Routine genetic testing for alpha-1 antitrypsin deficiency

In October 2003, the American Thoracic Society and the European Respiratory Society issued state of the art standards for the diagnosis of alpha-1 antitrypsin (AAT) deficiency [1]. These are the first comprehensive guidelines for the diagnosis of a genetic disease.

AAT deficiency is one of the most common lethal genetic diseases worldwide. Patients with this condition have insufficient amounts of serum AAT, a plasma protein with antiproteolytic activity. AAT deficiency leads to chronic obstructive airway disease, chronic liver disease, and occasionally to skin and vasculitic disorders.

Mutations in the PI gene, located on chromosome 14, are associated with this genetic disorder. The most common deficiency alleles are PiS (1:9 - 1:12 carrier frequency among Caucasians) and the PiZ allele (1:30 - 1:40 carrier frequency among Caucasians). New data on the worldwide prevalence of AAT deficiency support the scientific community’s impression that AAT deficiency is not a rare disease, but rather a disease that is rarely diagnosed [2,3]. The frequency of the phenotypic classes of the PiZ and PiS deficiency alleles varies considerably between different population groups and is shown in Figure 1 [3].

Disease origin: from neolithic times to the third millennium
Experts believe that the PiZ allele arose in northern Europe and was spread by the Vikings during their explorations in the Neolithic period. The PiS allele is much older and is thought to have originated in the Iberian Peninsula. AAT deficiency was discovered in 1963 by two Swedish physicians. Since this time, much has been learned about the disorder. The most common manifestation of AAT deficiency is emphysema, which is a significant cause of disability and death. Other lung diseases associated with AAT deficiency include chronic obstructive pulmonary disease (COPD), asthma, chronic bronchitis and bronchiectasis. The second most frequent clinical manifestation is liver disease, including hepatitis, cirrhosis, hepatocarcinoma and liver failure.

AAT deficient individuals are uniquely susceptible to exposure to chemical and particulate agents in the environment. Such exposure leads to lung and liver disease, as well as other adverse health effects. Cigarette smoking has a major effect on both the age at which pulmonary symptoms present and the course of pulmonary deterioration.

Early diagnosis is clearly important since prompt identification of the deficiency, particularly prior to the onset of lung disease, can induce the individual to modify his/her lifestyle, especially with respect to cigarette smoke. Moreover, early diagnosis allows effective treatment. The current treatment approach is augmentation therapy, in which human or recombinant AAT is infused or inhaled [4].

Genetic testing for AAT deficiency
The recent guidelines published by the American Thoracic Society and the European Respiratory Society provide a benchmark for the genetic diagnosis of AAT deficiency. These guidelines directly address who should be tested and why, and also distinguish between three different types of genetic testing:

1. Differential diagnosis allows for appropriate therapy and prevents unnecessary procedures for symptomatic individuals with AAT deficiency. Since AAT deficiency is not the first suspected cause of lung and liver disease, symptomatic individuals are frequently misdiagnosed or remain undiagnosed by health care providers. The new guidelines recommend that people with emphysema, COPD, unexplained liver disease, necrotising panniculitis, vasculitis, and bronchiectasis undergo genetic testing for AAT deficiency.
2. Genetic predisposition testing identifies asymptomatic individuals at high risk of AAT deficiency and allows treatment to be administered to prevent or delay the onset of the disease. Predisposition testing is recommended for family members of AAT deficient individuals, and to those with a family history of unexplained COPD or liver disease.

3. In countries where the prevalence of AAT deficiency is high, carrier screening can be performed to relieve anxieties during pregnancy, whereas a positive test may allow prospective parents to become emotionally prepared for parenting a child with AAT deficiency or to consider other options.

In view of these new guidelines, several commercial genetic tests for the diagnosis of AAT deficiency have been introduced to the market. However, there is growing demand for a simple, high-throughput, cost-effective screening tool.

A new SNP-based genetic test for AAT deficiency
Pronto Diagnostics has developed a multiplex single nucleotide primer extension assay for the identification of the PiZ and PiS deficiency alleles, based on the patented Pronto technology [Figure 2]. ProntoPlex AAT is a simple, high throughput screening method to detect the alleles most frequently associated with AAT deficiency. This assay tests for both the PiZ and PiS alleles in two wells of the ProntoPlex AAT 96-well microtiter plate. One well tests simultaneously for both mutant alleles, while the other detects the normal PiM allele. A complete AAT genotype of positive samples (PiSS, PiSZ, PiSM or PiZM) can be obtained using the Pronto AAT Verification Strip [Figure 3]. In this test every mutation is detected separately in two wells of an 8-well strip.

Each detection well contains 5′ labelled primers complementary to the mutation sites, as well as a single biotinylated nucleotide. The primers are extended, depending on the tested individual’s genotype, and thus become labelled with biotin. Reaction products are transferred to a streptavidin-coated ELISA plate and treated according to a standard ELISA protocol. Results are determined either visually (substrate turns blue) or colourimetrically (O.D. 450 nm).

Applications
The novel ProntoPlex AAT kit is designed to meet the new standards for the genetic diagnosis of AAT deficiency. It provides a simple, reliable and economical means for multiplex mutation detection. Using this test, molecular laboratories can easily adhere to the new guidelines for all three types of recommended genetic tests: differential diagnosis, predisposition testing and carrier screening.

In light of the high prevalence of the severe PiZ allele found among the Ashkenazi Jewish population (1:92),

AAT deficiency screening is now offered to these individuals. With the development of this AAT kit, Pronto Diagnostics now provides a 33-mutation panel which is a complete, uniform diagnostic tool for Ashkenazi Jewish carrier screening.

References
3. de Serres FJ. Alpha-1 antitrypsin deficiency is not a rare disease but a disease that is rarely diagnosed. Environ Health Perspect. 2003; 111(16): 1851-1854.
4. www.alpha1.org

Molecular Diagnostics

Figure 3. Complete genotyping of positive samples with the Pronto AAT Verification Strip.