Alpha-1 Antitrypsin Deficiency: From the Phenotype to Genotype

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Historical view

- Alpha-1 Antitrypsin Deficiency is a genetic condition arose in the Swedish population around 2000 years ago.
- Homozygous state for the Z allele has been suggested to increase fertility.
- By that time, life expectancy was short, smoking and pollution were not relevant.
Clinical History

- First clinical observation described in the 1960s in Sweden by Laurell and Eriksson. Makes Sense !!!
- In reviewing serum protein electrophoreses they noticed the absence of the band of Alpha-1 Antitrypsin protein in 5 of approximately 1,500 serum protein electrophoreses.
- These patients had emphysema at young age.
Since these defining descriptions less than 50 years ago, much has been learned about AAT and AATD including:
- The full structure of the Protein AAT.
- AAT molecular function.
- Pathological pathways of AATD.
- Genetics of AATD.

AATD Prevalence
- 1:1500 to 1:3500 in individuals with European ancestry
- 1:5000 in USA
- Uncommon in people of Asian descent.
Alpha-1 Antitrypsin Deficiency

• Alpha-1 Antitrypsin Deficiency (AATD) is a genetic condition that predisposes to
  – Chronic Obstructive Pulmonary Disease (COPD) characterized by early-onset emphysema.
  – Liver disease, especially cirrhosis and hepatocellular carcinoma.

What is Alpha-1 Antitrypsin?

• Alpha-1 Antitrypsin (AAT) is a serine Protease Inhibitor.
• Synthesized **Mainly** in the liver and produced in smaller quantities by macrophages, monocytes and possibly lung epithelial cells.
• Travels to lung by passive diffusion from circulation.
Alpha-1 Antitrypsin Function

• Its main function is to protect the lung against proteolytic damage from neutrophil elastase, which is secreted by neutrophils and macrophages during inflammation to destroys bacteria and host tissue.
• AAT keeps neutrophil elastase under control.
Alpha-1 Antitrypsin

Despite its name, AAT reacts with neutrophil elastase much more avidly than with Trypsin.
Alpha-1 Antitrypsin Deficiency

- The liver doesn’t produce enough functional AAT, and neutrophil elastase gets out of control.

<table>
<thead>
<tr>
<th>AAT serum level</th>
<th>Consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>20–53 uM</td>
<td>Normal</td>
</tr>
<tr>
<td>3-7 uM</td>
<td>AATD</td>
</tr>
<tr>
<td>11uM</td>
<td>Protective threshold</td>
</tr>
</tbody>
</table>

Protease-Antiprotease Balance

Normal

AAT Deficiency

- Neutrophil Elastase Burden
- Anti-Neutrophil Elastase Protection
- Neutrophil Elastase Burden
- Anti-Neutrophil Elastase Protection
Alpha-1 Antitrypsin Deficiency

- The AAT protein is 394 amino acids in size, with the active site residue being methionine at position 358.
- The molecule has a globular tertiary structure, and its active site to inhibit enzymes is on a surface protrusion (a part that sticks out).

Alpha-1 Antitrypsin Structure
**Mechanism of Neutrophil Elastase Inhibition**

Reaction results in destruction of both AAT and NE

AAT flings the tethered NE to the opposite end of the AAT molecule. This distorts the NE active site and alters its structure so it can be destroyed.

*Courtesy of James A. Huntington, PhD; University of Cambridge.*
Mechanism of Neutrophil Elastase Inhibition

Courtesy of James A. Huntingon, PhD; University of Cambridge.

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Mechanism of Neutrophil Elastase Inhibition

[Image of a molecular structure]

Courtesy of James A. Huntingon, PhD; University of Cambridge.
Mechanism of Neutrophil Elastase Inhibition

Courtesy of James A. Huntington, PhD, University of Cambridge.
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AATD Clinical features

• Patients usually develop the first signs and symptoms of lung disease between ages 20 and 50.
• Severity varies between patients AKA phenotypic variability. (Think Genetics ???)

