Alpha-1 Antitrypsin Deficiency

A future NBS candidate?

Robert A. Sandhaus, MD, PhD
Alpha-1 Antitrypsin Deficiency

**Condition:** Alpha-1 antitrypsin deficiency, alpha-1 proteinase inhibitor deficiency, Alpha-1, AATD

**Alpha-1 antitrypsin:** Protein that is potent serine proteinase inhibitor (SERPIN) and major anti-inflammatory protein

**Genetics:** Single gene mutation on lung arm of chromosome 14 in PI locus. Over 100 mutations identified about 1/3 of which lead to deficiency of dysfunction of alpha-1 antitrypsin protein; 18 Null mutations

**Manifestations:** Neonatal fulminant liver disease in 2%, childhood liver disease, adult liver and lung disease

**Prevalence in US:** Between 100,000 and 500,000 individuals with severe deficiency
The “Good” PiM Phenotype

The “Bad” PiZ Phenotype
The Genome and Alpha-1

~ 14,000 base pairs

Glu 342 GAG to Lys AAG

394 amino acids

Co-dominant allelic expression
Mass Screening of Newborn Swedish Infants for
\( \alpha_1 \) Antitrypsin Deficiency

C.-B. Laurell\(^1\) and Tomas Sveger\(^2\)

Alpha\(_1\) antitrypsin is the predominant extracellular protease inhibitor. It occurs in a series of genetic variants, the Pi system [1], determined by multiple codominant alleles. Pi ZZ is the main type which causes \( \alpha_1 \) antitrypsin deficiency with a concentration of 10\%–20\% of the normal mean adult level [1]. The existence of the Pi\(^r\) (null) allele with total deficiency for \( \alpha_1 \) antitrypsin was recently suggested by Talamo [2] and corroborated by others [3, 4]. The Pi\(^s\) allele causes a slight depression of the \( \alpha_1 \) antitrypsin level. Therefore, Pi SZ and Pi SS individuals have about 40\% and 60\% of the mean normal \( \alpha_1 \) antitrypsin concentration, respectively.

Genetic variants with severe deficiency are associated with certain diseases. Juvenile cirrhosis [5] and clinical or subclinical hepatic fibrosis and cirrhosis in adults [6] have been described in cases of severe \( \alpha_1 \) antitrypsin deficiency. Clinical pulmonary disease very rarely begins in childhood [7]. The first symptoms of pulmonary emphysema usually occur in young adults and in middle-aged people.
Neonatal screening for alpha$_1$-antitrypsin deficiency

The Oregon State Public Health Laboratory screened 107,038 newborn infants between 1971 and 1974 to determine the frequency and clinical characteristics of alpha$_1$-antitrypsin deficiency. The screening program was based upon an assay of the total trypsin inhibitory activity in dried blood specimens collected on filter paper from infants during the first week of life and again from 75% of the same infants at four to six weeks of age. Twenty-one homozygous-deficient (PiZ) infants were identified, an incidence of one in 5,000. Of the 18 infants studied and followed, only one had neonatal hepatitis; five had hepatomegaly or biochemical abnormalities or both, indicating hepatic damage. Presently, the children range from three to six years of age; all are asymptomatic. Four of them have mild hepatomegaly and biochemical evidence of liver damage. As a result of family studies, four homozygous-deficient (PiZ) siblings were identified. One child had evidence of mild hepatic dysfunction, but the other three were clinically and biochemically normal. Nine of the 21 PiZ infants detected were missed on the initial sample, but identified on the four to six week sample. If a screening method based upon TIA is to be utilized, these results indicate that a repeat screening specimen should be obtained at four to six weeks of age. Newborn screening for alpha$_1$-antitrypsin deficiency is not warranted at this time in view of the low frequency of significant pulmonary or hepatic involvement in childhood and the absence of specific therapy for this condition.
Preventive pediatrics

Neonatal screening for alpha-1-antitrypsin deficiency

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Abstract. The results of a neonatal screening programme for alpha-1-antitrypsin deficiency are presented. Cord blood samples with an alpha-1-antitrypsin concentration below 1.628 mg/ml, as measured by an enzyme-linked immunosorbent assay method, were phenotyped by isoelectric focusing in polyacrylamide gels. Abnormal phenotypes were found in 51% of this group as compared with 11.3% in a control group (\(P \ll 0.0001\)). Twenty subjects detected by the initial quantitative alpha-1-antitrypsin determination had a highly pathogenic phenotype (PiZZ, PiSS, PiSZ). In the control group only moderately affected individuals were found (PiMS, PiMZ).

Key words: Alpha-1-antitrypsin – Enzyme-linked immunosorbent assay – Isoelectric focusing – Neonatal screening

Introduction

disorder may be warranted. The main objective of this study was to evaluate quantitative cord blood alpha-1-antitrypsin levels as a screening procedure for abnormal phenotypes.

