A Family with Deficiencies of Both $\alpha_1$-Antitrypsin and Haptoglobin

To the Editor:

The association of $\alpha_1$-antitrypsin ($\alpha_1$AT) deficiency with disease is well described (1, 2). Deficient variants of $\alpha_1$AT are mainly confined to Europeans. The heterozygote for the severe Z protein is present in about 3% of Europeans, whereas a less-severe variant, the S protein, occurs in about 7% of Europeans (3). The Z variants have 5–10% of the concentrations of the normal (M) allelic product (4). The inheritance of $\alpha_1$AT is autosomal codominant.

Two alleles, Hp$^1$ and Hp$^2$, determine the phenotype of another plasma protein, Hp$^1$–$^1$, Hp$^2$–$^1$, or Hp$^2$–$^2$ (5). The haptoglobin of individuals homozygous for Hp$^1$ migrates as a single component on electrophoresis in polyacrylamide gel. Individuals homozygous for Hp$^2$ alleles synthesize two polypeptide chains, which form a series of stable polymers of increasing molecular mass and decreasing electrophoretic mobility on gels. Heterozygous individuals also form a series of components, the mobilities of which differ from those of the Hp$^2$–$^2$ polymers (6). Hp$^2$–$^1$ is the commonest phenotype.

Here we describe the unusual case of a white family in whom variants of $\alpha_1$AT deficiency are associated with a deficiency of haptoglobin. The daughter was presented for examination at age seven weeks, after a month of jaundice, dark urine, and pale stools. This history, together with the biochemical findings, suggested neonatal hepatitis. At that time, infections and likely metabolic causes were excluded. Over the next few weeks her jaundice resolved but the activities of her aspartate and alanine transaminases remained high, and she developed splenomegaly. When she was 19 months old, a liver biopsy showed a histological picture of cirrhosis, suggestive of $\alpha_1$AT deficiency. This diagnosis was confirmed by measurement of $\alpha_1$AT in serum (15% of normal) and by proteinase inhibitor (Pi) typing, which showed her to be homozygous for the Z protein variant.

Now six years old, she is growing well but still has some liver dysfunction and portal hypertension. Measurement of other serum proteins shows that in addition to low $\alpha_1$AT the concentration of haptoglobin is markedly low (10% of normal).

Immunoperoxidase studies on the girl’s liver biopsy specimen involved a “sandwich” antibody technique (10). Sections of the biopsy specimen were separately equilibrated with rabbit monospecific antiserum to $\alpha_1$AT and haptoglobin. Excess swine anti-rabbit immunoglobulins were the second antibody. We used rabbit peroxidase-antiperoxidase for the third antibody and stained the tissue for peroxidase (EC 1.11.1.7) activity, with diaminobenzidine tetrahydrochloride as substrate. The antisera were all obtained from Dako Immunochemicals Ltd., Copenhagen.

The staining pattern for $\alpha_1$AT (Figure 1) was consistent with a ZZ phenotype and a total absence of intracellular haptoglobin (data not shown).

We also measured other serum proteins—orosomucoid, prealbumin, albumin, transferrin, and immunoglobulins A, G, and M—by rocket immunoelectrophoresis (7). $\alpha_1$AT and haptoglobin concentrations were measured on at least three separate occasions.

The other members of her family are asymptomatic and have no evidence of hemolytic disease, as shown by repeatedly normal reticulocyte counts.

To determine haptoglobin phenotypes, we added excess hemoglobin to each serum and subjected the mixture to polyacrylamide gel electrophoresis, using a discontinuous buffer of pH 8.6. The haptoglobin/hemoglobin bands were identified by staining for peroxidase activity (8) and by immunofixation of haptoglobin bands separated by zone electrophoresis on agarose. The $\alpha_1$AT phenotypes were determined by isoelectric focusing (9) of plasma obtained from whole blood treated with EDTA and stored at $-20\,^\circ$C.

All members of the family showed a variable degree of deficiency of the two serum proteins (Table 1). Values for the other serum proteins were all within our reference limits.

Deficiency of two serum proteins occurring in one family is extremely rare. Moreover, deficiency of $\alpha_1$AT is reportedly more common in Europeans and haptoglobin deficiency in blacks; therefore the occurrence of the deficiency of both proteins in a Caucasian family is quite unusual.

It is unlikely that the $\alpha_1$AT and haptoglobin genes are linked (11). On the other hand, a secretory defect of haptoglobin, similar to the Z variant of

### Table 1. Phenotypes and Concentrations of $\alpha_1$AT and Haptoglobin

<table>
<thead>
<tr>
<th>Patient</th>
<th>$\alpha_1$AT conc. (%)</th>
<th>Haptoglobin conc. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient</td>
<td>ZZ</td>
<td>15</td>
</tr>
<tr>
<td>Father</td>
<td>ZZ</td>
<td>2-2</td>
</tr>
<tr>
<td>Mother</td>
<td>ZZ</td>
<td>2-2</td>
</tr>
<tr>
<td>Brother</td>
<td>ZZ</td>
<td>2-2</td>
</tr>
</tbody>
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$^a$ Haptoglobin concentrations of the patient and the brother were too low to phenotype accurately by polyacrylamide gel electrophoresis. Immunofixation showed the father, mother, and patient to be type 2-2.

