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Two Dutch families with hereditary hyperferritinaemia-cataract syndrome and heterozygosity for an HFE-related haemochromatosis gene mutation

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ABSTRACT

Hereditary haemochromatosis is an autosomal recessive disorder, leading to progressive iron overload, which is very common among the Caucasian population. In the vast majority of the cases, the hereditary iron overload is caused by mutations in the *HFE* gene. Most prominently this is the homozygous Cys282Tyr mutation. We report two Dutch families in which both propositi were found to be heterozygous for Cys282Tyr in the work-up of hyperferritinaemia. Frequent phlebotomies had no effect on the ferritin level, but led to microcytic anaemia. Finally, the family history with bilateral cataracts was the clue for the correct diagnosis.

Hereditary hyperferritinaemia-cataract syndrome (HHCS) is an autosomal dominant disease characterised by elevated serum ferritin levels and bilateral cataracts in the absence of iron overload. Several point mutations and deletions within the iron-responsive element (IRE) in the 5' noncoding region of the L-ferritin gene have been found in HHCS families. In the first Dutch family a G to C transition at position 32 was found and a G to A mutation at the same location was found in the second Dutch family.

In individuals with an isolated hyperferritinaemia (normal transferrin saturation), the presence of early onset (familial) cataract should raise the possibility of HHCS, even when Cys282Tyr heterozygosity is found.

INTRODUCTION

Hereditary hyperferritinaemia-cataract syndrome is a well-described disorder characterised by a combination of elevated levels of serum ferritin and early-onset bilateral cataracts.[1,2] Ferritins are heteropolymers of two different types of subunits (H-ferritin and L-ferritin), assembled to form a shell of 24 subunits with an internal core where iron can accumulate for storage purposes. The synthesis of both subunits is regulated by the interaction between a cytoplasmatic protein capable of binding iron, the so-called iron regulatory protein (IRP) and the highly conserved stem-loop motif, known as the iron responsive element (IRE) that is present at the 5' untranslated region (UTR) of the ferritin mRNA. When there is limited iron, the IRP binding to IRE inhibits the binding of the ferritin mRNA to ribosomes, and thus prevents the translation of the ferritin mRNA (see *figure 1*, also reviewed by Cazzola and Skoda).[3] Under conditions of abundant iron, the IRP cannot bind IRE because of the formation of 4Fe-4S clusters, leading to efficient synthesis of ferritin. In HHCS, mutations in the IRE make the L-ferritin mRNA translation independent of the iron status, since the IRP is not able to bind IRE.[2,4-9] HHCS is inherited in an autosomal dominant manner, although *de novo* mutations have also been reported.[10,11] The severity of cataract has been found to be related to the serum level of ferritin.[3,12] However, the location of the nucleotide substitution can lead to different levels of serum ferritin as well as to variable phenotype, even in individuals of the same family.[3,5,7]

Hereditary haemochromatosis is an autosomal recessive disorder characterised by an increased uptake of dietary

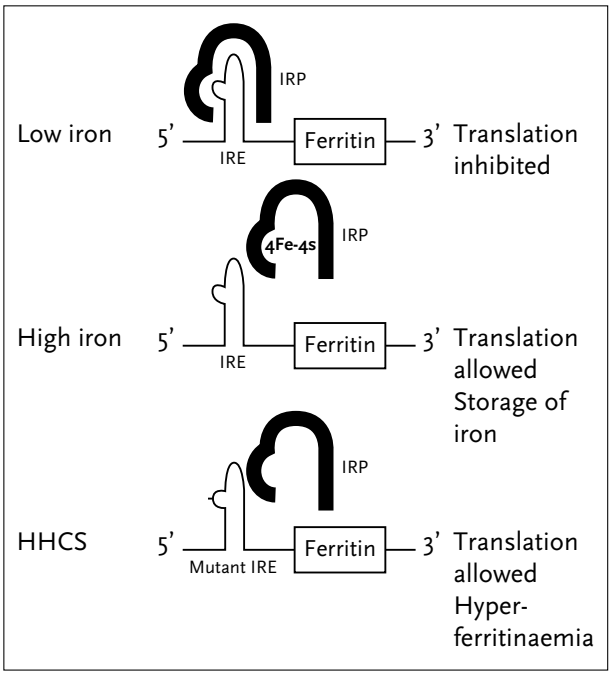

Figure 1

Schematic representation of the regulation of ferritin synthesis

This translational regulatory mechanism applies to the L- as well as the H-ferritin subunit. Upper panel: the situation when there is low iron, allowing the iron regulatory protein (IRP) to bind strongly to the iron responsive element (IRE) located at the 5' noncoding region of the ferritin gene. This prohibits the translation of the ferritin mRNA. Middle panel: when cellular iron is abundant, formation of 4Fe-4S clusters within IRP prevents binding to IRE, leading to efficient synthesis of ferritin.

