Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis

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Animal models indicate that the antimicrobial peptide hepcidin (HAMP; OMIM 606464) is probably a key regulator of iron absorption in mammals. Here we report the identification of two mutations (93delG and 166C→T) in *HAMP* on 19q13 in two families with a new type of juvenile hemochromatosis.

The regulation of intestinal iron absorption is crucial to avoid toxicity. Disruption of this regulation in hereditary hemochromatosis leads to iron overload, cirrhosis, cardiomyopathy, arthritis and endocrine failure. The most common form of hereditary hemochromatosis (OMIM 235200) is caused by mutations in the gene *HFE*[1], which may encode a duodenal crypt body iron sensor. Type 3 hereditary hemochromatosis (OMIM 604250) is associated with mutations of a low-affinity transferrin receptor (*TFR2*; refs. 2,3) and type 4 hereditary hemochromatosis (OMIM 606069) associated with the iron exporter ferroportin 1 (refs. 4,5). Juvenile or type 2 hereditary hemochromatosis (OMIM 602390) has the most severe phenotype and can be lethal at a young age[6,7]. Although the gene associated with this disorder has not been identified, the locus maps to chromosome 1q21 (ref. 8). A single inbred pedigree with juvenile hereditary hemochromatosis that is not linked to 1q was recently reported[9].

Animal models of iron overload include mice deficient in *Usf2* that do not express the antimicrobial peptide Hamp[10]. Constitutive overexpression of hepcidin in *Hamp* transgenic mice leads to iron-deficient anemia[11]. These findings indicate a key role for HAMP in regulation of iron absorption in mammals and make *HAMP* a functional candidate for association with juvenile hereditary hemochromatosis that is not linked to 1q.

HAMP is a pro-peptide of 84 amino acids that undergoes enzymatic cleavage into mature peptides of 20, 22 and 25 amino acids[12,13]. Active peptides are rich in cysteines that form intramolecular bonds and stabilize the β-sheet structure[14]. The peptide of 25 amino acids, isolated from human blood[12] and urine[13], has prevalent hepatic expression[14].

To determine whether *HAMP* was associated with juvenile hereditary hemochromatosis not linked to 1q, we genotyped affected individuals from two families. All affected individuals were less than 30 years old at the onset of clinical symptoms and