Patients and methods

Cord blood samples from 10,329 consecutive newborns were examined over a period of 23 months (1 February 1984–31 December 1985). The children were born in the community hospitals of Genk, Bree, Waterschei, Heusden, Tongeren, Bilzen, Hasselt and Neerpelt. All these hospitals are located in Limburg, a province in North Eastern Belgium, having 730,000 inhabitants and a birth rate of 12 per thousand. Nearly 60% of all newborns are currently enrolled in this screening program.
Molecular Confirmation of $\alpha_1$-Antitrypsin Genotypes in Newborn Dried Blood Specimens

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Deficiency of $\alpha_1$-antitrypsin ($\alpha_1$AT), a common hereditary disorder of Caucasians, is associated with an increased risk for early-onset chronic obstructive pulmonary disease and childhood liver dysfunction. The two most common deficiency variants, Pi* and Pi‡, are both single base-pair substitutions causing amino acid modifications, although neither mutation creates or destroys a naturally occurring restriction site. Dried blood specimens (DBS) submitted to the New York State Department of Health for mandated newborn screening tests were tested for $\alpha_1$AT activity using a fluorometric elastase inhibition assay. A second DBS from specimens determined to be $\alpha_1$AT deficient was phenotyped on an agarose isoelectric focusing gel. Genotypic confirmation was performed by amplifying, directly from a DBS, the regions of the DNA containing the S and Z mutation. The Z mutation was analyzed with a modified primer designed to create an artificial restriction site in the normal
Alpha-1-antitrypsin deficiency. High prevalence in the St. Louis area determined by direct population screening.

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Considerable attention has been focused upon alpha-1-antitrypsin deficiency because of the insights into the pathogenesis of human pulmonary emphysema that may derive from study of deficient subjects, and because of evolving therapeutic strategies that may slow the progression of lung disease in affected persons. We have applied an automated immunoassay for alpha-1-antitrypsin to plasma samples from 20,000 blood donors. Seven PI Z antitrypsin-deficient persons were identified and confirmed; this is more than twice the number predicted from previous estimates of the Z allele frequency in the St. Louis area. Five of the subjects were further evaluated. We anticipate that this assay, if utilized to screen large populations, could identify many alpha-1-antitrypsin-deficient persons for study of the natural history of lung and liver disease associated with the deficiency. These subjects would be potential candidates for early intervention strategies to prevent the development of lung disease. The surprisingly high prevalence of deficient persons indicates that direct screening is the best method for prevalence estimation of genetic disorders.
## Summary of Screenings

<table>
<thead>
<tr>
<th>Location</th>
<th>Year Population</th>
<th>Number screened</th>
<th>Prevalence of PiZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweden</td>
<td>1975 NBS</td>
<td>108,000</td>
<td>1 : 1,433</td>
</tr>
<tr>
<td>Oregon</td>
<td>1978 NBS</td>
<td>107,038</td>
<td>1 : 5,000</td>
</tr>
<tr>
<td>Belgium</td>
<td>1988 NBS</td>
<td>10,329</td>
<td>1 : 1,721</td>
</tr>
<tr>
<td>St. Louis</td>
<td>1989 Blood donors</td>
<td>20,000</td>
<td>1 : 2,857</td>
</tr>
<tr>
<td>New York</td>
<td>1993 NBS</td>
<td>11,081</td>
<td>1 : 3,694 (1 : 2,019)</td>
</tr>
</tbody>
</table>
1999 Alpha-1 Screening Workshop

- New Born Screening was rejected for the following reasons:
  - No infant or childhood intervention thought to be required or available
  - Family psychological ramifications (Sveger paper)
  - Risk of genetic discrimination in health insurance, life insurance, employment
  - May label an infant with a genetic disease who will never have any clinical manifestations due to their Alpha-1
1991 Alpha-1 Screening Workshop

- Adult Screening was rejected for the following reasons:
  - Giving a healthy individual a new “disease”
  - No intervention required for healthy adults
  - Low yield in screening programs done to that time
  - Risk of genetic discrimination
  - Psychological risks
1991 Alpha-1 Screening Workshop

- Targeted Detection was recommended because:
  - Not giving an individual a new “disease”; rather giving an individual with a known disease and explanation for that disease (and perhaps a more appropriate therapy)
  - High ‘hit rate’ in individuals with diseases associated with Alpha-1, therefore more cost-effective
• Recommended reconsideration of NBS
  – No infant or childhood intervention thought to be required or available
    • Liver treatment for neonates
    • Chaperone therapy
    • Gene therapy
    • Smoking cessation, especially parental
  – Family psychological ramifications (Sveger paper)
    • Follow-up paper discounted this
  – Risk of genetic discrimination in health insurance, life insurance, employment
    • GINA
  – May label an infant with a genetic disease who will never have any clinical manifestations due to their Alpha-1
    • But can provide long-term regular follow-up
Current testing methods

- Antibody-based level testing
- Pi-typing (phenotyping) by isoelectric focusing between pH 4.0-5.0
- Genotyping by PCR and probes for the two most common deficient genotypes
- Algorithm-based testing protocol in a standards document from 2003
What is needed?

- Agreement on what result should be targeted
  - Most common severe deficiency alleles?
  - All deficiency alleles?
  - Heterozygotes?
- Appropriate, high throughput testing methodology
- Documentation that proposed interventions can effectively improve outcomes
- Pilot studies
What have I learned by coming to this meeting?

- Alpha-1 could be the first to target early childhood screening rather than NBS
- Alpha-1 has some unique features compared with other conditions considered for NBS
- We’d better work fast if we want to use existing DBS for proof of concept