Identification of New BMP6 Pro-Peptide Mutations in Patients with Unexplained Iron-Overload

Chiara Piubelli, Annalisa Castagna, Giacomo Marchi, Monica Rizzi, Fabiana Busti, Monia Marchetti, Marco De Gobbi, Antonella Roetto, Luciano Xumerle, Alejandro Giorgetti, Massimo Delledonne, Oliviero Olivieri and Domenico Girelli. BLOOD. Volume: 128. Pages: 264. Published: DEC 2016

Identification of New BMP6 Pro-Peptide Mutations in Patients with Unexplained Iron-Overload

Chiara Piubelli 1-2, Annalisa Castagna 1-2, Giacomo Marchi 1-2, Monica Rizzi, 1-2 Fabiana Busti 1-2, Monia Marchetti 3, Marco De Gobbi 4, Antonella Roetto 5, Luciano Xumerle 6, Alejandro Giorgetti 6, Massimo Delledonne 6, Oliviero Olivieri 1-2 and Domenico Girelli

1 Department of Medicine, University of Verona, Verona, Italy
2 Veneto Region Referral Centre for Iron Disorders, Azienda Ospedaliera Universitaria Integrata di Verona, Verona, Italy
3 Oncology Unit, Ospedale Cardinal Massaia, ASTI, Italy
4 Department of Clinical and Biological Sciences, University of Turin, Orbassano (TO), Italy
5 Dept. Of Clinical and Biological Sciences, University of Turin, Orbassano (TO), Italy
6 Department of Biotechnology, University of Verona, Verona, Italy

Background

Hereditary Hemochromatosis (HH) is a genetically heterogeneous disorder caused by mutations in at least 5 different genes (HFE, HJV, TFR2, SLC40A1, and HAMP) involved in the production and function of the liver hormone hepcidin, a key regulator of iron metabolism. Nevertheless, patients with a HH-like phenotype that remains unexplained, despite extensive sequencing of the known genes, are not infrequently seen at referral centres, implicating the role of still unknown genetic factors. A compelling candidate is Bone Morphogenetic Protein 6 (BMP6), a member of TGFb superfamily, whose expression is stimulated by increased iron stores in the liver. BMP6 acts as a major activator of the BMP-SMAD signalling pathway, ultimately leading to the upregulation of hepcidin gene transcription. Indeed, early this year French Authors have described 3 heterozygous missense mutations in BMP6 (p.Pro95Ser, p.Leu96Pro, and p.Gln113Glu) in 6 unrelated patients with mild to moderate, late onset, unexplained iron overload (Daher R, Gastroenterology 2016).

Methods

We recently updated our next generation sequencing (NGS)-based second level genetic test for the molecular diagnosis of non-HFE HH (Badar S, Am J Hematol 2016), by adding a number of novel potential candidate genes, including BMP6, to the panel of the 5 known HH genes. This test was applied to 38 patients evaluated at our tertiary referral centre for iron disorders, because of an unexplained iron overload phenotype.

Results

We found 3 heterozygous missense mutations in BMP6 gene in 4 patients with unexplained, late-onset, iron overload, from 3 different families. Their relevant clinical data are summarized into Table 1. Of note, 1 mutation (p.Leu96Pro) was the same recently described by Daher et al. and proven to be functional. The other two mutations (p.Glu112Gln, p.Arg257His) were novel, predicted damaging by bioinformatic tools, and both located in the pro-peptide domain, known to be crucial for appropriate BMP6 processing and secretion. They were further studied by in silico modelling, based on the available 3D structure of the TGFb, which also resulted to be consistent with their pathogenetic role.

Conclusions

To the best of our knowledge, our results provide the first independent confirmation of the likely causal role of BMP6 mutations in late onset, moderate iron overload phenotype, unrelated to mutations in the established 5 HH genes.

Table 1.

Table 1: summary of patients' data

Pt. ID	Age/Sex	1ST level genetic test	TS (%)	Ferritin (ng/ml)	LIC* (µmol/g)	BMP6 sequencing (NGS targeted panel)	Cofactors	Notes
1	54/M	HFE H63D (+/-)	95	1.901	100	Heterozygous for rs200573175 (p.Leu96Pro). Already reported (Daher R., Gastroenterology 2016)	Metabolic syndrome. Former alcohol consumption up to 48 g/die.	
2	71/M	wt**	70	763	95	Heterozygous for rs201486498 (p.Glu112Gln). New	β-thalassemia minor. Former alcohol consumption up to 36 g/die.	
3	65/M	wt**	36	413	130	Heterozygous for rs148916269 (p.Arg257His). New	Metabolic syndrome. Former alcohol consumption 24 g/die.	
4	62/M	HFE H63D (+/-)	33	1.175	91	Heterozygous for rs148916269 (p.Arg257His). New	Metabolic syndrome	Brother of Pt. 3

* Liver Iron Content by MRI or SQUID (n.v. <36). ** HFE wild type