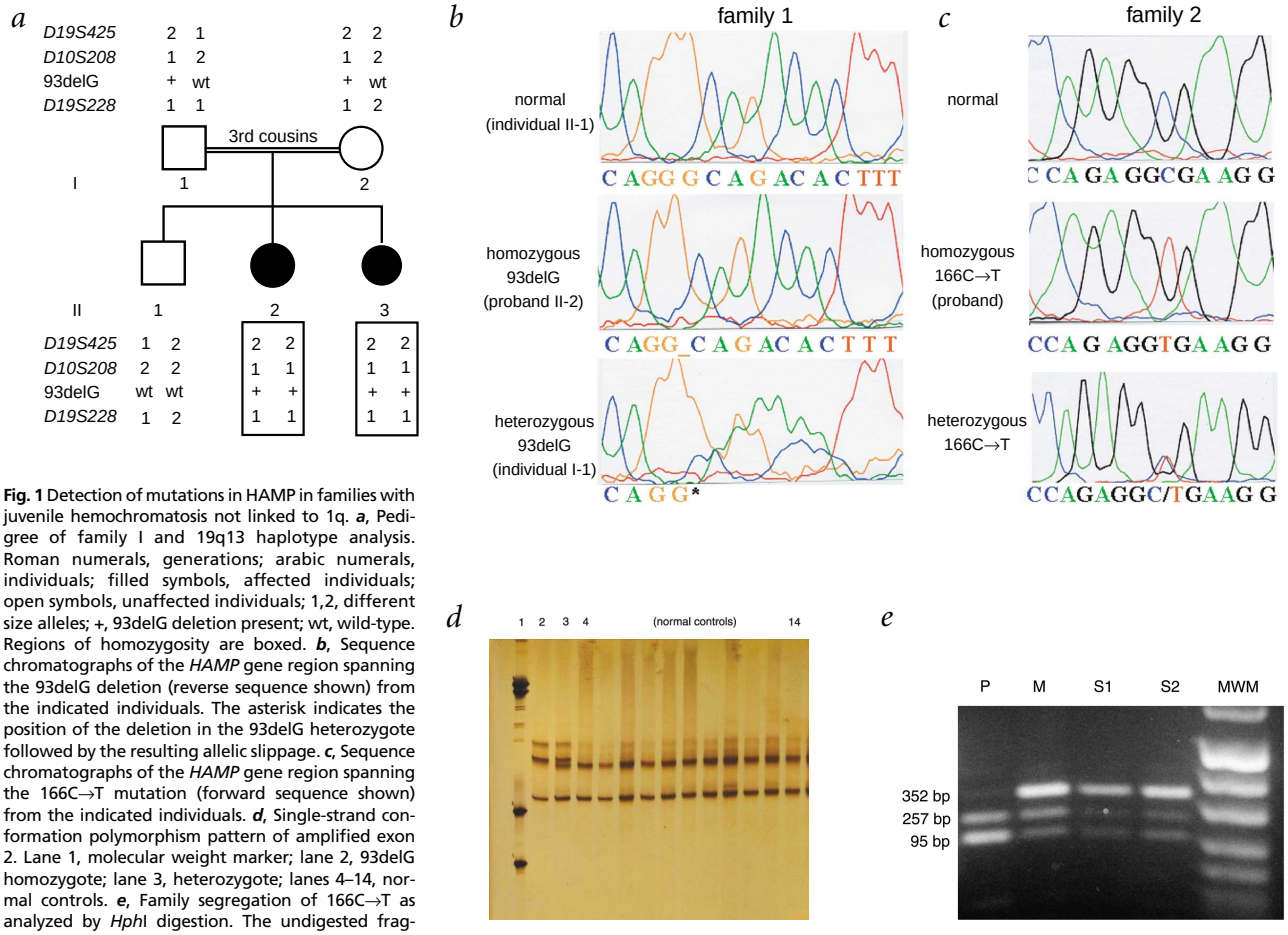

Fig. 1 Detection of mutations in HAMP in families with juvenile hemochromatosis not linked to 1q. ***a***, Pedigree of family I and 19q13 haplotype analysis. Roman numerals, generations; arabic numerals, individuals; filled symbols, affected individuals; open symbols, unaffected individuals; 1,2, different size alleles; +, 93delG deletion present; wt, wild-type. Regions of homozygosity are boxed. ***b***, Sequence chromatographs of the *HAMP* gene region spanning the 93delG deletion (reverse sequence shown) from the indicated individuals. The asterisk indicates the position of the deletion in the 93delG heterozygote followed by the resulting allelic slippage. ***c***, Sequence chromatographs of the *HAMP* gene region spanning the 166C→T mutation (forward sequence shown) from the indicated individuals. ***d***, Single-strand conformation polymorphism pattern of amplified exon 2. Lane 1, molecular weight marker; lane 2, 93delG homozygote; lane 3, heterozygote; lanes 4–14, normal controls. ***e***, Family segregation of 166C→T as analyzed by *Hph*I digestion. The undigested fragment is 352 bp. Two fragments (257 and 95 bp) are produced from the mutated allele. P, proband; M, mother; S1 and S2, heterozygous siblings; MWM, molecular weight marker.