Lower panel: the condition when the IRE is mutated in HHCS patients, meaning that irrespective of the iron status the IRP is not capable of binding IRE, allowing the translation and synthesis of ferritin, leading to hyperferritinaemia.

iron.[13,14] This eventually leads to iron loading in parenchymal organs, resulting in dysfunction of these organs, especially the liver, heart and pancreas. Two major mutations in the *HFE* gene have been described, Cys282Tyr and the His63Asp. Most haemochromatosis patients are homozygous for the Cys282Tyr mutation.[15] The role of the His63Asp mutation in iron overload is less clear, however. Even in the so-called compound heterozygotes (heterozygosity for both Cys282Tyr and His63Asp mutations) iron overload is usually mild and only present in 1 to 2% of the cases.[16] The iron overload can be treated by (repeated) phlebotomies.

We describe two heterozygous mutations in the IRE of the 5'-UTR of the L-ferritin gene co-inherited with a heterozygous mutation in the *HFE* gene. The co-inheritance of both mutations may be misleading, as is illustrated by our study.

PATIENTS AND METHODS

Family 1

A 53-year-old man was seen at our outpatient department after he had been investigated elsewhere. He was initially seen by his family doctor because of symptoms in his joints (backache and pain in the hips). The physical examination was unremarkable: body mass index (BMI) of 26.5 kg/m^2 and a normal blood pressure. Initial blood tests, performed elsewhere, showed a normal erythrocyte sedimentation rate, haemoglobin, leucocyte, and platelet counts, as well as normal liver and renal function. The glucose level was normal. The patient had a plasma cholesterol level of 5.9 mmol/l with normal triglycerides. Further analysis revealed a high serum ferritin level of 1750 μg/l (normal: 10-250 μg/l). Serum iron transferrin and transferrin saturation were normal. Despite the persistently normal values of the transferrin saturation, the patient was suspected of having HFE-related haemochromatosis. Genetic analysis of the *HFE* gene showed a heterozygosity for the G to A nucleotide substitution at position 845, responsible for the Cys282Tyr mutation (*figure 2A*). Thereafter, the patient was subjected to repeated phlebotomies, which had no effect on his ferritin level or his symptoms. On the contrary, the patient developed microcytic anaemia. Finally, the patient was referred to our hospital for further analysis of the hyperferritinaemia. The patient had an unremarkable medical history, except for operations because of bilateral cataracts. The first operation was when he was 27 years old (left eye), the second at the age of 38 (right eye). The family history was also positive for bilateral cataracts at a young age (see *figure 2A*). This prompted us to analyse the 5'-UTR of L-ferritin. The close relatives (first, second and third degree relatives) of the proband were tested for serum ferritin level, and those with a high ferritin level had undergone operations for bilateral cataracts. To confirm the phenotype of hereditary hyperferritinaemia-cataract syndrome, molecular analysis was performed.

Family 2

A 58-year-old man was found to have elevated serum ferritin level (1454 μg/l) during a routine examination of body iron status, with normal transferrin saturation. This patient was found to be heterogyzous for the Cys282Tyr mutation of the *HFE* gene (*figure 3A*). His BMI was 27.8 kg/m^2, he had a blood pressure of 140/80 mmHg, with a fasting plasma cholesterol level of 5.9 mmol/l, normal triglycerides, and a normal glucose level. His sister (BMI 29.9 kg/m^2, blood pressure of 130/75 mmHg, plasma cholesterol 6.3 mmol/l, normal triglycerides, normoglycaemic), who was also analysed because of hyperferritinaemia, was also found to be a carrier of the Cys282Tyr mutation, for which she was subjected to frequent phlebotomies, without any effect on the serum ferritin level. However, she developed

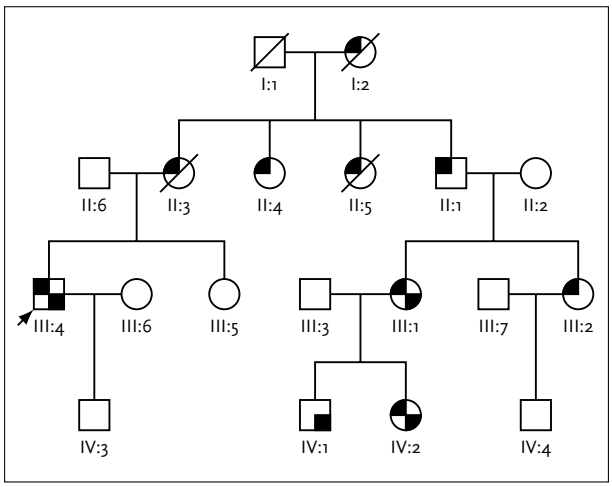

Figure 2A

Pedigree of the family 1 with the presence of cataract as well as heterozygosity for the Cys282Tyr mutation in the HFE gene

Cataract indicated by symbol with filled upper left quadrant, mutation in the *HFE* gene indicated by symbol with filled lower right quadrant, diagonal lines indicate deceased members and the proband is indicated by the arrow.

