Ned Tijdschr Klin Chem Labgeneesk 2005; 30: 276-277

Short communications

The first Dutch family with juvenile Hemochromatosis caused by a Gly320Val mutation in the HFE2 gene

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Introduction

Hereditary hemochromatosis is an autosomal recessive disorder of iron metabolism resulting in accumulation of excess iron. The excess iron is deposited in a variety of organs, leading to organ failure and serious illness. Two specific point mutations of the HFE gene (C282Y and H63D) have been described and they are in general the main cause of hereditary hemochromatosis in the Northern European population (1). High prevalence of non-HFE gene associated hemochromatosis has been reported (2) and several other genes than HFE have been identified to be responsible for iron overload or hyperferritinemia: the hepcidin gene (HAMP) or hemojuvelin gene (HJV) is responsible for type 2 hemochromatosis (3), the transferrin receptor-2 gene (TFR2) is responsible for type 3 hemochromatosis (4), ferroportin (SLC40A1) is responsible for type 4 hemochromatosis (5), H-ferritin (FTH1) is responsible for type 5 hemochromatosis (6) and L-ferritin (FTL) is responsible for type 6 hemochromatosis (hyperferritinemia) (7) (see table 1). Mutations in these genes are reported in only a small number of families (8).

A Dutch family was presented with hereditary hemochromatosis, only one of three brothers (Son 1 in table 2) was symptomatic; he suffered from recurrent infections, anemia, hypogonadism and liver insufficiency. All three brothers had very high levels of ferritine (1570, 2934 and 3571 μg/L) and very high transferrin saturation (> 90%) in their second decade of life, and therefore suspicious for juvenile hemochromatosis (table 2). Juvenile hemochromatosis is an early onset autosomal recessive disorder of iron overload and is also called type 2 hemochromatosis.

Juvenile hemochromatosis has been linked to the centromeric region of chromosome 1q and recently the gene crucial to iron metabolism has been identified (hemojuvelin). The hemojuveline gene is localized on chromosome 1q21 and consist of 4 exons (9). Hemojuvelin (HJV) is transcribed from a gene of 4,265 bp into a full-length transcript with 5 spliced isoforms. The putative full-length protein from the longest transcript is 426 amino acids. Hemojuvelin contains multiple protein motifs consistent with a function as a membrane-bound receptor or secreted polypeptide hormone.

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An other gene responsible for juvenile hemochromatosis is the gene encoding hepcidin antimicrobial peptide (HAMP). The hepcidin gene has been proposed as a key regulator of iron absorption in mammals (3). Loss of function of hepcidin leads to severe iron overload. Overexpression leads to macrophage iron retention and an iron deficient phenotype (anemia of chronic disease). The two forms of juvenile hemochromatosis (HFE2) are designated HFE2A and HFE2B respectively and the clinical and biochemical phenotype of both forms are indistinguishable, both showing hyperabsorption of intestinal iron, early onset of iron overload associated with macrophages that do not load iron and hypogonadism (9-11). In their study, Papanikolaou et al, noticed that urinary hepcidin levels were depressed in patients with juvenile hemochromatosis. Therefore it is suggested that HFE2 seems to modulate the hepcidin expression.

Materials and methods

DNA of all subjects was isolated from peripheral blood. PCR was performed on all exons of the hemochromatosis (HFE), hepcidin (HFE2b) and hemojuveline (HFE2a) genes, including their flanking regions. Primer sequences and amplification protocols are available on request. The known HFE mutations, C282Y, H63D, H63H, S65C and T281M were checked by RFLP to exclude HFE related hemochromatosis. Only one of the asymptomatic brothers carried a heterozygous H63D mutation. Mutation detection for the HFE, HAMP and HJV gene for the three brothers was performed by CEL I heteroduplex mutation detection analysis of PCR products of all exons of these genes on a polyacrylamide gel. Heteroduplexes caused by a heterozygous mutation are cut by the CEL I enzym, which will cause a shift in the electrophoretic pattern or an increase or decrease of a specific fragment. To be sure that homozygous mutations would not be missed, patient samples were also analysed in a 1:1 dilution with normal DNA.

Results

The CEL 1 heteroduplex mutation analysis showed a change in the electrophoretic pattern for PCR products of some exons of the HFE gene and exon 4 of the HFE2a gene. The change in electrophoretic pattern was confirmed by Primer Dye Cycle sequencing. In the HFE gene, only some polymorphisms were found.

Table 1. Overview of the genetic causes of hemochromatosis, iron overload and hyperferritinemia and their responsible genes

Type	Inheritance	Clinical Characteristics	Gene	Gene symbol	References
I	recessive	classical hereditary hemochromatosis	HFE	HFE	
II	recessive	juvenile hemochromatosis early onset, hypogonadism, cardiac complications, liver disease (less prominent)	Hemojuvelin Hepcidin	HFE2A HFE2B	3, 8, 9
III	recessive	similar to classical hereditary hemochromatosis (rare, only 8 family's described)	Transferrin receptor 2	TFR2	10, 12
IV	dominant	high ferritin levels, increased reticuloendothelial iron deposition, mild anemia. minimal iron deposition in the liver	Ferroportin	SLC40A1 (former SLC11A3)	5, 8, 13
V	dominant	rare, only 1 family described	H-Ferritin	FTH1	6
VI	dominant	high levels of ferritin with bilateral congenital cataract, no iron deposition 'heriditary hyperferritinemia-cataract syndrome'	L-Ferritin	FTL	7, 14

Table 2. Overview of the iron status and HFE mutations of the Dutch family with the HFE2A Gly320Val mutation. NA = not assessed

Subject	*Age (years)*	*C282Y mutation*	*H63D mutation*	*Tf (g/L)*	*Fe (µmol/L)*	*Fe Sat (%)*	*Ferritin (µg/L)*	*ASAT (U/L)*	*ALAT (U/L)*	*LDH (U/L)*	*GGT (U/L)*	*AP (U/L)*
Father		wildtype	heterozygous	2.9	23	35	200	NA	NA	NA	NA	NA
Mother		wildtype	wildtype	2.6	30	52	120	NA	NA	NA	NA	NA
Son 1	20.0	wildtype	wildtype	2.6	58	98	2934	66	87	454	50	126
Son 2	18.8	wildtype	heterozygous	2.4	58	107	3571	93	178	268	52	133
Son 3	11.9	wildtype	heterozygous	2.1	45	96	1570	66	78	340	18	NA

In codon 320 (exon 4) of the hemojuveline gene (HFE2a) a homozygous glycine to valine mutation was observed for all three brothers. Both parents, who showed consanguinity, are heterozygous for this mutation. The mutation was confirmed by digestion with restriction enzyme Nla IV.

Discussion

We characterized the first Dutch family in which juvenile hemochromatosis was diagnosed, being caused by a homozygous Gly320Val mutation in the recently described hemojuveline gene. This mutation was the same mutation that was observed in Greek, Canadian and French families and accounted for two third of the mutations found in these families (9).

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