



Recurrent mutations in the iron regulatory element of L-ferritin in hereditary hyperferritinemia-cataract syndrome

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ABSTRACT

Background and Objective. Hereditary hyperferritinemia-cataract syndrome (HHCS) is an autosomal dominant disorder characterized by bilateral cataracts and increased serum and tissue L-ferritin, in the absence of iron overload. The deregulation of ferritin production is caused by heterogeneous mutations in the iron regulatory element (IRE) of L-ferritin that interfere with the binding of iron regulatory proteins.

Design and Methods. We have identified several patients from three unrelated Italian families with HHCS. Iron parameters were assessed by standard methods. The IRE element of L-ferritin was amplified by PCR using appropriate primers and directly sequenced.

Results. Ferritin levels ranged from 918 µg/L to 2490 µg/L in the patients studied. In one family bilateral cataracts were diagnosed early in life, whereas in the others cataracts were diagnosed around 40-50 years. The female proband of family 3 presented with a severe iron deficiency anemia, which was unrecognized because of the increased ferritin values. Sequencing of the IRE element of L-ferritin in the probands of the three families identified three different nucleotide substitutions (+32 G→A, +40 A→G and +39 C→T) in the IRE of L-ferritin. These mutations have already been reported in unrelated subjects of different ethnic origins.

Interpretation and Conclusions. Our findings are consistent with recurrent mutations associated with HHCS and underline the importance of this syndrome in the differential diagnosis of unexplained hyperferritinemia. In addition, the findings highlight the role played by transferrin saturation in the diagnosis of iron deficiency in these patients.

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Key words: iron, ferritin, cataract, iron regulatory element, iron regulatory proteins

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Hyperferritinemia-cataract syndrome (HHCS) is a condition characterized by constitutively increased L-ferritin production in the absence of iron overload and by bilateral cataracts. The disease is related to an abnormality of the mechanism which controls, at the translational level, the cellular availability of ferritin.¹⁻³ A linkage was established between hyperferritinemia and congenital cataracts in 1995.⁴ Subsequently several mutations in the stem loop structure of the iron regulatory element (IRE) located in the 5' untranslated (5'UTR) region of L-ferritin have been described in this syndrome.⁵⁻¹¹ It has been demonstrated that these mutations interfere with binding of the iron regulatory proteins (IRPs) and abolish or greatly reduce the resulting repression of L-ferritin synthesis, which becomes non iron-regulated.^{3,12} Increased L-ferritin is found in several tissues and in the lens,^{5,13} although the mechanism of cataract formation is still unclear.

During the evaluation of Italian patients suspected to have genetic hemochromatosis, we identified several subjects from 3 unrelated families with this syndrome. Sequencing of the IRE element of L-ferritin in the probands identified three different nucleotide substitutions already reported in unrelated subjects of different ethnic origins affected by the same disorder.

Design and Methods

Case Report

Family 1. The proband (III-1 in Figure 1) is a 20 year old woman who had routine blood examinations showed a slightly decreased serum iron and a remarkably elevated serum ferritin (Table 1). The patient had an unremarkable past history, except for congenital bilateral cataracts, diagnosed at 7 years of age because of impaired visual acuity. For this reason she had surgery at 17 years of age. Her mother (II-2) had bilateral cataract and hyperferritinemia and underwent surgery for bilateral cataracts at the age of 19. A maternal aunt (II-3) had bilateral cataracts removed at 50 years of age. The maternal grandfather (I-1) also had cataracts, but had never had his ferritin

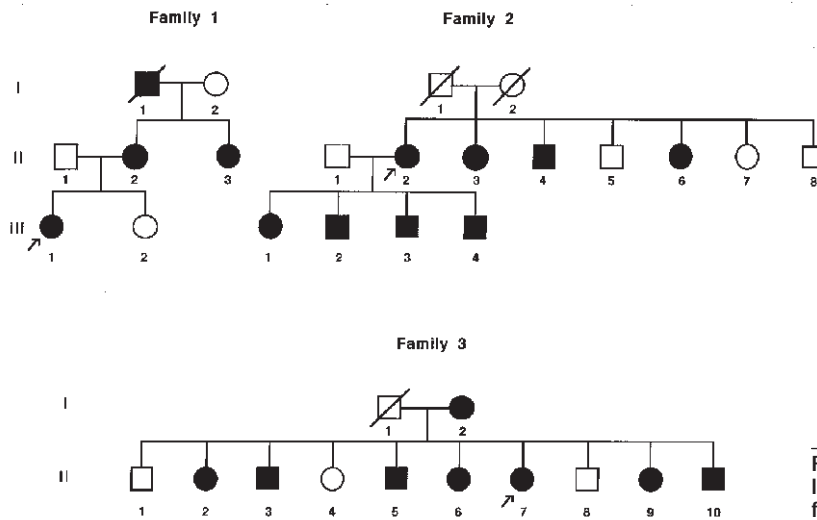


Figure 1. Pedigrees of the HHCS families studied. The probands are identified by the arrows.