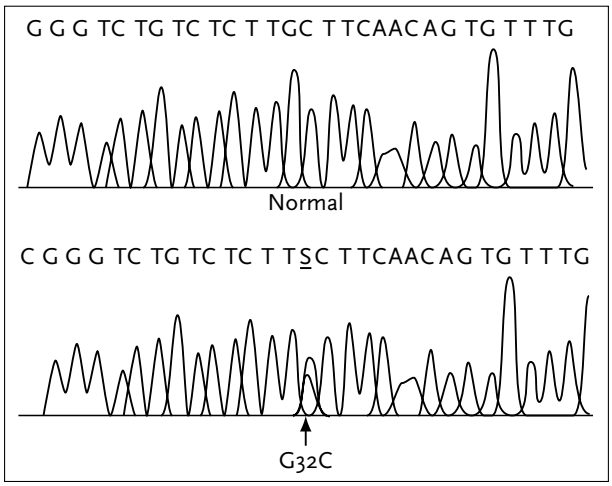

Figure 2B

Identification of the G to C mutation at position 32 in the IRE of the L-ferritin gene – sequence analysis of genomic DNA of a normal individual and of the proband

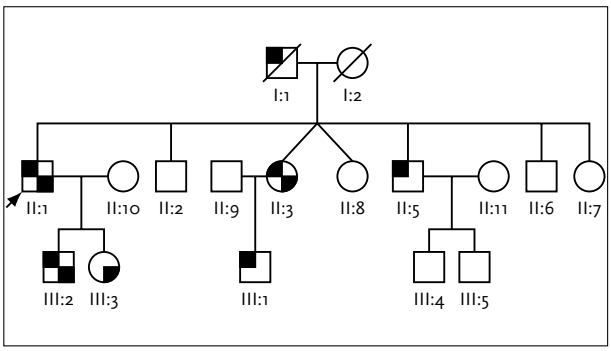

Figure 3A

Pedigree of the family 2 with the presence of premature bilateral cataracts as well as heterozygosity for the Cys282Tyr mutation in the HFE gene

Premature bilateral cataracts is indicated by symbol with filled upper left quadrant, Cys282Tyr mutation in the *HFE* gene is indicated by symbol with filled lower right quadrant, diagonal lines indicate deceased members and the proband is indicated by the arrow.

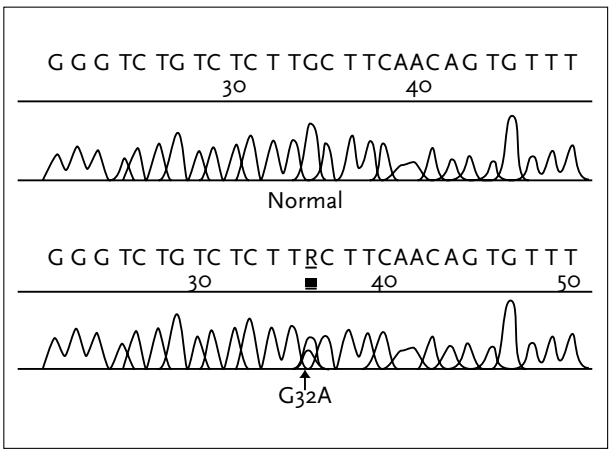

Figure 3B

Sequence analysis of genomic DNA of a normal individual and of the proband, showing the G to A mutation at position 32

microcytic anaemia. Both patients had a medical history of premature bilateral cataracts. The pedigree (*figure 3A*) shows the inheritance pattern of the Cys282Tyr mutation, as well as the family members with a history of premature bilateral cataracts necessitating surgery.

Molecular analysis of the 5'untranslated region of the L-ferritin gene genomic DNA was isolated from blood leucocytes according to standard methods. Primers were used to amplify the entire IRE of the L-ferritin gene.

