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Alpha-1 antitrypsin Null mutations and severity of emphysema

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Summary
Background: Alpha-1 antitrypsin (AAT) deficiency is an autosomal-codominant disorder, caused by mutations in the SERPINA1 gene on chromosome 14. Individuals affected by the most common mutations, SZ and ZZ, have serum AAT concentrations of 25% and 15% of normal levels, and present a higher risk of emphysema. Mutations causing total absence of serum AAT (Null mutations) were suggested to be associated with very early onset emphysema but their clinical phenotype is poorly known.
Hypothesis: Absence of AAT in Null mutations results in more severe emphysema as compared to ZZ and SZ.
Methods: We genotyped all known Dutch subjects ($n = 12$) with absent serum AAT, and compared their lung function values (FEV_1 and K_{CO}) with those of individuals with ZZ and SZ genotype, matched for age and smoking history.
Results: All subjects with absent serum AAT presented homozygous Null mutations. In three subjects, a new mutation in exon 2 of the SERPINA1 gene was found. Subjects with Null mutations showed significantly lower lung function values than SZ and ZZ individuals ($p = 0.000$ and 0.001 for FEV_1 and K_{CO}, respectively). In all groups, there was a positive correlation between serum AAT and lung function values ($p = 0.025$ and 0.014 for FEV_1 and K_{CO}, respectively).
Conclusions: Serum levels of AAT are correlated with the severity of pulmonary phenotype. Subjects with Null mutations should be considered a subgroup at particularly high risk of emphysema within AAT deficiency (AATD). Early detection of carriers of this genotype would be important for preventive and therapeutic interventions.

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Introduction

Alpha-1 antitrypsin (AAT) is the most prevalent serine protease inhibitor in serum; it is synthesized by hepatocytes and belongs to the family of serpins (*ser*ine *p*rotease *in*hibitors).[1] Its main role is that of inhibiting neutrophil elastase (NE), an enzyme that degrades several components of the extra cellular matrix in the lungs. Insufficient inhibition of NE in AAT deficiency (AATD) can cause severe, early age onset pulmonary emphysema, resulting in high incidence of lung transplantation and reduced life expectancy.[2]

AATD is one of the most common autosomal-codominant genetic disorders in the Caucasian population.[1,3] To date, about 100 genetic variants of AAT have been identified. The normal AAT genotype is Pi MM, (94–96% of the white population),[4–6] characterized by protein serum levels of 150–350 mg/100 ml (20–48 μM). The S variant, originated in the Iberian Peninsula, and the Z variant, arisen in the Viking population, account for more than 95% of the mutations in patients with severe AATD.[7,8] Both variants are missense mutations in the serpina1 gene (SERPINA1) on chromosome 14q32.1.[9–11] In contrast to the S, the Z phenotype is characterized by polymerization of AAT, which can result in liver cirrhosis. Individuals with SS, SZ, and ZZ genotypes have serum AAT concentrations of approximately 85%, 25%, and 15% of normal levels, respectively.[12] Homozygosis for S mutation has not been associated with disease. A very limited number of studies investigated the different risk of developing emphysema between ZZ and SZ genotypes.[13,14] Most studies concluded that the SZ genotype is less important than the ZZ in the development of emphysema and SZ patients develop emphysema at an older age than ZZ patients.[1] However, it is not know whether the different risk is linked to the different AAT serum levels between the two populations or to other, unknown, factors.

Among the variants within the SERPINA1, several mutations have been described leading to total absence of AAT production (Null mutations). Although the number of these mutations is large, they are rare within the population, thus very little is known about their clinical phenotype. In 1988, Cox and Levison showed that lung disease associated with Null homozygosis (Null Mattawa: Q0mat) was more severe than that associated with ZZ genotype.[15] The very small sample size of that study (three homozygous sisters) prevents generalizability of the findings; however, this study and other sparse clinical reports seem to point to a higher risk and severity of emphysema in subjects with absence of AAT in serum. If this would be the case, early detection of the Null homozygous genotype should be promoted and should lead to stronger educational intervention (e.g. smoking avoidance) and to a possible use of the replacement therapy as preventive treatment. Recently, Ferrarotti et al.[16] reported lower values of FEV_1 in subjects with rare variants of AAT as compared to ZZ; however, in their study ZZ and rare variants had similar AAT plasma levels and the lung function results were confounded by smoking. Thus, the authors could not discriminate between the importance of genetic factors (type of mutation), protein serum levels, and environmental factors (smoking) in determining the severity of the disease.

Aim of our study was to analyze whether homozygous Null genotype is related to severity of emphysema in AATD and whether protein serum levels are the major determinants of severity. To this aim, we characterized the SERPINA1 mutations in the Dutch Null population, we compared lung function of Null subjects with that of ZZ and SZ subjects matched for age and smoking history, and correlated the functional data with AAT serum levels.

Methods

Subjects

All subjects included in the study were recruited from the Alpha-1 International Registry database (AIR, www.aatregistry.org). AIR is the largest international database of individuals with AATD, containing lung function data, clinical history, and AATD phenotype of more than 2600 individuals with severe deficiency from 21 different countries, collected in the years 1997–2006. A review with a detailed description of the AIR database development and methodology has been recently published.[17] The Dutch part of the AIR database includes a total of 290 subjects, and one of the largest Null populations ever detected, composed by 12 subjects from 7 families. All 12 Null subjects were included in our analysis.

Study design

The study had a matched-paired design.[18] For each Null case, a subject with ZZ and a subject with SZ genotype were selected from the AIR Registry, matched on the basis of age ($\pm$5 years) and smoking ($\pm$7 pack-years). Due to the young age of the Null subjects, only one-to-one matches with complete lung function data were found within the ZZ and SZ population. The study was approved by the Ethics Committee of the Leiden University Medical Centre and patients gave their written informed consent.

Lung function

Lung function tests to determine the severity of emphysema were performed according to the European Respiratory Society guidelines.[19,20] All tests were performed after nebulization of 5 mg of salbutamol and 500 mcg of ipratropium bromide. Among the lung function measurements, forced expiratory volume as percentage of predicted ($ppFEV_1$) and the coefficient of diffusion of carbon monoxide as percentage of predicted (ppK_{CO}) were included in the analysis, since they are currently considered as the most relevant for determining emphysema severity in AATD.[21,22]

Serum AAT levels

Serum AAT levels were measured using a completely automated immunoassay, as previously described.[23] The lower limit of detection of the assay is 10 nM. As confirmation of absence of an AAT band observed in the gel of isoelectrophoresis of serum samples, all subjects with AAT serum values lower than 1.5 μM were considered as having