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Haptoglobin Polymorphism and Iron Hemostasis

To the Editor:

We read with interest the article of Beutler et al. *(1)* on the haptoglobin (Hp) polymorphism and its influence on iron metabolism in hereditary hemochromatosis. In contrast to our results *(2–4)*, they found no overrepresentation of the Hp 2-2 phenotype in hemochromatosis patients and the Hp polymorphism did not influence the iron status of patients and healthy individuals.

In healthy individuals, Beutler et al. *(1)* found no influence of the Hp phenotype on serum iron indices. They state that "there is no reason to believe that the haptoglobin pathway is an important limiting factor in iron hemostasis". This is supported by the facts that they found no Hp-type-dependent influence on serum ferritin and transferrin saturation values in healthy individuals and that in anhaptoglobinemia, no major disturbances in iron metabolism have been found. We agree that the rate of iron uptake through haptoglobin-hemoglobin (Hp-Hb) complexes is relatively small compared with major iron regulatory pathways. As in hemochromatosis, however, a long-term effect may have important consequences for iron status. In liver transplant patients, donor Hp phenotype determines the iron status post transplantation *(5)*. Because of the limited numbers of healthy individuals and a higher degree of ethnic variation in the observational trial reported by Beutler et al. *(1)* compared with ours *(4)*, their trial may have missed the statistical power needed to detect the Hp-type-related influence on iron metabolism. Our group investigated and needed more than 700 individuals to clearly demonstrate the higher serum iron indices in Hp 2-2 individuals. In young and middle-aged males, we saw a doubling of the serum ferritin concentrations between Hp 1-1 and 2-2 individuals *(3)*.

The acute-phase-regulated and signal-inducing macrophage protein CD 163 has been identified as a receptor that scavenges Hb by mediating endocytosis of Hp-Hb complexes *(6)*. Complexes of Hb and multimeric Hp 2-2 exhibit higher functional affinity for CD 163 than do complexes of Hb and dimeric Hp 1-1. In anhaptoglobinemia, serum hemopexin acts as a back-up scavenger molecule to protect the individual from the toxic effects of free Hb.

In contrast to our findings *(4)*, Beutler et al. *(1)* found no overrepresentation of Hp 2-2 in hemochromatosis and no influence of this genotype on serum iron indices. However, the set-up of their study differed from that of our study. We chose patients with clinical characteristics of hereditary hemochromatosis who were homozygous for the Cys282Tyr missense mutation. This is clearly demonstrated by the younger age of our cohort and the higher serum iron indices observed. Beutler et al. examined a group of individuals homozygous for the Cys282Tyr missense mutation without requiring the presence of the clinical and biochemical characteristics of hereditary hemochromatosis. This is reflected by the wide range of serum ferritin concentrations and transferrin saturation. In the cohort studied by Beutler et al. *(1)*, low serum ferritin concentrations ($<$100 μg/L) and serum transferrin saturation ($<$50%) were observed in a large number of cases. In individuals homozygous for the Cys282Tyr missense mutation *(7)*, higher values would be expected.

Recently, Beutler et al. *(8)* estimated that $<$1% of individuals homozygous for the Cys282Tyr missense mutation will develop frank clinical hemochromatosis. Thus, only one to two patients in the cohort described by Beutler et al. *(1)* will develop clinical hemochromatosis. This clearly illustrates that their cohort and ours cannot be compared and consist of two different populations. We agree that it seems unlikely that the Hp polymorphism (having only a mild influence on iron metabolism) would be the major determining factor explaining why only 1% of the population homozygous for the Cys282Tyr missense mutation will develop clinical hemochromatosis. We could clearly demonstrate, however, that in clinical hemochromatosis, the Hp 2-2 phenotype is overrepresented and that patients with this

phenotype have evidence of higher serum iron markers and a higher amount of blood donation before iron depletion. Our conclusion remains that in hereditary clinical hemochromatosis, the Hp polymorphism influences the biochemical presentation of the condition *(4)*. This does not contradict the findings of Beutler et al. *(1)* because the cohorts cannot be compared.

