CASE REPORTS

Hyperferritinaemia in the absence of iron overload

J D Arnold, A D Mumford, J O Lindsay, U Hegde, M Hagan, J R Hawkins

Abstract

Background—Serum ferritin is normally a marker of iron overload. Ferritin genes are sited at chromosomes 19 and 11. Regulation of ferritin synthesis involves an interaction between an iron regulatory protein (IRP) and part of the ferritin mRNA designated the iron regulatory element (IRE). A disorder of ferritin synthesis resulting in hyperferritinaemia in the absence of iron overload has been described recently.

Patients and methods—Hyperferritinaemia in the absence of iron overload was detected in a patient who was investigated for possible haemochromatosis. Serum iron, transferrin saturation, and ferritin concentration were studied in 11 members of this patient's family from three generations. Eight members had DNA samples analysed by direct cycle sequencing of the 5' untranslated region of the L ferritin gene.

Results—Six of the family members studied had serum ferritin concentrations greater than 900 µg/l. However, serum iron and transferrin saturation were normal in these subjects who all had evidence of cataracts. Three affected family members who had genetic studies of the L ferritin gene on chromosome 19 had an A to G point mutation which was not found in unaffected members.

Conclusions—There was complete concordance between a mutated IRE, cataracts, and hyperferritinaemia in three generations of this family. This family study confirms the finding that hereditary hyperferritinaemia in the absence of iron overload is an autosomal dominant inherited disorder.

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Keywords: ferritin; hyperferritinaemia; hereditary haemochromatosis; cataract; iron overload

The iron storage protein ferritin is synthesised in the liver and different proportions of the component L (19 kDa, light) and H (21 kDa, heavy) subunits give rise to isoferritins with tissue specific distributions.¹ The L ferritin and H ferritin genes are sited at chromosomes 19 and 11, respectively.²

Transferrin is the main iron transport protein. Cellular iron homeostasis is maintained by regulation of ferritin synthesis in coordination with transferrin receptor expression. Ferritin synthesis can also be stimulated as part of an acute phase response by cytokines such as tumour necrosis factor α and interleukin 1 α .³

Regulation of ferritin synthesis involves an interaction between an iron binding protein termed the iron regulatory protein (IRP) and the ferritin mRNA.⁴ IRP contains an iron-sulphur complex and in iron deficient cells it functions as an RNA binding protein; in iron replete cells it functions as an aconitase, an enzyme in the Krebs cycle.

A constant region of the mRNA molecule, termed the iron responsive element (IRE),⁵⁻⁷ binds with IRP. The resultant IRE-IRP complex inhibits ribosomal binding to mRNA and prevents translation of the ferritin coding sequence. A critical CAGUGU sequence within IRE is important for binding with IRP.

When there is iron overload, as in hereditary haemochromatosis, the IRE-IRP inhibitory system is suppressed and ferritin synthesis is increased. When this form of iron storage is saturated, iron is deposited as haemosiderin in tissue.⁸ Relatives of patients with hereditary haemochromatosis may have elevated serum ferritin values without clinical evidence of tissue iron deposition.⁹ In hereditary haemochromatosis about 83% of affected individuals have a mutated gene on chromosome 6 designated the HLA-H gene.¹⁰

We report on a family with high serum ferritin values associated with early onset cataracts. Affected family members showed no clinical evidence of hereditary haemochromatosis or iron overload.

TABLE 1 Relation between serum ferritin concentrations and cataracts

Family member	Serum ferritin (µg/l)	Transferrin saturation	Cataract
I1	938	18	Yes
I2	1449	17	Yes
II1	1420	22	Yes
II4	988	17	Yes
II5	1452	26	Yes
III1	902	37	Yes
II2	37	21	No
II3	49	NA	No
III2	15	16	No
III3	19	NA	No

Departments of Gastroenterology, Haematology, and Ophthalmology, Ealing Hospital, Southall, UK

J D Arnold
A D Mumford
J O Lindsay
U Hegde
M Hagan

Department of Paediatrics, Addenbrooke's Hospital, Cambridge, UK
J R Hawkins

Correspondence to: Dr J Arnold, Consultant Gastroenterologist, Ealing Hospital NHS Trust, Uxbridge Road, Southall, Middlesex UB1 3HW, UK.

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Figure 1: Family tree of the patients with hyperferritinaemia. Solid symbols indicate those with ferritin in excess of 900 µg/ml; symbols with crosses indicate ferritin concentrations within the normal range; clear symbols indicate spouses in whom the ferritin concentrations have not been measured.

Case report

A 25 year old woman was referred to the gastroenterology clinic for investigation of possible haemochromatosis following the detection of raised serum ferritin at 1420 µg/l. She was born with cerebral palsy and complained of tiredness and brittle nails. She normally attends a learning disability clinic and since her symptoms suggested anaemia, haemoglobin and ferritin were measured. The unexpected ferritin result had prompted referral to our gastroenterology clinic. She had a history of cataracts, as did several members of her family. She was found to have a raised serum ferritin on three separate occasions despite normal haemoglobin, liver and renal function, and acute phase reactants (serum ferritin was measured using a standard solid phase, two site chemiluminescent immunometric assay (Immulite)). Her karyotype was normal and HLA haplotype was A2B44. Initial screening investigations in other family members revealed that her mother (I1) and her maternal aunt (I2) had notably elevated serum ferritin with no other markers of iron overload (table 1 and fig 1).