Direct sequencing was carried out in both orientations of genomic DNA PCR products, performed on an ABI 3100 automated sequencer using a version 3 dye termination sequencing kit (Applied biosystems Torrence, CA, USA). Molecular analysis of the *HFE* gene for the G845A nucleotide substitution was performed based on the findings by Feder *et al.* and taking into account the 5569 G/A polymorphism in the choice of the nucleotide primers.[15]

RESULTS AND DISCUSSION

We found a heterozygous G to C transition at position 32 of the L-ferritin gene of proband 1 and the close relatives

(first, second and third degree) with high ferritin level and a medical history of surgery because of premature bilateral cataracts (*figure 2B*). The youngest member with bilateral cataracts was subjected to surgery at the age of seven, but she had already suffered from blurred vision at the age of four. The G32C substitution has previously been described in French and Italian pedigrees.[6,7,17] At the same position of the 5'-UTR of the L-ferritin gene, a G to A substitution was found in the second family (*figure 3B*). This mutation has also been reported previously in two Italian families.[5,8]

It is remarkable that the highly conserved guanine at position +32 is prone to mutations, and thus can be considered as a hot spot for mutations, since it has been found to be mutated in Italian, French and Dutch families.[5-8,17] The level of hyperferrritinaemia in the two Dutch families in this study is comparable with those previously described in the Italian and French families. However, as noted previously, there is a phenotypic diversity among subjects with the same mutation.[7] Because some of the described mutations in the IRE of L-ferritin in patients with HHCS are *de novo*, and in some patients with hyperferritinaemia the cataract is asymptomatic, this syndrome should be included in the differential diagnosis of hyperferritinaemia even in the absence of a positive family history. The exact mechanism causing hyperferritinaemia in HHCS subjects to lead to cataracts (and not to pathological conditions in other tissues/organs) remains unclear. Possible theories have been proposed. An abnormal deposition of L-ferritin (approximately tenfold accumulation of ferritin in HHCS patients, compared with normal individuals)[18] may cause alteration of local iron turnover, or modify other lens proteins, leading to oxidative lens damage.[19-21]

The next remarkable finding is the co-inheritance of G32C and G32A mutation of the L-ferritin gene with the Cys282Tyr mutation in the *HFE* gene in an heterozygous manner in both families in this study. Co-inheritance of alleles associated with HFE-related haemochromatosis and HHCS has been reported previously.[22] Heterozygous substitution of an A40G of the IRE of the L-ferritin gene together with His63Asp mutation of the *HFE* gene was found. However, the combination of mutations in two genes involved in iron metabolism, the Cys282Tyr in the *HFE* gene and mutation in the L-ferritin gene, have not been reported until now. Altogether, because of the high frequency of the Cys282Tyr mutation in a heterozygous state in persons from North-European descent (10% of the Caucasian population), the co-inheritance of this mutation with the mutation in the IRE of the L-ferritin gene as described in this study, could be regarded as coincidental. The propositus of both families were found to be heterozygous for the Cys282Tyr mutation of the *HFE* protein, meaning that *HFE*-related haemochromatosis was excluded in both propositi. However, another hereditary iron overload syndrome, primary haemochromatosis type 4 associated with mutations in the ferroportin-1 gene, remains possible.[23,24] This autosomal dominant disorder is characterised by relatively low serum iron level with an elevated serum ferritin level. One of the features in haemochromatosis type 4 is that the ferritin level increases prior to elevation of the transferrin saturation. Unlike the situation in HHCS, iron accumulates in reticuloendothelial cells. During phlebotomy (indicated in haemochromatosis type 4), haemoglobin as well as transferrin saturation may reach low levels despite high-normal serum ferritin. This finding may lead to confusion with HHCS (phlebotomy *not* indicated). In theory, there is a very small chance that in our patients the ferroportin-1 gene is also mutated, which can be investigated in future studies.

In 1997 Moirand *et al.* described a hepatic iron overload syndrome (also designated as dysmetabolic iron syndrome) characterised by hyperferritinaemia with a normal transferrin saturation.[25] The authors showed on the basis of HLA typing that this syndrome was not related to haemochromatosis. However, this syndrome was associated with various metabolic disorders; 72% of the patients (*vs* 35% of matched genetic haemochromatosis [GH] subjects) had a body-mass index of >25kg/m^2, 65% of the patients had hyperlipidaemia (*vs* 17% of GH subjects), 43% of the patients with an abnormal glucose metabolism (*vs* 8% of GH subjects), and 19% of the patients had hypertension (*vs* 12% of GH subjects).[25] Notably, these conditions are all components of the insulin-resistance syndrome. In our patients a clear association with the so-called dysmetabolic iron syndrome could not be demonstrated.

In conclusion, physicians should be aware that an isolated hyperferritinaemia (with a persistent normal transferrin saturation) does not per se imply that there is an iron overload and that the presence of an early onset cataract should raise the possibility of HHCS, even if there is a heterozygosity for a mutation in the *HFE* gene.

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