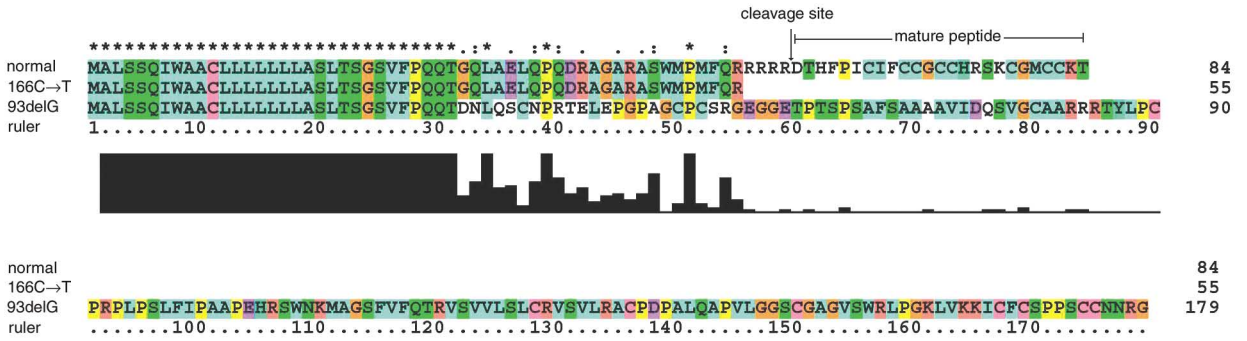

Fig. 2 Comparison of the predicted mutant peptides resulting from the 93delG and 166C→T mutations with normal pro-hepcidin.

had severe iron overload with liver fibrosis or cirrhosis and hypogonadism[6,9], meeting the diagnostic criteria for juvenile hemochromatosis[7]. One affected individual also had cardiomyopathy[6].

We analyzed microsatellites encompassing a region of 2.7 cM on chromosome 19q13 in family I (Fig. 1*a*) and identified a region of homozygosity identical in both probands. We then sequenced the *HAMP* coding region, exon-intron boundaries and 5′ and 3′ untranslated regions in probands of family I and in a previously reported affected individual[6] in family II and identified two mutations. The first was deletion of a guanine in exon 2 at position 93 of *HAMP* cDNA (93delG). Probands of family I were homozygous with respect to this deletion, and obligate carriers were heterozygous (Fig. 1*a*,*b*). Single-strand conformation polymorphism analysis excluded the mutation in 50 unaffected controls (Fig. 1*d*). The 93delG deletion results in a frameshift, and, if mutated RNA reaches translation, generates an elongated (179 residues) abnormal pro-hepcidin peptide. Because the frameshift occurs after residue 31, the active peptides and the cysteine motif are completely disordered.

The second mutation, which we identified in the proband of family II, was a C→T substitution at position 166 in exon 3 of *HAMP* cDNA (166C→T), which changes arginine at position 56 to a stop codon (R56X; Fig. 1*c*). *Hph*I restriction analysis showed the expected intrafamilial segregation of 166C→T (Fig. 1*e*) and its absence in 50 controls (data not shown). The R56X amino-acid change occurs in a penta-arginine (residues 55–59) basic domain, which is probably the recognition site for pro-hormone convertases[12], and produces a truncated pro-hepcidin lacking all mature peptide sequences (Fig. 2).

The iron overload resulting from mutations in *HAMP* suggests that HAMP plays a role in maintaining iron balance in humans, and adds *HAMP* to the list of genes associated with hereditary hemochromatosis. The severity of the phenotype of juvenile hemochromatosis relative to the other types of hereditary hemochromatosis[7] suggests that HAMP may very well be a principal component of the iron regulatory machinery. This conclusion is supported by the fact that wild-type HFE and TFR2 in individuals with mutations in *HAMP* are unable to inhibit iron absorption. Our results are in agreement with recent data on hypotransferrinemic mice indicating that Hamp may mediate the regulation of iron absorption according to the needs of both erythropoeiesis and body iron stores[15]. Hemochromatosis associated with *HAMP* inactivation is the first example of a genetic disorder associated with an antimicrobial peptide, but the apparent lack of susceptibility to infections in affected individuals suggests that the antimicrobial role of HAMP is not critical for staving off infection.

Our results identify a new form of juvenile hereditary hemochromatosis, increasing the genetic heterogeneity of iron-overload diseases. They may also facilitate identification of the gene on 1q that is associated with juvenile hereditary hemochromatosis, as it may encode a molecule involved in the HAMP signaling pathway.

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Competing interests statement

The authors declare that they have no competing financial interests.

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