A novel (Leu183Pro-)mutation in the HFE-gene co-inherited with the Cys282Tyr mutation in two unrelated Dutch hemochromatosis patients


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Abstract

We describe a novel heterozygous mutation in exon 3 of the HFE-gene that was co-inherited with Cys282Tyr in two unrelated Dutch men both presenting a classical form of hereditary hemochromatosis. Heterozygosity for this mutation was also found in one out of 100 healthy controls of Dutch descent. This c.548T>C mutation converts a leucine to a proline residue at position 183 in the α2-helix of the HFE-protein (Leu183Pro).

Standard bioinformatics analysis shows that the mutation is likely to disturb the HFE interaction with TfR1. This disrupting role of the mutation in the iron regulatory pathway is further corroborated by the familial co-occurrence of the observed compound heterozygosity with increased serum iron parameters. Haplotype analysis strongly suggests that this novel mutation arose from a common ancestor in the distant past. These findings may have implications for HFE-testing of iron overloaded heterozygous Cys282Tyr-patients of Northern European origin and their relatives.

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Introduction

Hereditary hemochromatosis (HH) patients of European origin are homozygous for the Cys282Tyr mutation in 64–100%, another 4–7% are Cys282Tyr/His63Asp compound heterozygotes. In Caucasians, 4–35% of HH patients carry at least one chromosome without any of the known HFE-mutations (Cys282Tyr, His63Asp or Ser65Cys) [1]. In addition to these common mutations, there is a scattering of rare mutations usually found in only one family [1].

HFE is an atypical major histocompatibility complex class I molecule that interacts with the transferrin receptor 1 (TfR1), a type II transmembrane glycoprotein that is the primary effector of cellular iron uptake [2,3]. The HFE-protein is expressed in duodenal crypt cells, Kupffer cells and hepatocytes, and has been thought to play its role in the iron uptake of these cells [4]. Recently, however, it has been proposed that HFE plays its most important role in iron sensing in the liver by conveying the whole-body iron status, reflected by diferric transferrin from the HFE–TfR1 complex to TfR2, resulting in potential signalling of downstream events [5]. HFE-mutations are supposed to exert their effect by disrupting either the interaction with β2-microglobulin (β2M) and/or TfR1 (Fig. 1A). The Cys282Tyr alteration in HFE-related hemochromatosis disrupts the sulfide bridge in the HFE α3-domain, impairing the β2M binding and the HFE cell surface expression [6,7]. The His63Asp mutation in a loop of the α1-domain is another frequent, but much less severe HFE variant. It probably induces a minor rearrangement that slightly influences the structure of the TfR1

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interacting domain, but this has been an area of controversy [3,7,8]. In this study we describe a novel missense mutation in exon 3 of the HFE -gene and the α2-helix of the HFE-protein, Leu183Pro that was co-inherited with Cys282Tyr in two unrelated males presenting a high transferrin saturation and clearly elevated ferritin levels at an age of 44 and 34 years, respectively. We propose that compound heterozygosity for this novel mutation on one DNA-strand and Cys282Tyr on the other resulted in classic hemochromatosis. Here we report the phenotype of the probands and their relatives and assess the functional consequences of the Leu183Pro mutation by visualizing its effect on the interaction with TfR1 in the crystal structure of the HFE–TfR1 complex.

Materials and methods

Case summaries

Proband T was a 44 year old male of Dutch descent, who presented with pain in the small joints of both his hands and in the ankles for several years. He had one sister; she and his mother were in good health. His father died at the age of 66 years due to carcinoma of the larynx. Physical examination revealed limited flexion of the metacarpophalangeal and proximalinterphalangeal joints at both hands without signs of arthritis or tendinitis. No other abnormalities were found. His blood counts as well as his serum chemistry profile (glucose, albumin) were normal, except for the slightly increased alanine aminotransferase of 67 U/l (upper limit reference range is 40 U/l). Iron indices revealed that serum iron level was 37.7 μmol/l (210 μg/dl) transferrin saturation was 72.5% and serum ferritin concentration was 2070 μg/l. The patient was heterozygous for the Cys282Tyr mutation; the His63Asp mutation was absent. He refused a diagnostic liver biopsy, but an MRI showed heavy iron deposition in the liver, without collaterals, but with a slightly dilated portal vein. A total of 34 weekly 500 ml phlebotomies normalized ferritin levels to 112 μg/l, but did not significantly improve his complaints. The patient blood samples were then shipped to our hemochromatosis referral center for further analysis.

Proband G was a 34 year old asymptomatic male of Dutch descent, who was referred to the outpatient clinic due to an elevated serum ferritin level found by a military sports
examination. He had no physical complaints, except muscle spasm during stretching in calves and underarms. He used 1–2 glasses of alcohol weekly, and no medication. There were no liver or rheumatic diseases in his family. His father and two sisters were healthy. His mother was previously diagnosed with Cys282Tyr homozygous hemochromatosis and on maintenance phlebotomies. On physical examination no abnormalities were found. His blood counts as well as his serum chemistry profile (glucose, albumin) were normal. His serum iron level was 44 μmol/l (245 μg/dl), the transferrin saturation was 83% and serum ferritin concentration was 544 μg/l. The patient was found to be heterozygous for the Cys282Tyr mutation, the His63Asp alteration was absent. After a total of 9 weekly 500 ml phlebotomies, the ferritin level was normalized to 34 μg/l, since then the phlebotomy interval has been reduced to once every three months.

Patient blood and urine samples were then sent to our hemochromatosis referral center for further analysis. Two months after the most recent phlebotomy, we found urine and serum hepcidin [9] that were decreased with 0.01 Mint/mmol creatinine (normal range is 0.52–7.83) and 0.41 Mint/l (normal range is 0.58–9.95 Mint/l), respectively, with a transferrin saturation of 42%, serum ferritin of 22 μg/l and Hb of 10.6 mmol/l (17.1 g/dl).