Table 1. Clinical and hematologic findings in HHCS patients.

	Family #1		Family #2		Family #3		
	II-2	III-1	II-2	III-4	II-6	II-7	II-9
Age at diagnosis (yrs)	55	20	61	20	44	41	39
Sex	F	F	F	M	F	F	F
Hb (g/L)	137	142	143	151	134	77° 133*	122
MCV (fL)	84	83	84	87	91	63° 82*	86
Serum iron (µg/L)	980	400	1230	1270	1110	280° 640*	960
Serum ferritin (µg/L)	1275	1100	2000	1070	1615	2490° 1080*	918
Transferrin saturation (%)	26	15	30	32	31	6° 23*	30
Cataract onset (yrs)	18	7	55	19	39	37	40
Cataract surgery (yrs)	19	17	NO	NO	40	NO	NO

Data obtained before (°) and after (*) parenteral iron treatment.

levels measured. The family originated from Apulia, a region of Southern Italy.

Family 2. The proband (II-2 in Figure 1) is a 61 year old woman with a history of iron deficiency and parenteral iron treatment for menorrhagias twenty years ago. At that time her ferritin levels were not determined. Bilateral cataracts were diagnosed at the age of 55, because of visual impairment (Table 1). At diagnosis ferritin was found to be increased, while serum iron and hemoglobin levels were normal. Her family members are as follows: a 49 year old sister (II-3) has bilateral cataracts and hyperferritinemia (1070 µg/L). Of the other five living siblings two had cataracts and underwent surgery at 45 and 51 years, but never had their ferritin levels determined. All four of the proband's children were affected. Data concerning III-4 are given in Table 1. The parents died very old; they had never had ferritin determinations but both had cataracts, which in the father were

removed at 70 years of age. The family is from Apulia in Southern Italy.

Family 3. The proband (II-7 in Figure 1) is a 41 year old woman with a long history of anemia of unknown origin. Hematologic data are summarized in Table 1. Although the patient had remarkably reduced serum iron and low transferrin saturation, the persistently high serum ferritin hindered the diagnosis. The patient was intensively studied to exclude chronic inflammatory disorders or neoplastic conditions and had two bone marrow biopsies to exclude a myelodysplastic syndrome. Bilateral cataracts were diagnosed at 37 years of age. When HHCS was recognized, parenteral iron treatment restored normal hemoglobin levels in the proband (Table 1). At that time the patient was found to have hypermenorrhea and a positive fecal occult blood due to erosive gastritis. Among nine siblings three were normal and six had bilateral cataracts. Two of them (II-6 and II-9)

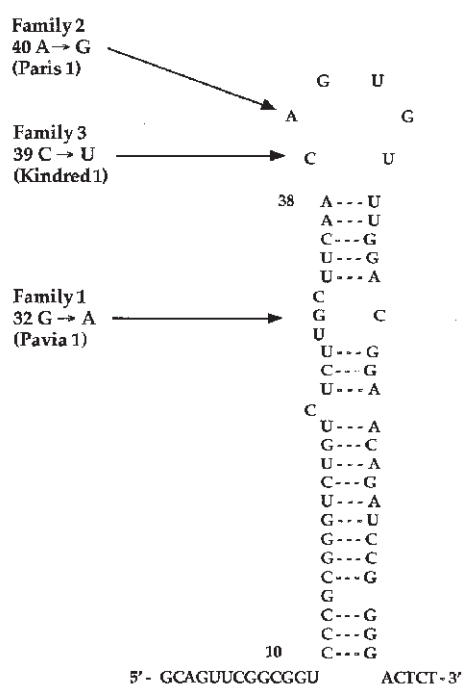


Figure 2. Schematic illustration of the IRE stem loop. The position of the mutations identified in the three families are indicated.

had elevated serum ferritin levels; the others were not evaluated. The family originated from Calabria, a region in Southern Italy.

Molecular studies

Genomic DNA was isolated from peripheral blood buffy coats. The entire IRE sequence of the L-ferritin gene was amplified by PCR using flanking primers (forward primer: 5'-TCCTTGCCACCGAGATTG-3'; reverse primer 5'-TTGGCAAGAAGGAGCTAACC-3').⁷ PCR was performed in an automated Thermal Cycler (Perkin Elmer) in 100 μ L final volume, using 25 pmol of each primer and 1U of Taq polymerase for 30 cycles.

