Homozygous mutation of the 5'UTR region of the L-Ferritin gene in the hereditary hyperferritinemia cataract syndrome and its impact on the phenotype

Hereditary hyperferritinemia cataract syndrome (HHCS) is an autosomal dominant disorder characterized by hyperferritinemia without iron overload and early-onset bilateral cataract induced by L-ferritin accumulation in the lens.[1] Affected patients harbor heterozygous mutations within the iron responsive element (IRE) located in the 5' untranslated region (UTR) of the L-Ferritin gene (*FTL*).[2-3] These mutations disrupt the IRE sequence binding with iron regulatory proteins (IRPs), altering the iron-mediated down-regulation of *FTL* translation. Here, we report the first proven case of homozygosity in a female affected with HHCS.

A 54-year old female was referred for unexplained hyperferritinemia (initially 1960 μg/L). Her medical record showed that she had been submitted to empirical twice-a-month phlebotomies for one year. Ferritin levels remained high (roughly 1500 μg/L) and the patient developed microcytic anemia. No other clinical manifestations were noted and main causes of elevated ferritin levels had been previously excluded. However, careful questioning revealed that she had bilateral cataract diagnosed at the age of 35 years and she recently underwent surgery at 56 years of age. We, therefore, suspected HHCS. Several family members, including both possibly consanguineous parents and at least 2 of the proband's siblings, had visual impairment or known cataract (Figure 1). All family members except the proband lived in Canada and could not be genetically tested before. Sequence analysis of the 5'UTR of *FTL* identified the +51G>C mutation relative to transcription initiation signal, (NM_000146.3: c.-149G>C using the Human Genome Variation Society nomenclature). Unexpectedly for this autosomal dominant disorder, the mutation was at the homozygous state. This initial result was checked using a different set of primers. To exclude a deletion in the allele in *trans*, we looked for heterozygous large rearrangements of the 5'UTR of *FTL*. Analysis by semi-quantitative multiplex fluorescent polymerase chain reaction (PCR) (Figure 1) confirmed that the mutated +51G>C allele was present in double dose, ruling out a false homozygosity. To our knowledge, this is the first proven case of homozygosity in HHCS ever described. A single other case of homozygosity has been mentioned but the authors did not provide any proof for this unusual status and no clinical data were reported.[5]

The +51G>C mutation has already been described in 2 father-child pairs with moderate hyperferritinemia and clinically silent bilateral cataract.[6,7] The first father-daughter pair (50 and 15 years old, respectively) were Canadians, like our proband. Both had serum ferritin levels below 1000 μg/L and only fine bilateral lenticular changes were observed by slit lamp examination.[6] The second father-son pair (46 and 19 years old, respectively) was of Caucasian origin. They displayed serum ferritin levels of 1291 and 1251 μg/L, respectively. Slit-lamp and dilated funduscopic examinations revealed bilateral white breadcrumb-like nuclear and cortical lens opacity, whereas visual acuity