Fig. 2. Family trees. The figure shows the 6p21.3 region of chromosome 6, comprising the HLA-A and -B haplotypes, the allele size (in base pairs) of 6 short tandem repeats flanking HFE, and single nucleotide polymorphisms at 3 locations within the HFE-gene (c.340+4, c.548 and c.845). Family trees represent family T and family G. Also the haplotype of the Leu183Pro mutated control is shown. Markers are positioned in order of their position relative to the HFE-gene and given at the position of the deceased parent of family T. The p.Leu183Pro mutation is reflected in the c.548T→C transition and the p.Cys282Tyr in the c. 845G→A transition. Different colors represent the various haplotypes; shaded areas refer to the shared haplotype of the p. Leu183Pro alteration. The 2 probands and the control share a 5 marker haplotype of approximately 1 megabase. The distance between HLA-B and D6S2239 is around 5 megabases [14]. P, proband; C282Y, Cys282Tyr; L183P, Leu183Pro; WT, wild type; mb, megabases; n.d, not determined.

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Laboratory methods

The classic presentation of HH of both patients as well as the moderately decreased hepcidin levels in proband G led us to search for private mutations in the TfR2- and the HFE-gene. The latter was performed using the primers reported by Mattman et al. [10] and Barton et al. [11], but with newly developed primers for exons 4 and 5 (HFE ex4 6838R: TACCTCCTCAG-GCACCTCCTC; HFE ex5 6819F: GAGGAGTGCCCTGAG-GAGGTA). The HAMP and the HJV genes were sequenced to exclude modifying mutations. To test for a common ancestor, a low-intermediate resolution DNA typing for HLA-A and -B was performed by a PCR-Sequence Specific Oligonucleotide multiplex technique (Tepnel Lifecodes, Stamford, CT, USA), according to ASHI standards. Additional search for a founder effect was done by typing Short Tandem Repeats (STR) closer to the HFE-gene by PCR exploiting fluorescent primers in two multiplex reactions (probe mix 1: D6S2239, D6S105, D6S2222; probe mix 2: D6S265, D6S1683, D6S1621). PCR products were analysed on an ABI 3130 DNA sequencer.

Results

Patients

We detected a c.548T>C transition changing a leucine to a proline at position 183 in the α2-helix of the HFE-protein (Leu183Pro). The mutation created an Msp I® (New England Biolabs) restriction site in exon 3 detectable by 5067F and 5494R primers. Mutations in the genes TfR2, HAMP and HJV that encode for proteins previously shown to be involved in the pathogenesis of HH [12], were found to be absent. Subsequent family studies revealed the Cys282Tyr and Leu183Pro to be present in trans, e.g. on the two different DNA-strands. For proband T this was confirmed by the genotype of his mother and sister: CysCys/LeuLeu and CysTyr/LeuLeu, respectively and for proband G by the TyrTyr/LeuLeu and CysCys/LeuPro genotype of his mother and father, respectively. Moreover, by HLA-typing we found that in family T the Leu183Pro and Cys282Tyr segregation with the respective HLA-α1, B haplotypes. In family G the Cys282Tyr mutation with iron overload in two unrelated Dutch men. Haplotype analysis supports the hypothesis of this Leu183Pro alteration in the compound heterozygous state with the Cys282Tyr allele in 200 chromosomes is far too small for a proper statistical evaluation. However, even with this small sample size it is likely that the Leu181Pro mutation is less frequent than the Cys282Tyr or His63Asp mutation. Obviously, it is much more important for clinical practice to know if the co-occurrence of the new mutation with the Cys282Tyr mutation is correspondingly low in suspected hemochromatosis patients. We therefore, also tested a selection of 100 DNA samples from patients that appeared to be heterozygous for the Cys282Tyr mutation upon referral to our laboratory for HFE-testing, but did not find the Leu183Pro alteration in any of these samples.

Discussion

The novel Leu183Pro mutation is situated in the α2-helix of the HFE-protein. This mutation is expected to disturb the intermolecular contacts that involve the HFE α1–α2-platform and the helical domain of TfR1 (Figs. 1B and C). However, in the absence of evidence that this mutant HFE molecule is actually expressed on the hepatocyte surface, we cannot fully exclude the possibility that the mutant protein is unstable and that the phenotypic effect is simply due to its absence from the membrane. Also some other HFE missense mutations in the α1- and α2-domain in the compound heterozygous state with Cys282Tyr are shown to result in the classic hemochromatosis phenotype (reviewed in [1]), e.g. the Ile105Thr and Gly93Arg mutation in the C-terminal region of the HFE α1-helix [3,11]. The same holds for the Italian nonsense mutations Glu168X and Tyr169X, that, situated in the kink of the α2-helix of HFE, give rise to truncated proteins [3,13]. To the best of our knowledge, this is the first HFE-mutation that clearly affects the stability of the α2-helix of the HFE-protein, thereby disrupting the interaction with the TfR1 protein that is essential to exert the iron sensing function of the HFE-protein. The functional relevance is corroborated by the association of this Leu183Pro alteration in the compound heterozygous state with the Cys282Tyr mutation with iron overload in two unrelated Dutch men. Haplotype analysis supports the hypothesis of Leu183Pro being an ancestral mutation and provides a molecular haplotype that is as old if not older than that of the Cys282Tyr mutation [14]. This novel HFE-mutation is probably too scarce to include in routine DNA-analysis of Cys282Tyr-heterozygous patients, but prevalent enough to be tested for in tertiary HH referral centers.

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References