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C29G in the iron-responsive element of L-ferritin: a new mutation associated with hyperferritinemia-cataract

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Abstract

Hyperferritinemia-cataract syndrome (HHCS) is a dominant disorder characterized by high serum ferritin and early onset of bilateral cataract. The disorder is caused by mutations in the iron-responsive element (IRE) of L-ferritin, which disrupt the postranscriptional control of L-ferritin synthesis. Here, we report a new (C>G) mutation which affects base 29 in the loop (c.-169C>G), previously unrecognized as essential for the stem loop stability. The mutation was identified in two members of an Italian family. Computer modeling and electrophoretic mobility shift assay (EMSA) confirm a decreased affinity of the C29G IRE for IRPs control proteins.

Keywords: Hyperferritinemia-cataract syndrome; L-Ferritin synthesis; c.-169C>G

Introduction

Hyperferritinemia-cataract syndrome (HHCS) is a genetic dominant disorder associated with bilateral cataract and increased serum ferritin in the absence of iron overload. Mutations in iron-responsive element (IRE) of the L-ferritin promoter result in constitutive, iron-independent ferritin expression [1,2]. Several nucleotide substitutions [3] and rare deletions [4,5] affect the L-ferritin IRE in this syndrome. The vast majority of mutations cluster in the highly conserved terminal loop (5′CAGUG), especially positions 39–41 of the IRE or close to the unpaired cytosine 32 (positions 32, 33) that forms a lateral bulge in the IRE stemlike structure [3].

As we are in a hemochromatosis referral center, we occasionally identify subjects with this disorder, who are referred for hyperferritinemia [3]. In the last years, 14 HHCS families were diagnosed out of 1100 samples referred for hemochromatosis. Here, we describe a family of central Italian ancestry, presenting a new IRE mutation.

Patients and methods

The proband (I-1) is a 40-year-old male with an unremarkable history. He had a reduction of visual acuity, because of bilateral lens opacities discovered since many years. Blood tests were performed because of the occasional finding of elevated serum ferritin in his infant child (II-1). Transferrin saturation, hemoglobin levels, and hematological parameters were normal. He had total bilirubin of 1.29 (indirect 0.94 mg/ml), ALT 57 IU/l, γGT 141 IU/l, hypercholesterolemia (276 mg/dl), and hypertrygliceridemia (244 mg/dl). Liver ultrasound showed slightly enlarged liver with mild steatosis.

The proband's child had the first ferritin determination (>1000 μg/l) at 38 days of age, concomitant with acute respiratory infection. Serum ferritin, measured at 6-month interval, was ranging between 520 and 734 μg/l. Slit lamp

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examination at 18 months of age did not reveal lens opacities. Liver iron concentration (LIC) was obtained by using a superconducting quantum interference device (SQUID) and was normal in the proband and at the upper limits for age (587 ± 250 μg/g tissue wet weight) in II-1 (Table 1). The mother, I-2, had normal iron parameters.

The proband sister, aged 36, had low serum iron and low ferritin levels. The paternal father had surgery because of cataract when aged 68. His ferritin was 365 μg/l. The paternal mother had a familial history of cataract, but her serum ferritin was 65. Both grandparents refused molecular tests.

Molecular studies were performed on peripheral blood DNA of the family. Hemochromatosis mutations were determined using the Hemochromatosis Strip assay (Nuclear Laser Settala, Milan, Italy). PCR and direct sequencing were performed by standard methods. Primers used for PCR were as described [4]. DHPLC was carried