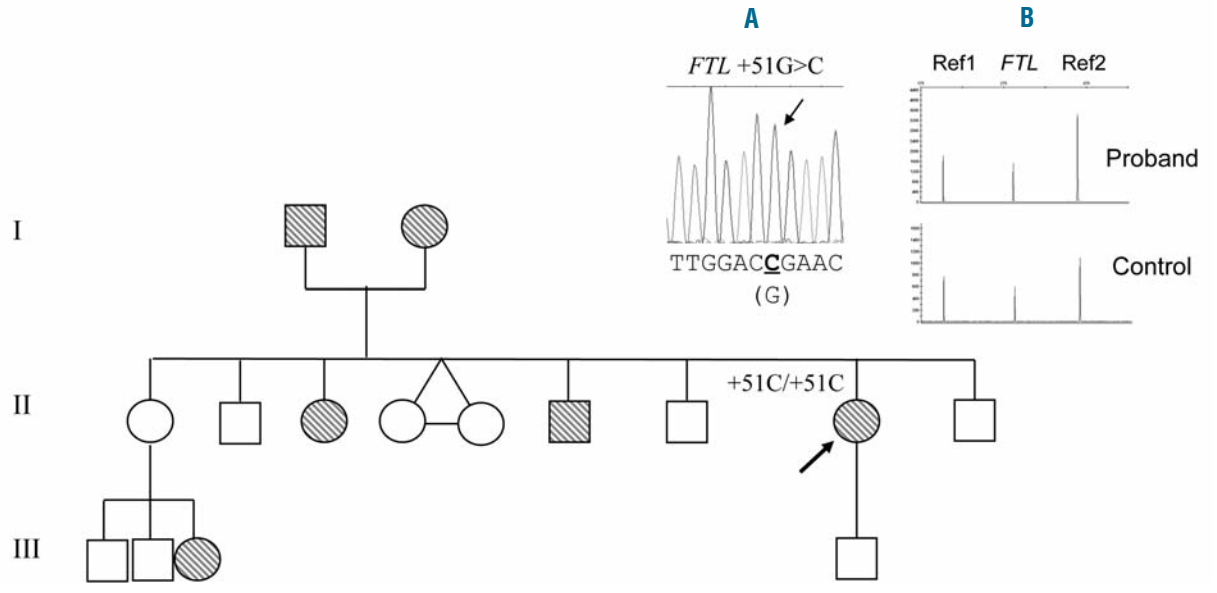

Figure 1. Patients with visual impairment or cataract are denoted in hatch drawing. The proband is indicated with a black arrow. Both proband's parents are probably consanguineous. (A) The 5'UTR region of the *FTL* gene was sequenced as previously described.[4] The proband gave informed written consent for genetic studies. (B) Gene dosing of the 5'UTR of the *FTL* gene was performed using an optimized semi-quantitative multiplex fluorescent PCR (SQF-PCR) method. Briefly, a 288 bp fragment of the 5'UTR of the *FTL* gene (using sense 6-Fam labeled primer 5'-TCCTGCCACCGCAGATTGG-3' and antisense primer 5'-TTGGCAAGAAGGAGCTAAC-3') was co-amplified by PCR with two additional fragments corresponding to exon 5 of the *HFE* gene (Ref1) and to exon 6 of the *F7* gene (Ref2) as controls. DNA fragments were then separated on an ABI 3130XL DNA sequencer. Scoring of the 5'UTR fragment corresponded to the ratio of the fluorescent peak areas obtained for the sample compared to the controls [sample *FTL*/sample Ref]/[normal *FTL*/normal Ref]. A heterozygous deletion would result in a ratio of 0.5, whereas a normal pattern gives a ≈1 ratio. The calculated ratio was normal, at 1.04 and 1.14, respectively, for Ref1 and Ref2, ruling out a deletion or a large rearrangement and thus a false homozygosity.

was not affected.[7] In our case, the proband displayed higher ferritin levels (roughly twice the levels observed in heterozygotes) but as for heterozygous affected patients, visual symptoms were mild and ophthalmological examination revealed bilateral cataract in the third decade only. However, it is noteworthy that serum ferritin levels may vary among patients harboring the same mutation, and also in the same patient.[8]

Mutations within the IRE may variably affect the binding of IRPs, leading to different degrees of hyperferritinemia.[8] Mutations altering the CAGUG sequence in the loop, the highly conserved UCG motif of the bulge or the IRE upper stem are associated with high serum ferritin levels and early-onset bilateral cataract. By contrast, mutations modifying the lower stem are less critical for the IRP-IRE interaction and are often associated with moderately high ferritin levels and mild to asymptomatic cataract. Consequently, the +51G>C mutation, located at the frontier between these two parts of the IRE, is difficult to classify.[8,9] Nevertheless, the related phenotypic expression for heterozygotes with plasma ferritin levels ranging from 800 to 1200 μg/L and silent cataract,[6,7] appears to be less severe than for loop mutations. Therefore, we hypothesize that, even if +51G>C homozygotes have a more severe biological expression than heterozygotes, their clinical phenotype remains relatively mild. In our proband, the phenotype is close to that observed in the common form of the HHC syndrome, or even milder as cataract did not lead to early visual impairment.

With the exception of Huntington's disease or some autosomal dominant neoplasia syndromes,[10] autosomal dominant disorders classically result in more severe clinical phenotypes when the mutation is present in double rather than in single dose. The homozygous state is typically not compatible with life.[11] The present case shows that HHCS is another exception to this rule. Heterozygous IRE mutations are sufficient to induce aggregation and crystallization of L-ferritin chains in the lens and the clinical expression of the syndrome, whereas in the case of mutation +51G>C, a double dose does not worsen significantly the phenotype. Indeed, the correlation between serum ferritin levels and cataract severity is poor.[12] However, we cannot exclude the possibility that patients with mutations located in the upper part of the IRE, such as the upper stem, the bulge or the apical loop, may display a more severe phenotype.

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