Purified PCR products (287 bp) were used for direct sequencing using the Sequenase 2.0 DNA sequencing kit (USB-Amersham, Cleveland, Ohio, USA). Restriction enzyme analysis of the PCR product was performed by using the enzyme Tsp RI (New England-Bio Lab), according to the manufacturer recommendations.

Results

Haematologic data from the patients studied are reported in Table 1. The proband of family 1 had iron deficiency without anemia. The proband of family 2 had a past history of iron deficiency too, due to hypermenorrhoea. The proband in family 3 had a long history of iron deficiency with a severe degree of anemia, which was corrected by iron administration, as shown in Table 1.

Molecular studies showed that the mutation in family 1 is a G→A transition at position +32 (Figure 2). This nucleotide change was identified at the heterozygous state in two subjects. The substitution at position +32 occurs in the highly conserved three nucleotide motif forming the IRE bulge and corresponds to the previously described Pavia 1 mutation.⁶ The genetic change in family #2 occurs in the invariant CAGUG sequence of the IRE loop and is an A→G substitution at position +40. This mutation, identified in two family members, has already been described in a French family and is known as Paris 1.⁵ In family #3 there is a C→T substitution occurring at position +39 in the CAGUG element of the loop, a mutation described in a single English patient (kindred 1).⁹ The +39 C→T change was identified by using the enzyme Tsp RI, whose recognition site is abolished by mutation. The same enzyme was used also to recognize the mutation in family #2, since it occurs within its cleavage site (not shown).

Discussion

During a study of Italian patients suspected to have genetic hemochromatosis we identified three families with HHCS. Hematologic analysis showed that no patient had iron overload, but on the contrary three women had a history of iron deficiency, which in two cases required parenteral iron treatment. Since iron deficiency is extremely common, especially in females, cases presenting in the way that II-7 in family #3 did are expected in subjects with HHCS. An increased level of ferritin associated with a reduction of circulating iron and a low transferrin saturation is an unusual finding that, as in the case of family #3, may mask the correct diagnosis and delay correct treatment. Patient II-7 went undiagnosed, notwithstanding two bone marrow biopsies that showed absent hemosiderin deposits, another common feature of iron deficiency. In these cases determination of transferrin saturation becomes the most convenient diagnostic tool. Evidence of increased circulating serum transferrin receptor¹⁴ may also be a useful diagnostic tool in these patients.

Sequencing of the IRE element demonstrated three different mutations in the three families. In two families the mutation involves the CAGUG invariant motif in the loop and in one the three-nucleotide motif in the bulge. Both these sequences have been demonstrated to play an important role in the binding of the regulatory proteins.^{15,16} Since our families are ethnically unrelated to those previously reported with identical mutations, they represent examples of recurrent mutations in functionally important domains of the IRE element.

Once the mutation was identified in the index case, enzymatic digestion was employed to identify the mutation of families 2 and 3 using the endonuclease TspRI. It is noteworthy that this restriction enzyme is theoretically able to identify all the mutations in the

loop from position +39 to +43, since its cleavage site corresponds to the sequence NNCAGTGNN. Thus the use of this enzyme analysis could be proposed as a first screening of all the mutations occurring at the CAGUG level in patients with HHCS showing severe hyperferritinemia.

A relationship has been suggested between the position of the mutation in the stem loop and the phenotypic severity of the syndrome. It has been proposed that mutations occurring within the loop of the IRE structure result in higher serum ferritin concentrations and denser cataracts, whereas mutations in the lower part of the stem loop cause a milder phenotype.⁶ This relationship is not, however, absolute. It is of interest that, while ferritin values are remarkably high and similar in the three families studied, there are both inter-familial and intrafamilial variations in the cataract severity. Since all the mutations reduce the IRE affinity for IRE-binding proteins the finding of similar ferritin levels in all our patients is not unexpected. The onset of cataract formation occurred early and required surgery before 20 years in two out of the three cases in family #1, who had mutations in the bulge. Families #2 and #3 are examples of a split phenotype with severe hyperferritinemia and mild cataract. It is likely that the process of cataract formation is the result of several factors and that other gene products may modulate the phenotype.⁷

Contributions and Acknowledgments

CC was responsible for the study design and writing the paper, in collaboration with UM. MC was responsible for mutation identification. GZ contributed to data analysis and review of the literature. AR and SB were involved in molecular studies. VI and SS were involved in recruitment of the patients and family members and discussion of the project.

All the authors gave their critical contribution to the paper and approved the final version. The name order was a joint decision, considering the role of each Author.

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Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

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