AATD Symptoms

The earliest symptoms are:
• Shortness of breath following mild activity
• Reduced ability to exercise
• Wheezing

Other signs and symptoms can include:
• Unintentional weight loss
• Recurring respiratory infections
• Fatigue
• Rapid heartbeat upon standing
• Vision abnormalities
Clinical Features: Lung Disease

Advanced lung disease leads to a condition known as Chronic Obstructive Pulmonary Disease (COPD) or emphysema of the lungs. Characteristic features of emphysema include:

- Difficulty breathing,
- Hacking cough, and
- Barrel-shaped chest.

Clinical Features: Liver Disease

- **Liver Disease**
  - AAT secreted from the liver
  - The improperly folded protein cannot be secreted, and buildup causes liver damage.

- **Clinically**
  - Cirrhosis may develop in patients with alpha-1-antitrypsin deficiency
  - Reduced liver function
  - Inflammation of liver → severe fibrosis of the liver
  - Liver failure.
  - Increased risk of developing hepatocellular carcinoma.
Clinical Features: Panniculitis

In some cases, people with AATD develop a skin condition called panniculitis, which is characterized by hardened skin with painful lumps or patches.

AATD phenotypic variability

- Severity of disease manifestations varies between patients. For example, not all patients with AATD develop liver disease. WHY?
SERPINA1 gene

- SERpin Peptidase INhibitor, clade A member 1.
- Mutations in the SERPINA1 gene cause AATD.
- Located on the long (q) arm of chromosome 14 at position 32.1. (14q32.1)
- The gene has 4 coding exons (II, III, IV, V), 3 non-coding exons (IA, IB, IC) and 6 introns; the region coding for the reactive loop is within exon V.

SERPINA1 and AATD

- It provides instructions for making a protein called alpha-1 antitrypsin.
- Mutations in the gene can lead to a shortage (deficiency) of alpha-1 antitrypsin or an abnormal form of the protein that cannot control neutrophil elastase causing AATD.
AATD mode of inheritance

- This condition is inherited in an **autosomal co-dominant pattern**. In which two alleles of a gene pair in a heterozygote both have full phenotypic expression.

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Co-dominance vs. Incomplete dominance

<table>
<thead>
<tr>
<th>Co-dominance</th>
<th>Incomplete dominance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-dominance occurs when the contributions of both alleles are visible in the phenotype.</td>
<td>Occurs when the phenotype of the heterozygous genotype is distinct from and often intermediate to the phenotypes of the homozygous genotypes.</td>
</tr>
</tbody>
</table>

![Co-dominance vs. Incomplete dominance diagram](image)
AAT Alleles and Phenotypes

- Phenotypes are classified by a PI coding system.

- We write them as PI*MZ or PI*MM where PI means protease inhibitor and the letters following the * referring to the alleles.
AAT Gene Coding Region

SERPINA1 Alleles

• The most common version (allele) of the SERPINA1 gene, called M, produces normal levels of alpha-1 antitrypsin.
• Most people in the general population have two copies of the M allele (MM) in each cell.
Other versions of the SERPINA1 gene lead to reduced levels of alpha-1 antitrypsin. The S allele produces moderately low levels of this protein.
SERPINA1 Alleles

Deficient Alleles

• Z allele produces very little alpha-1 antitrypsin.
• Characterized by a single amino acid substitution of lysine for glutamic acid at position 342, is the most common, accounting for approximately 95% of cases of clinically recognized AATD.

SERPINA1 Alleles

Null Alleles

• Rare alleles.
• Null variants are characterized by absent circulating AAT due to transcriptional or translational errors that interrupt protein synthesis and dysfunctional variants which are characterized by abnormal function of AAT.
Range of Serum Levels by Phenotype

Adapted from The Alpha-1 Foundation slide set. www.alphaone.org.
Courtesy of H. Ari Jaffe, MD
Range of Serum Levels by Phenotype

<table>
<thead>
<tr>
<th>Genetic PI Type</th>
<th>MM</th>
<th>MS</th>
<th>SS</th>
<th>MZ</th>
<th>SZ</th>
<th>ZZ</th>
<th>Null</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk of lung disease</td>
<td>“normal”</td>
<td>low</td>
<td>low</td>
<td>low</td>
<td>high</td>
<td>high</td>
<td>high</td>
</tr>
</tbody>
</table>