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Negative Thyrotropin Assay Interference Associated with an IgGκ Paraprotein

To the Editor:

Human anti-animal immunoglobulin or "heterophile" antibodies interfere in many immunoassays *(1, 2)*. Anti-animal immunoglobulin antibodies can occur idiopathically or after treatment with monoclonal antibodies. Those heterophilic antibodies that bind murine immunoglobulins are often referred to as human anti-mouse antibodies *(1)*. Two-site (sandwich) immunoassays are the assays most frequently reported to be subject to positive interference *(1)*. False-negative heterophile interferences, to our knowledge, have all been cases of endogenous anti-analyte (not anti-immunoglobulin) antibodies or anti-idiotype antibodies in patients treated with monoclonal antibodies *(3–6)*. Here we describe a negative assay interference associated with an IgGκ paraprotein.

The AxSYM Ultrasensitive hTSH II assay (Abbott Laboratories) is a sandwich assay for thyrotropin (TSH) that uses mouse monoclonal anti-TSH-coated microparticles and goat anti-TSH-alkaline phosphatase conjugate. In our laboratory, the functional detection limit of this assay is 0.1 mIU/L with a day-to-day CV of 12% (mean, 0.17 mIU/L).

Inconsistent thyroid function test results in an 80-year-old male patient suffering from a myelodysplastic syndrome (IgGκ monoclonal protein, 38 g/L) prompted our attention. TSH by the AxSYM was 0.1 mIU/L. In our laboratory, samples with TSH results ≤0.1 mIU/L are routinely retested on the DPC Immulite 2000 Third Generation TSH assay [Diagnostic Products Corp.; day-to-day (total) CV at 0.077 mIU/L is 6.2%]. The TSH value obtained with this assay was 5.9 mIU/L (reference interval, 0.4–6.2 mIU/L). Further testing revealed nonpathologic free thyroxine (fT_4; 10.9 ng/L; reference interval, 7–18 ng/L) and free triiodothyronine (fT_3; 2.41 ng/L; reference interval, 1.4–3.5 ng/L). The lack of symptoms and the nonpathologic fT_4 and fT_3 results suggested that the Immulite 2000 result of 5.9 mIU/L was correct and that the AxSYM result was falsely low.

On a second sample, we performed dilution experiments with the AxSYM TSH diluent and a heterophilic blocking reagent (HBR; Scantibodies Laboratory, Inc.). The TSH diluent contains a proprietary mixture of Tris buffer and "bovine stabilizers", and the HBR contains a proprietary mixture of "immunoglobulins of murine origin with specific binders that neutralize by active attachment to the heterophilic antibody". The addition of 10 μL of AxSYM TSH diluent or HBR to 290 μL of sample had little effect on the TSH results. The addition of 30 μL of HBR produced a nonpathologic TSH result (4.0 mIU/L), whereas 30 μL of diluent had no effect. The addition of 50 μL of diluent or HBR brought the TSH into the nonpathologic reference interval. We were unable to repeat similar dilution experiments on the Immulite 2000 because of insufficient sample quantity and the refusal of the patient to provide additional material.

Immunoglobulins in the patient sample and a control sample were precipitated by the use of 40 g/L ammonium sulfate *(7)*. The precipitated immunoglobulins were then washed with phosphate-buffered saline and concentrated to 138 g/L (patient sample) and 100 g/L (control sample) by use of an Amicon YM10 filter unit (Millipore Corp.). Serum protein electrophoresis was performed on the concentrated immunoglobulins. The electrophoretic profile of the patient's proteins (Fig. 1 in the Data Supplement, available with the online version of this Letter at http://www.clinchem.org/content/vol49/issue4/) demonstrated a prominent monoclonal peak in the γ region consistent with this patient's previous analyses and known IgGκ paraprotein. The concentrated proteins were then added serially to a sample from a patient with a known TSH concentration, keeping the final volume at 300 μL. When a final immunoglobulin protein concentration