Juvenile hemochromatosis caused by a novel combination of hemojuvelin G320V/R176C mutations in a 5-year old girl

During a screening program we identified a 5-year old girl with elevated iron parameters. The child was found to have a combination of a novel R176C mutation together with the G320V mutation in the juvenile hemochromatosis gene (*HJV*). The girl was also homozygous for the H63D mutation in *HFE*. The possibility of detecting juvenile hemochromatosis before the onset of clinical manifestations raises questions about the management of such young children in order to prevent iron overload.

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Juvenile hemochromatosis (JH) is a severe form of hereditary hemochromatosis (HH) and is characterized by an early age of onset.[1] The disease is genetically heterogeneous since it can be associated with mutations in two distinct genes: *HAMP*[2] and *HJV*[3] encoding hepcidin and hemojuvelin, respectively. The majority of cases of JH are due to mutations in *HJV*.[4,5] Digenic inheritance of mutations in *HFE*[6] and *HAMP* may result in either JH or adult HH depending upon the severity of the mutation in HAMP.[7] A cascade screening program designed for family studies when a family member is affected by a major complication possibly due to hemochromatosis detected a 5-year girl with elevated iron indices and allowed us to characterize the genetic defect responsible.

The proband was referred in 2003 for investigation of repeatedly raised transferrin saturation (90%) associated with elevated serum ferritin (199 µgL). Iron parameters had initially been measured in all family members following the death of the paternal grandfather with a diagnosis of hepatocellular carcinoma. Serum iron parameters of the grandfather were not available. The proband's parents and two brothers had normal iron indices (Table 1). Written informed consent to all the investigations was obtained from the parents for themselves and their children. The girl had a normal blood count, no elevated liver transaminases and no clinical signs of hemochromatosis. Her father originated from north-east France and her mother had Spanish and Italian ancestry. The girl was found to be homozygous for the H63D mutation in *HFE* as was her older brother 2 who did not, however, have any abnormal iron indices (Table 1). We then sequenced the *HAMP* gene, the only gene known to be related to JH in early 2003. None of the exons or exon-intron junctions were mutated. In an attempt to determine a possible digenic inheritance of mutations in *HAMP* and *HFE*, the latter was also sequenced and found to be normal. We then added *TFR2* and *SLC40A1* to the panel. A new *TFR2* c.2085G→C variant was identified, although with no resulting amino acid change (p. S695S). It is very unlikely that this nucleotide change could have played a role in the elevated iron parameters in the proband as it was also present in her father who had normal serum iron parameters. We did not identify variants in the coding or flanking intronic sequences of the gene encoding for ferroportin from the proband. With the discovery of the *HJV* gene we asked for fresh informed consent from the parents for *HJV* analysis in the family. The proband was heterozygous for a new missense mutation at position c.526 (C→T) in exon 3 of *HJV* resulting in a substitution R176C. The other allele carried the G→T common transition at position 959 in exon 4 that leads to the G320V substitution in hemojuvelin (Figure 1). Both of the mutations were confirmed by restriction fragment length polymorphism analysis. The c.526 (C→T) and the c.959 (G→T) substitutions abolish *Hha*I and *Nla*IV enzyme restriction sites respectively (*data not shown*). The c.526 (C→T) transition was not detected in a control group of 61 individuals.

Now aged 8 years old, the girl is still clinically asymptomatic with an elevated transferrin saturation of 100% and a ferritin concentration of 235 ng/L. Her hepatic iron concentration was found to be 260 µmol/g by magnetic resonance imaging (normal <36 µmol/g).

The new R176C substitution is located in the partial von Willebrand type D domain of the protein; this amino acid is highly conserved phylogenetically.[3] The mutation occurred two amino acids downstream from a highly conserved GDPH motif, a consensus site for hemojuvelin and mRGMc (mouse repulsive guidance molecule c), which undergoes acid sensitive autocatalytic cleavage.[8] By introducing the C176 residue in a molecular modeling program as already described[9] we found that this substitution destabilizes a short helix made of the RSF residues

Table 1. Biochemical data and genotypic results in the family members.

Data/family members	*Father*	*Mother*	*Proband*	*Brother 1*	*Brother 2*
Age	40	38	5	13	15
Serum Iron (µmol/L)/N:9-29	12.7	13.6	39.2	10.6	18.8
Transferrin saturation (%) /N:20-40	20	26	90	19	32
Serum Ferritin (µg/L)*	194	41	199	46	32
HFE mutations					
p.C282Y	-/-	-/-	-/-	-/-	-/-
p.H63D	+/-	+/-	+/+	-/-	+/+
HJV mutations					
p.R176C	-/-	+/-	+/-	+/-	-/-
p.G320V	+/-	-/-	+/-	-/-	-/-
TFR2 polymorphism p.S695S	+/-	-/-	+/-	-/-	-/-
HAMP exons 1, 2, 3 /	ND	ND	WT	ND	ND
HAMP promoter polymorphism g.-582 A→G	-/-	+/-	-/-	-/-	-/-
SLC40A1 /	ND	ND	WT	ND	ND

