The corresponding trypsin inhibiting capacity is between 1.0 and 1.3 mg of trypsin inhibited by one ml of serum. During recent years, different phenotypes of alpha-1-at have been identified and a genetic model with two genes (Pi) has been established in more than 70 cases that have been reported in the literature. Stewart commented that he is currently growing pulmonary vascular endothelial cells in tissue culture and that they demonstrate some of the same enzyme activity as the intact pulmonary vasculature.

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SESSION IV. ALPHA1-ANTITRYPSIN DEFICIENCY

Alpha1-Antitrypsin: 1. Evidence for two genes causing low concentrations in serum; 2. Association of heterozygosity and Chronic obstructive lung disease

Friedrich Kueppers, M.D.

Alpha1-antitrypsin (alpha-1-at) is one of several proteinase inhibitors in human serum. It is a glycoprotein with molecular weight of approximately 50,000. The normal concentration in serum is 213±32 mg/100 ml. The corresponding trypsin inhibiting capacity is between 1.0 and 1.3 mg of trypsin inhibited by one ml of serum. During recent years, different phenotypes of alpha-1-at have been identified and a genetic model with several codominant alleles at one locus has been developed. Two phenotypes can be distinguished quantitatively and electrophoretically while others can be recognized by electrophoresis only. I consider here only the phenotypes with lower than normal alpha-1-at concentrations in serum. The method most commonly used to determine the total trypsin inhibiting capacity of serum is an inhibition assay with H₂O₂-benzoyl-arginine-p-nitroanilide as substrate for trypsin. A convenient method for a specific determination of alpha-1-at is the radial immunodiffusion employing an antiserum against alpha-1-at. For the electrophoretic distinction of the different phenotypes starch gel electrophoresis at an acidic pH (4.95) in combination with a second electrophoresis at an alkaline pH (8.65) in agarose gel that contains an antiserum for alpha-1-at is a sensitive method. With this technique, the alpha-1-at phenotypes of heterozygotes and homozygotes for the two genes, Pi¹ and Pi² (nomenclature of Fagerhol), that cause lower concentrations of serum alpha-1-at, can be recognized as a characteristic pattern of bands. The protein that is determined by the gene Pi¹ moves on electrophoresis more slowly towards the anode than that determined by Pi², but both are moving slower than the normal protein controlled by the most common gene Pi⁰. In heterozygotes, both patterns of alpha-1-at can be recognized. The resulting phenotypes are called: M/M, S/S, Z/Z, M/S, M/Z, etc. The alpha-1-at concentrations for these phenotypes are in mg/100 ml of serum: M/M, 213±32, S/S, 122±10, Z/Z, 25±6, M/S, 159±39, M/Z, 125±46. These concentrations are only found under normal physiologic conditions. In the presence of an inflammation, during pregnancy, and due to certain steroid hormones, the alpha-1-at concentration of normal individuals can rise to levels 100 percent above the starting value, and heterozygotes can show values in the normal range of 200 mg percent. The electrophoretic characteristics are, however, independent of concentration and for that reason the electrophoretic determination of the alpha-1-at phenotype is more reliable than the quantitative one, particularly in patients with inflammation.

The association of the Z/Z phenotype and chronic obstructive lung disease (COPD) has been well established in more than 70 cases that have been reported in the literature.

To test the hypothesis of an association of heterozygosity

Table 1.

<table>
<thead>
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</thead>
<tbody>
<tr>
<td>COPD</td>
<td>+/−</td>
<td>+/+</td>
</tr>
<tr>
<td>COPD</td>
<td>Total</td>
<td>+/−</td>
</tr>
<tr>
<td>COPD</td>
<td>25; 25.5%</td>
<td>73; 74.5%</td>
</tr>
<tr>
<td>N</td>
<td>14; 14.0%</td>
<td>86; 86.0%</td>
</tr>
</tbody>
</table>

COPD: Patients with chronic obstructive pulmonary disease
N: Normal individuals
+/−: Heterozygotes for a deficiency gene; Pi⁰/Pi² and Pi⁰/Pi⁰
+/+: Homozygotes for the most common gene; Pi⁰/Pi⁰
Alphal-Antitrypsin Deficiency: Heterozygosity, Intermediate Levels, and Pulmonary Disease

C. A. Guenter, M.D.; M. H. Welch, M.D.; S. Ferguson, M.D.; L. Henderson, M.D.; and J. F. Hammarsten, M.D.

Homozgyous inheritance of alpha-antitrypsin deficiency results in very low serum trypsin inhibitory capacity (TIC) and is associated with a high prevalence of a characteristic form of pulmonary emphysema. Homozygous inheritance of the trait results in intermediate serum TIC values, but may be difficult to identify with certainty by serologic methods. Because of this, the relation between the heterozygous state, serum TIC values and the development of lung disease is difficult to assess. In a previous report we attempted to evaluate the prevalence of intermediate antitrypsin levels in a population of patients with pulmonary disease. In contrast, the present paper correlates the prevalence of pulmonary disease and antitrypsin levels in three different, carefully selected populations.

First, family studies were initiated among the relatives of 12 subjects with homozygous antitrypsin deficiency and pulmonary emphysema. The purpose was to identify persons with the obligate genetic heterozygous state to circumvent the uncertainty of identification by serology alone. Histories, spiromograms and TIC's were obtained on 41 heterozygous members of these families. Of the family members studied, 12 were over 40 years of age. Only one had clearly established symptomatic obstructive lung disease with abnormal spirometry. Abnormal spiromograms were obtained in three additional symptomatic individuals. This prevalence of abnormal spirometry was no greater than that obtained in a local population study. TIC values in these obligate genetic heterozygotes were similar to the values generally accepted as charac-

Discussion

Kuepper's criteria for heterozygous deficiency were: quantitative, radial immunodiffusion and antigen-antibody crossed electrophoresis. The most common heterozygous type is M/S, not M/Z. Due to infection and consequently elevated a1-antitrypsin levels, present in many patients with chronic obstructive pulmonary disease, one might misclassify some heterozygotes as normals if one depended on the radial immunodiffusion alone. He stated further that the method he used was undesirable to search for a buffer which does not interact in this fashion with the protein, eg, phosphate, barbital or tris buffer.

Kueppers commented that if you use a buffer of alkaline pH, it is hard to distinguish the phenotypes: M/S, M/Z, S/S and Z/Z.

P. Stevens asked what percent of the San Francisco population would be heterozygous if quantitative immunodiffusion alone is used. Kueppers answered that it would be approximately 8 percent. The Hamburg population represents people coming in for paternity determination in the Hamburg Genetic Institute. The emphysema group was derived from people applying for retirement benefits from the State Insurance Corporation.