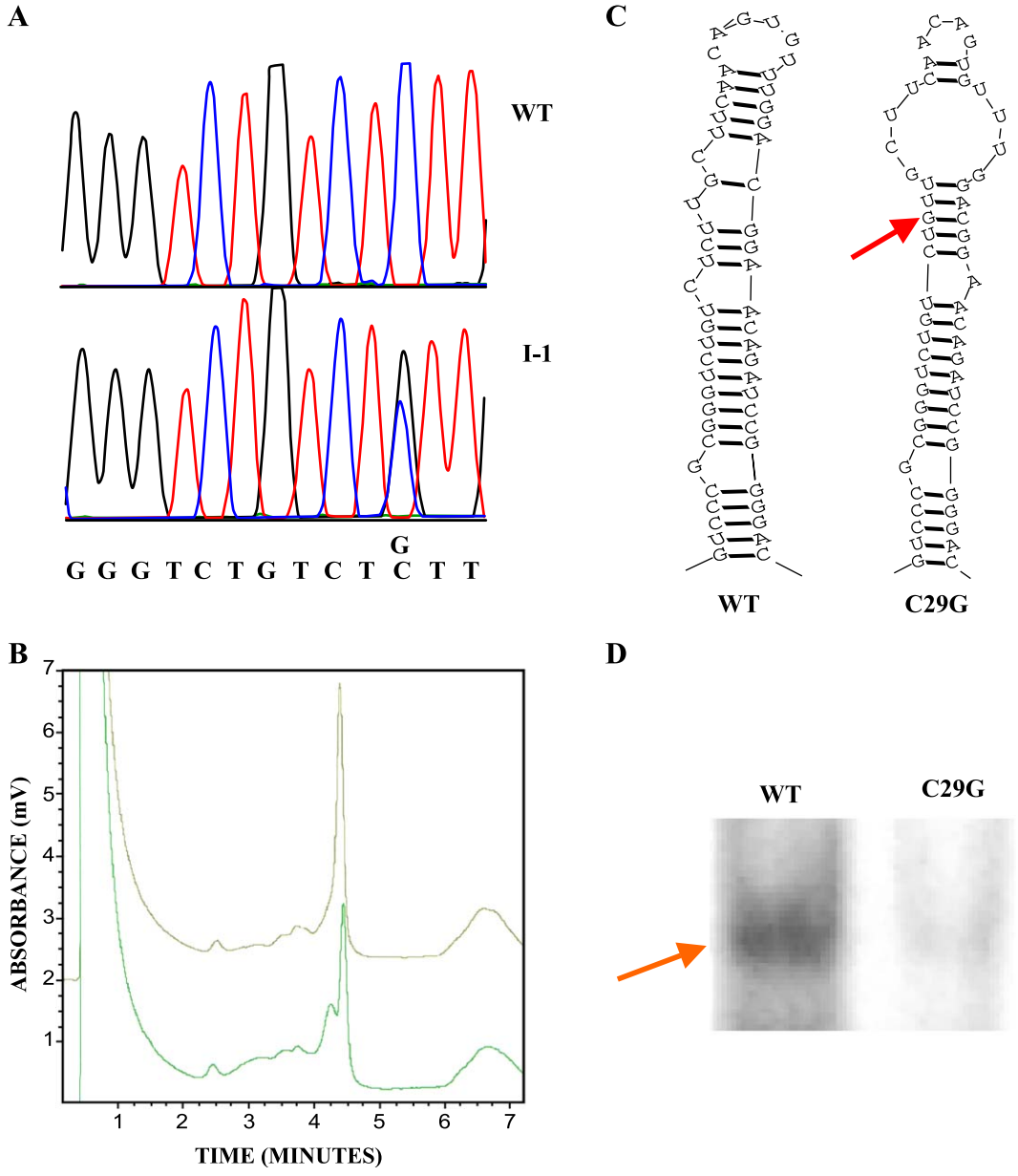

Fig. 1. (A) Sequence analysis of the iron-responsive element of L-ferritin. Sequence analysis of the IRE of L-ferritin in the region encompassing the mutation in I-1 and a wild-type (WT) control. The mutation is indicated under the chromatographs. It is located at c.-169C>G with the nucleotice preceding the initiator ATG counted as −1. (B) DHPLC profiles of the amplified IRE sequence of a normal case (gray) and of a carrier of C29G (I-I) (green). (C) Predicted IRE structure of WT and C29G mutant ferritin mRNA according to Ref. [12]. The arrow indicates the 29-position. The rearrangement of base paring caused by the mutation is shown. (D) EMSA of WT and C29G iron-responsive elements of ferritin L-chain: 2 μg of HeLa cell extracts were incubated for 10 min at 37°C with 100,000 cpm ^{32P}IREs. WT^{32P} IRE and C29G^{32P} IRE are shown. The samples were run on 6% native PAGE and IRPs binding ability was revealed by autoradiography.

Table 1
Hematological data of the family studied

Case	Sex	Age	Hb g/dl	TS %	SF μg/l	LIC (SQUID) μg/g tissue ww	Cataract (age at diagnosis)	IRE sequence
I-1 father	M	40	15.7	34	1521	219 ±180	Yes (30)	G29C
I-2 mother	F	35	13.0	22	20			N
II-1 son	M	1.5	12.6	25	734	587±250	No	G29C
Normal values					<200	<400[a]		

TS indicates transferrin saturation; SF, serum ferritin; LIC, liver iron concentration; ww, wet weight; N, normal.
[a] Indicates normal values for adult patients.

out on a Transgenomic WAVE system as described [6]. EMSA experiments were performed as described [7]. Labeled RNAs were synthesized using T7 RNA polymerase and the following oligonucleotides as templates: 5′CCGGATCTGTTCCGTCCAAACACTGTTGAAGCAA-*G*AGACAGACCCGCTATAGTGAGTCGTATTA3′ for the wild type (WT) L-ferritin and the 5′CCGGATCTGTT-CCGTCCAAACACTGTTGAAGCAA*C*AGACAGACCC-GCTATAGTGAGTCGTATTA3′ for the mutated L-ferritin.

Results and Discussion

Hemochromatosis mutations were negative in all subjects. Nucleotide sequencing of IRE of L-ferritin identified a c.-169C>G (29C>G in the IRE) heterozygous mutation (position 169 from the ATG) (Fig. 1A) in I-1 and II-1. I-2 was normal. I-1 was also homozygous for the A(TA)7TAA pattern in the uridine 5′-diphosphate-glucuronosyl-transferase promoter (not shown), compatible with Gilbert disease [8]. The C29G mutation was never reported before in HHCS and was not found in a large series of HHCS patients studied by efficient screening procedures, as DHPLC [9–11] or DGGE [10]. DHPLC profiles of WT and mutant DNA shown in Fig. 1B confirm that it was easily recognizable by this technology.

The position of the nucleotide change is close the IRE lateral bulge, but only changes at positions 32–33 were previously reported to cause HHCS. We hypothesized that the C29G change results in a decreased IRE–IRP interaction through a destabilization of the stem loop structure. A modeling of the mutated IRE, compared to the wild type is in Fig. 1C. Computation of mRNA folding of the first 70 nucleotides of L-ferritin mRNA [12] predicts that the presence of 29G induces a significant rearrangement of base pairing, which indirectly modifies both the lateral bulge and the upper loop structure, affecting the residues in direct contact with IRPs. To assess the functional effect of C29G mutation, we measured the relative binding of the mutated IRE for the IRPs by EMSA. As shown in Fig. 1D, the binding of the mutated probe to IRPs at 37 °C was remarkably lower than that of the normal control. Densitometric analysis indicates that IRPs bind mutated IRE sevenfold lower than WT.

Our studies have biological and practical implications: they add a new mutation to the list of substitutions that cause HHCS and strengthen the irreplaceable role of specific nucleotides in the stem loop structure.

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