*Gene sequences were first scanned using denaturing high performance Liquid chromatography for all exons and sequenced when necessary using a fluorescent-tagged dideoxy chain termination method with an ABI prism Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) as previously reported. * The range of assumed normal values for ferritin in our laboratory hospital is 15-75 µg/L for children aged from 5 to 8 years old. It is 27-310 µg/L and 21-224 µg/L for adult males and adult females, respectively. ND: not determined. WT: wild type*

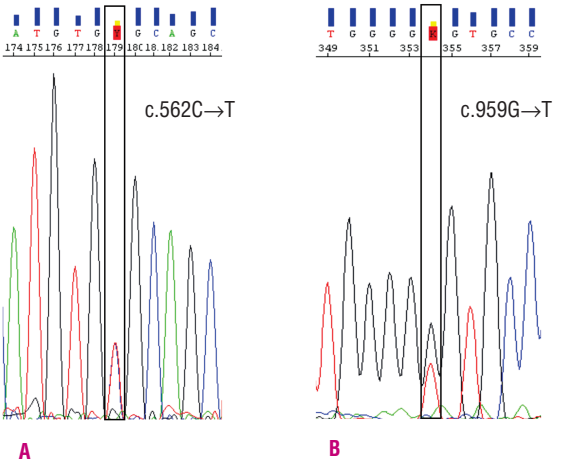

Figure 1. ***HJV*** **sequencing results. A. Proband's** ***HJV*** **mutation in position nt 526. B. Proband's** ***HJV*** **mutation in position nt 959.**

(from 176 to 178). The proband was also homozygous for H63D in *HFE*, but *HJV*-related JH does not seem to be aggravated when associated with *HFE* genotypes, including H63D homozygosity.[4,10] This confirms the need to search for other causes of disease in *HFE*-H63D homozygotes with clinical forms of HH.

In *HJV*-related JH clinical manifestations arise rapidly in teenagers. As it becomes possible to make an early diagnosis of JH before the onset of clinical manifestations, questions are being raised about the follow-up of such young patients in order to prevent them developing iron overload. Because of the increased iron accumulation in JH compared to type 1 hemochromatosis we have taken into account the projected severity of the disease and devised a phlebotomy program for the proband.

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Key words: hemojuvelin, HJV, juvenil hemochromatosis, infant.

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References

1. Cazzola M, Ascari E, Barosi G, Claudiani G, Dacco M, Kaltwasser JP, et al. Juvenile idiopathic haemochromatosis: a life-threatening disorder presenting as hypogonadotropic hypogonadism. Hum Genet 1983;65:149-54.
2. Roetto A, Papanikolaou G, Politou M, Alberti F, Girelli D, Christakis J, et al. Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. Nat Genet 2003;33:21-2.
3. Papanikolaou G, Samuels ME, Ludwig EH, MacDonald ML, Franchini PL, Dube MP, et al. Mutations in HFE2 cause iron overload in chromosome 1q-linked juvenile hemochromatosis. Nat Genet 2004;36:77-82.
4. Lanzara C, Roetto A, Daraio F, Rivard S, Ficarella R, Simard H, et al. Spectrum of hemojuvelin gene mutations in 1q-linked juvenile hemochromatosis. Blood 2004; 103: 4317-21.
5. Lee PL, Beutler E, Rao SV, Barton JC. Genetic abnormalities and juvenile hemochromatosis: mutations of the HJV gene encoding hemojuvelin. Blood 2004;103:4669-71.
6. Feder JN, Gnirke A, Thomas W, Tsuchihashi Z, Ruddy DA, Basava A, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996;13:399-408.
7. Merryweather-Clarke AT, Cadet E, Bomford A, Capron D, Viprakasit V, Miller A, et al. Digenic inheritance of mutations in HAMP and HFE results in different types of haemochromatosis. Hum Mol Genet 2003;12:2241-7.
8. Zhang AS, West AP Jr, Wyman AE, Bjorkman PJ, Enns CA. Interaction of hemojuvelin with neogenin results in iron accumulation in human embryonic kidney 293 cells. J Biol Chem 2005;280:33885-94.
9. Le Gac G, Dupradeau FY, Mura C, Jacolot S, Scotet V, Esnault G, et al. Phenotypic expression of the C282Y/Q283P compound heterozygosity in HFE and molecular modeling of the Q283P mutation effect. Blood Cells Mol Dis 2003;30:231-7.
10. De Gobbi M, Roetto A, Piperno A, Mariani R, Alberti F, Papanikolaou G, et al. Natural history of juvenile haemochromatosis. Br J Haematol 2002;117:973-9.