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**Fig. 1.** Liver cells of patient stained for $\alpha_1$AT (intra-cellular dark material)
α1AT, is also unlikely, because the liver biopsy specimen of the patient did not demonstrate the presence of intracellular haptoglobin. However, because the liver biopsy was done at an age when plasma haptoglobin concentrations may be low in normal infants (12) and because cirrhosis can be associated with decreased plasma concentrations of hepatic haptoglobin, the possibility of a secretory defect cannot be totally excluded.

The absence of intracellular haptoglobin in the liver biopsy specimen suggests that haptoglobin synthesis is reduced. That this is a physiological consequence of the α1AT deficiency may partly be ruled out by the lack of correlation between the degree of the two deficiencies in the family members investigated. The lowest proportion of haptoglobin (5%) is found in the healthy brother with 75% α1AT, which is close to the lower limit of the reference interval.

The only explanation for the combined deficiencies is the presence of two unlinked mutant genes in the same subject, which in turn presumably the presence of the same two mutant genes in two people: the mother and father.

An extensive family history excluded a consanguinous marriage. This leads us to speculate about a complex physiological relationship between α1AT and haptoglobin. The recent demonstration that haptoglobin has antiprotease activity (13) and the observation that haptoglobin and α1AT appear to increase in parallel in inflammation suggest that further extensive family studies may be useful to confirm or deny any physiological connection.

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Liquid-Chromatographic Identification of Acetaminophen in Cerebrospinal Fluid with Use of Electrochemical Detection

To the Editor:

In a recent study of the monoamine metabolites 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylglycol, homovanillic acid (HVA), and 5-hydroxyindoleacetic acid (5-HIAA) in cerebrospinal fluid (CSF) from infants and children (ms. in preparation), we used reversed-phase “high-pressure” liquid chromatography with electrochemical detection (1). We used a previously described mobile phase (2), modified for better separation of the first two metabolites. It consisted of sodium phosphate (0.1 mol/L, pH 4.9) and methanol (96/4 by volume). We frequently observed a large peak in the chromatogram where 5-HIAA ordinarily appears. This compound consistently co-eluted with 5-HIAA in 12.6 min with a flow rate of 2.0 mL/min and was observed in about 65% of all CSF specimens from the infants and children involved in the study.

A review of the medical records revealed the coincidence of acetaminophen (Tylenol) administration for the control of fever and the presence of the interfering compound in CSF specimens. Analysis of an acetaminophen standard solution (McNeil Labe., Ft. Washington, PA 19034) resulted in a peak with a retention time (12.6 min) similar to that observed in the CSF specimens. When we added acetaminophen to a CSF specimen that did not contain the interfering compound, injection of this acetaminophen-supplemented CSF specimen resulted in a chromatographic profile similar to that observed in the analysis for monoamine metabolites in CSF from the children receiving Tylenol.

Accordingly, to separate the presumed acetaminophen peak from HVA and 5-HIAA and to aid in further identifying the interfering compound, we developed a second mobile phase: citrate/phosphate buffer (0.1 mol/L, pH 3.0) and methanol (95/5 by volume), delivered at a flow rate of 2.7 mL/min. The interfering compound was satisfactorily separated from 5-HIAA in CSF, thus allowing accurate quantitation. The CSF specimens previously found to contain the interfering compound, chromatographed with use of the new mobile phase, showed a briefer retention time (5.5 min) for the interfering compound than for HVA (15 min) or 5-HIAA (10 min). On analysis of an acetaminophen standard with use of the new mobile phase, the resulting peak showed the same retention time as that observed for the interfering compound present in the CSF samples. We have successfully used the new mobile phase to isolate acetaminophen from HVA and 5-HIAA in more than 20 CSF samples. We have also quantitated acetaminophen by use of this method in several of these samples, finding ranging from 9 μg/L to well over 150 μg/L. Acetaminophen is a highly oxidizable compound with an output signal of 0.95 nA/ng when measured at an oxidation potential of +0.75 V in our system. Analyses for acetaminophen in plasma (3) and in microsomal incubation mixtures (4) have been described. We believe that ours is the first description of an acetaminophen assay in human CSF by liquid chromatography with electrochemical detection. Such an assay may prove useful in basic studies on the pharmacokinetics of acetaminophen entry and clearance from the central nervous system.

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References
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