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 ferritin (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 (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 contains multiple protein motifs consistent with a function as a membrane-bound receptor or secreted polypeptide hormone.

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 all 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.
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).

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

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

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References
7. Hetet G; Devaux I; Soufir N; Grandchamp B; Beaumont C. Molecular analyses of patients with hyperferritinemia and normal serum iron values reveal both L ferritin IRE and 3 new ferroportin (SLC11A3) mutations. Blood 2003; 102: 1904-1910.