role in neonatal FSP.

Materials and Methods

Patient Samples: Original serum samples for the twin infants were sent to the lab for routine phenotype testing. Upon realizing the complexity of the phenotype results, the physician was contacted and the parents were offered to be tested as controls. The parents and their twin boys submitted additional whole blood and serum samples. Consent for testing was obtained from both parents.

Total AAT: Serum AAT was quantified using an immunoturbidimetric assay on the Roche Modular Analytics P.

Isoelectric Focusing Electrophoresis: IEF was performed using the Hydragel A1AT Isofocusing kit (Sebia, Norcross, GA). Serum samples were applied to a 0.1% agarose (pH = 4.2-4.9) 7F gel and electrophoresis performed for 45 minutes at 20 °C using 700-1000V. AAT bands were visualized using an AAT-antiserum conjugated to peroxidase that produced a pigmented stain after addition of acidic dimethylformamide in hydrogen peroxide. The AAT phenotype is determined by visual inspection and comparison to known patterns (Figure 1).

DNA Sequencing: The coding exons 2-5 of the AAT gene were amplified and sequenced bidirectionally using Big-dye terminator chemistry on an ABI PRISM 3100 DNA Sequencer (Applied Biosystems, Foster City, CA). Data analysis was performed using Mutation Surveyor software (SoftGenetics, State College, PA). Sequence data was aligned to the published human chromosome 14 sequence containing the SERPINA1 gene (AL132708.3).

FIGURE 1: Examples of different AAT phenotypes using IEF

FIGURE 2: Twins’ AAT phenotypes are not consistent with any known AAT variants

FIGURE 3: Family study shows that each parent expresses one wild type and one rare AAT variant

Phenotype Results

Conclusions:

• Fraternal twin boys with a family history of respiratory problems were born with spontaneous pneumothorax.

• Isoelectric focusing electrophoresis analysis of serum revealed that the neonatal twins harbored an AAT phenotype that we were unable to interpret. Serum and whole blood were obtained from the family to elucidate the AAT results.

• DNA sequencing of the twins’ and their parents revealed that both parents were carriers of a rare allele and that the twins’ had inherited both of the rare alleles. One of these alleles (c.250G>A, c.457A>G) corresponding to the A60T;K129E variant had yet to be described in the literature. This variant was named ISalt Lake.

• The mechanism of FSP in neonates is poorly understood and is likely multifactorial, but our work suggests a role for AAT deficiency in the pathogenesis.

Genotype Results

TABLE 1: Gene sequencing of the SERPINA1 alleles and total AAT concentration for the infant twins and their parents. Reference interval for AAT is 100-200 mg/dL.

<table>
<thead>
<tr>
<th>Family Member</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>AAT concentration (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother</td>
<td>S330F (S_munich)</td>
<td>A60T;K129E (K129E)</td>
<td>R01H;E376D (M)</td>
</tr>
<tr>
<td>Father</td>
<td>no mutations (M)</td>
<td>A60T;K129E (K129E)</td>
<td>ISalt Lake</td>
</tr>
<tr>
<td>Twin A</td>
<td>S330F (S_munich)</td>
<td>A60T;K129E (K129E)</td>
<td>117*; 72*</td>
</tr>
<tr>
<td>Twin B</td>
<td>S330F (S_munich)</td>
<td>A60T;K129E (K129E)</td>
<td>132*; 92*</td>
</tr>
</tbody>
</table>

*First draw at 6 days old; Second draw at 17 days old

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References


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