Adapted from The Alpha-1 Foundation slide set. www.alphaone.org. Courtesy of H. Ari Jaffe, MD

AATD Clinical Features

Fregonese (2008)
Disease Mechanism
The PI*ZZ story

• Patients of AATD with PI*ZZ alleles Usually have problems in the lungs and liver.
• The substitution of lysine for glutamic acid at position 342 widens the b-sheet A and allows polymerization, which links the reactive loop of one AAT molecule to the b-sheet A of another molecule in an irreversible process.

Figure 6
**Disease Mechanism**
**The PI*SS story**

- PI*SS results in an instable protein that is easily degraded outside the hepatocyte and affects the half-life of the S variant AAT.
- Mutation: Glu264Val

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**Table 1. Effects of genetic variation within the Pi locus**

<table>
<thead>
<tr>
<th>Allele</th>
<th>Genetic defect</th>
<th>Cellular defect</th>
<th>Disease association</th>
</tr>
</thead>
<tbody>
<tr>
<td>Z</td>
<td>1-bp substitution</td>
<td>intracellular aggregation</td>
<td>lung, liver</td>
</tr>
<tr>
<td>S</td>
<td>1-bp substitution</td>
<td>intracellular aggregation</td>
<td>lung</td>
</tr>
<tr>
<td>Mmalton</td>
<td>3-bp deletion</td>
<td>intracellular aggregation</td>
<td>lung, liver</td>
</tr>
<tr>
<td>Siiyama</td>
<td>1-bp substitution</td>
<td>intracellular aggregation</td>
<td>lung</td>
</tr>
<tr>
<td>Mjrocl/a [42]</td>
<td>1-bp substitution</td>
<td>intracellular degradation</td>
<td>lung</td>
</tr>
<tr>
<td>Mheerlen [43, 44]</td>
<td>1-bp substitution</td>
<td>intracellular degradation</td>
<td>lung</td>
</tr>
<tr>
<td>QOgranite falls</td>
<td>1-bp deletion</td>
<td>unstable mRNA</td>
<td>lung</td>
</tr>
<tr>
<td>QOHong kong</td>
<td>2-bp deletion</td>
<td>truncated protein</td>
<td>lung</td>
</tr>
<tr>
<td>QOIsola di procida</td>
<td>deletion exons II–IV</td>
<td>no mRNA</td>
<td>lung</td>
</tr>
<tr>
<td>Pimlneral springs</td>
<td>1-bp substitution</td>
<td>poor inhibition of NE</td>
<td>lung</td>
</tr>
<tr>
<td>PIPittsburgh</td>
<td>1-bp substitution</td>
<td>antithrombin III activity</td>
<td>bleeding diathesis</td>
</tr>
</tbody>
</table>

Why liver disease is not associated with null alleles or S allele?

Wood (2007)
PI*SZ Phenotype

- A slower rate of polymer formation occurs with the PI*S allele (Glu264Val), because the structural change in Beta-sheet A is not as radical as in PI*Z, resulting in a milder serum deficiency but little evidence of clinical disease. If an individual has the genotype PI*SZ, then their clinical phenotype for liver disease and lung disease in smokers is usually similar of PI*Z and PI*S subjects, Remember co-dominance?

Genetic Counseling

When patients are identified as a new case of homozygous type Z alpha-1-antitrypsin deficiency, the issue of heritability for their children is frequently raised. It is less inconvenient for the children when first the other parent is investigated by isoelectric focusing or genotyping for alpha-1-antitrypsin. Since about 95% of individuals carry the MM phenotype, all children from parents with ZZ and MM type will carry the MZ type alpha-1-antitrypsin. If the parent is not MM, but is carrying a deficient allele next to the M allele (i.e. MZ), there is a 50% chance of ZZ genotype for every newborn from these parents and this can be confirmed in the child by iso-electic focusing of serum.