Figure 2: Cataract in patient I2.

Liver biopsies on these two family members (I1 and I2) were done to examine for tissue iron overload. These showed no evidence of excess iron. All three family members (I1, I2, and III1) had one unit of blood each venesected on successive weeks. They developed iron deficiency anaemia with reduced serum iron but continued to have raised serum ferritin concentrations.

Three other family members (fig 1 and table 1) also had raised ferritin values. Blood samples from family members were analysed for genetic mutation within the L ferritin IRE.

POLYMERASE CHAIN REACTION AMPLIFICATION AND DNA SEQUENCING

DNA (extracted from red and white blood cells) from patient I2 was amplified by using the polymerase chain reaction (PCR) as described previously.¹¹ Primer sequences were 5'-AGAAGCCGCCCTAGCCACGT-3' (forward) and 5'-GAGCTAACCACAAAA CGGTGC-3' (reverse). The PCR product was electrophoresed on an agarose gel and the amplification fragment electroeluted. The fragment was reamplified under the same conditions, but using a nested reverse primer: 5'-GGTCCCAGAAGCAGGAGATG-3'. The resulting amplification product was electroeluted and ethanol precipitated. The fragment was dissolved in water and sequenced on both strands using the ABI PRISM AmpliTaq FS dye terminator cycle sequencing ready reaction kit (Perkin Elmer) under the manufacturer's recommended conditions.

ALLELE SPECIFIC OLIGONUCLEOTIDE HYBRIDISATION

DNA samples were amplified with the forward and reverse primers. Amplification products were immobilised onto "Gene-Screen +" membrane (DuPont) and probed as described previously.¹² The primer sequences were 5'-TGCTTCAACGGTGTTTGGA-3' (mutant specific) and 5'-TGCTTCAACAGTGTTTGGA-3' (wild type).

Direct cycle sequencing of the 5' untranslated region of the L ferritin gene revealed that subject I2 was heterozygous for an A to G point mutation at position +40. This corresponded to position 2 of the CAGUGU motif within the L ferritin IRE. Allele specific oligonucleotide hybridisation confirmed the presence of the mutation in two further affected family members studied (subjects II1 and II5); this was absent from all of the five unaffected family members (subjects II2, II3, II6, III2, III3) who were studied.

Each family member underwent an ophthalmic examination by a consultant ophthalmologist (MH). Results were normal in those with normal ferritin levels, whereas all affected family members had anterior and posterior sutural opacities (fig 2). The older members (I1 aged 62, and I2 aged 54) also had peripheral opacities of varying extent and density. The peripheral opacities had a radial orientation and were more prominent near the equator of the lens. The appearance was as clear fluid vacuoles

between lens fibres in the cortical zone. They did not seem to interfere greatly with visual acuity.

Discussion

This is the first report of an English family with autosomal dominant inheritance of hyperferritinaemia associated with premature cataracts. Our patients had normal transferrin saturations and showed no histological evidence of increased iron deposition in the liver.

The abnormal phenotype in our family was associated with heterozygosity for an A to G point mutation in the L ferritin gene. This corresponded to position 2 of the conserved CAGUGU nucleotide motif in the mRNA IRE. This mutation was absent in unaffected family members. The base substitution in this region disrupts the site specific binding between IRE and IRP thereby reducing the suppresser influence on L ferritin mRNA translation. Increased ferritin synthesis occurs independent of cellular iron status.

Inherited hyperferritinaemia without iron overload has previously been reported in three continental families, all with similar inheritance pattern to our patients.^{13 14} In a family study reported from Italy, hyperferritinaemia was associated with a G to C substitution at position 3 of the CAGUGU IRE motif.¹⁵ In another report from France affected members were found to have an A to G substitution in position 2.¹⁶ This is an identical substitution to that in our patients.

The only clinical manifestation in these patients seems to be early onset cataracts. However, the structure of the sutural cataracts described here differs from the pulverulent type described in the French family.¹⁴ Hereditary cataracts are a heterogeneous group of disorders that may result from abnormal expression of genes encoding lens specific proteins.^{17 18} However, it is difficult to envisage how the point mutation in the L ferritin gene could result in abnormal expression of an unrelated lens protein gene. A more likely explanation is that the cataracts in our patients represent deposits of L ferritin subunits. Serum ferritin should be measured routinely in children with developmental cataracts.

"Sunflower" cataract due to deposition of copper is a rare but recognised finding in Wilson's disease.¹⁹ It is unclear as to why cataracts are common in hereditary hyperferritinaemia but a rare finding in hereditary haemochromatosis. It is possible that tissue specificity of iso-ferritins found in hereditary hyperferritinaemia may be different to that in hereditary haemochromatosis.

In contrast to patients with hereditary haemochromatosis, transferrin saturation is normal in hereditary hyperferritinaemia. A syndrome of non-hereditary liver iron overload in patients with modestly raised serum ferritin

but normal transferrin saturation was reported recently.²⁰ This disorder is distinct from both hereditary haemochromatosis and hereditary hyperferritinaemia and seems to be associated with hyperlipidaemia and impaired glucose tolerance.²⁰

Transferrin saturation and serum ferritin measurements are used as screening tests for hereditary haemochromatosis,⁹ but caution should be exercised in the interpretation of raised ferritin values.

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