Hereditary Hyperferritinemia-Cataract Syndrome: Two Novel Mutations in the L-Ferritin Iron-Responsive Element

To the Editor:

Cazzola et al.1 recently reported two kindreds with hereditary hyperferritinemia cataract syndrome (HHCS) associated with novel point mutations within a regulatory stem-loop motif in the L-ferritin mRNA termed the iron-responsive element (IRE). Affected individuals showed a characteristic clinical phenotype of elevated serum ferritin concentration and cataract developing early in life. The proposed pathogenesis of this disorder is that nucleotide substitutions within the IRE disrupt its specific interaction with the cytoplasmic iron regulatory protein (IRP). Failure of optimal IRP-IRE binding in turn leads to failure of suppression of L-ferritin translation.

There are now increasing numbers of reports that describe the genotype-phenotype relationship in kindreds with naturally occurring IRE mutations, and as Cazzola et al.1 report, the phenotype varies with the position of the mutation in the IRE. These descriptions now provide clinical data that support the structural model of the IRE-IRP interaction deduced from in vitro binding studies using artificially created IRE mutants.2-4

We have identified two further kindreds with HHCS and novel mutations in the L-ferritin IRE that further support this model.

Kindred 1. The 51-year-old male proband of English origin developed visual symptoms in his mid-thirties from cataracts, but was otherwise asymptomatic. Investigations revealed a serum ferritin of 1,389 µg/L but normal transferrin saturation. Similar abnormalities were noted in the proband’s sister, and liver biopsy specimens from both these individuals showed no iron overload. Sequencing of genomic DNA from the proband showed a heterozygous point mutation that corresponded to a C→U substitution in the L-ferritin mRNA.

Kindred 2. The 42-year-old female proband of English origin was investigated for anemia detected at one of her regular blood transfusion sessions. Although her red cell indices and transferrin saturation were consistent with mild iron deficiency, her serum ferritin was elevated at 1,020 µg/L. The proband herself had had previous surgical extraction of cataracts, and there were premature cataracts in 8 other family members. The son of the proband required cataract extraction at 5 years old. Hyperferritinemia was confirmed only in family members with cataract. Analysis of genomic DNA also showed a heterozygous point mutation that corresponded to a C→A substitution in the L-ferritin mRNA. This substitution created an Mse I restriction site within the amplified sequence, and restriction digests from additional family members confirmed that the substitution segregated with the hyperferritinemia-cataract phenotype.

The nucleotide substitutions detected in kindreds 1 and 2 lie in the apical loop and upper stem of the IRE, respectively (Fig 1). We note that in both kindreds individuals display a severe phenotype, and this is consistent with the observations of Cazzola et al. that mutations near the apex of the IRE result in higher serum ferritin concentrations and denser cataracts. These results also comply with data from in vitro binding studies; nucleotide substitutions in the apical loop of the IRE dramatically reduce IRP affinity, consistent with its putative role as the IRP binding site.2,3 Individuals from kindred 1 with a naturally occurring mutation at this site are therefore expected to have a severe defect in L-ferritin regulation. In the case of kindred 2, artificially created nucleotide substitutions in the IRE upper stem exert a profound effect on IRP binding in vitro, but only if complementary base pairing in the stem is disrupted.4 Pairing of nucleotides may facilitate IRE-IRP binding by maintaining an optimum secondary structure of the IRE. The
severe phenotype of kindred 2, who have a naturally occurring noncomplementary nucleotide substitution close to the IRP binding site, may therefore reflect a broader structural derangement of the IRE.

Our kindreds help clarify the relationship between genotype and phenotype in HHCS, and the description of two novel mutations illustrates the increasing genotypic diversity of this disorder. The severity of the phenotype of our patients and the position of the nucleotide substitution support the existing models of IRE-IRP interaction.

A.D. Mumford
T. Vulliamy
J. Lindsay
Imperial College School of Medicine
Hammersmith Hospital
London, UK
A. Watson
Stoke Mandeville Hospital NHS trust
Aylesbury, Bucks, UK

REFERENCES

β-Spectrin Promissão: A Translation Initiation Codon Mutation of the β-Spectrin Gene (ATG → GTG) Associated With Hereditary Spherocytosis and Spectrin Deficiency in a Brazilian Family

To the Editor:

Hereditary spherocytosis (HS) is a common inherited anemia characterized by the presence of spheroidal red cells and increased osmotic fragility of erythrocytes.1 This disorder is heterogeneous in terms of its clinical presentation, molecular basis, and inheritance.2 HS mutations have been ascribed to several genes,3 including the β-spectrin gene. So far 13 β-spectrin mutations have been described associated with HS.3-6

We have studied a Brazilian family with HS diagnosed in eight subjects from two generations and inherited in an autosomal dominant fashion (Fig 1). The propositus was a 28-year-old black man, who presented compensated hemolytic disease with splenomegaly, hyperbilirubinemia, increased osmotic fragility, and a regular number of spherocytes and acanthocytes in the blood smear (Fig 1). His recent hematological profile was: hemoglobin (Hb) 15.0 g/dL, red blood cell 4.49 × 10^12/L, mean corpuscular volume 88 fL, mean corpuscular hemoglobin concentration 38.0 g/dL, reticulocyte count 530 × 10^9/L (11.8%). His mother, uncle, and two cousins were splenectomized.

Densitometric scans of Coomassie blue–stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of the propositus membrane proteins showed an 18% reduction in spectrin content (Fig 1). This pointed to the β-spectrin gene as the most likely candidate for bearing the primary defect. Therefore, we started screening for mutations in the β-spectrin gene. This was performed through the amplification of the individual exons of the β-spectrin gene with intronic primers. The amplification products were submitted to nonradioactive single-strand conformation polymorphism (SSCP) technique in a PhastSystem apparatus (Pharmacia, Uppsala, Sweden) to detect sequence abnormalities. The DNA amplification products of exon 2 of the patient and his mother showed an identical band shift in two independent experiments (Fig 2). No such band pattern was observed in 2 independent controls, nor was it observed in 12 other HS patients with spectrin deficiency and acanthocytes in the blood smear, suggesting that this patient bore a unique or at least a rare sequence alteration in this region of the gene. Sequencing revealed an heterozygous A → G nucleotide substitution at the translation initiation codon of the β-spectrin

Fig 1. (Left) 3.5% to 17% exponential gradient SDS-polyacrylamide gel of total membrane proteins stained with Coomassie blue, showing a reduction of spectrin content in the patient (lane 2) compared with the control (lane 1). (Upper right) Blood smear of the propositus showing regular numbers of spherocytes and acanthocytes. (Lower right) Family pedigree showing all affected members from two generations. Splenectomized individuals are indicated by asterix and the proband is indicated by an arrow.
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A.D. Mumford, T. Vulliamy, J. Lindsay and A. Watson