HEPCIDIN GENE PROMOTER C.-1010T AND C.-582G VARIANTS ARE MODULATORS OF IRON OVERLOAD DEVELOPMENT IN INDIVIDUALS CARRYING THE H63D MUTATION IN THE HFE GENE

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Introduction

Hepcidin is a 25 amino-acid peptide hormone known to be a crucial regulator of iron homeostasis. It is able to decrease the absorption of dietary iron in the duodenum and the release of recycled iron from macrophage, as well as the export of the stored iron from hepatocytes.

It is known that the deficiency in hepcidin levels leads to the development of iron overload and that, in contrast, its overproduction causes iron deficiency/anemia.

Several mutations located in the hepcidin gene (HAMP) have already been associated to the development of iron overload or hereditary hemochromatosis (HH). Additionally, some HAMP promoter variants were described, however their functional consequences remain unclear. One of them is the polymorphism c.-582 A>G, recently described to be in association with an increased iron overload phenotype in beta-thalassemia major patients, but that has no effect on the iron status in the healthy population. Also, functional assays have revealed that hepcidin expression becomes slightly reduced under the promoter 6 variant when transactivated by the upstream stimulatory factors 1 and 2 (USF1/USF2) in HepG2 cells.

The aims of this study were to determine: i) the frequency of the c.-582 A>G HAMP polymorphism in patients presenting the common HFE mutations (H63D and/or C282Y); ii) if it modulates iron overload in these patients and, if so, which are the upstream stimuli that are impaired by the polymorphism.

Methods

HAMP promoter variants screening

Two polymorphisms were found in the proximal region of the HAMP promoter, the c.-582 A>G and c.-1010 C>T.

These polymorphisms appear to be in linkage disequilibrium in our sample.

In our study sample, the c.-582 A>G HAMP promoter polymorphism seems to be in linkage disequilibrium with the c.-1010 C>T HAMP polymorphism.

In individuals that present H63D or C282Y mutations in HFE gene, the CT/AG and TT/GG genotypes are significantly more frequent than in the control caucasian population (NCBI database).

However, when analysing the allelic frequencies we only found significant differences in the group of individuals that have SF higher than 400ng/mL along with one or two H63D alleles (HD/CC and DD/CC individuals). We also observe that the allelic frequency is also significantly different when comparing individuals having the same HD/CC or DD/CC background, but with different SF levels (higher polymorphism frequency found in individuals with SF levels higher than 400ng/mL).

In silico studies show that both polymorphisms can disrupt highly predictable transcription factor binding sites, such as USF2 and TATA. We tried to find which stimuli are impaired by these variants, however after performing luciferase assays we found that neither holo-Tf, ferric citrate, IL-6, hypoxia nor GDF-15 seem to be the stimuli that become unable to trigger the HAMP promoter activity.

In conclusion, c.-1010 C>T and c.-582 A>G polymorphisms seem to be a risk factor to iron overload development in individuals that by their H63D or C282Y background are already prone to develop this phenotype.

Arguments

Higher frequency of polymorphism c.-582 A>G in patients with SF higher than 400ng/mL, compared to healthy controls.

The frequency of polymorphism c.-1010 C>T was also higher in the same group.

The allelic frequency is also significantly different when comparing individuals with the same HD/CC or DD/CC background, but with different SF levels, especially in those with SF levels higher than 400ng/mL.

In silico studies suggest that both polymorphisms can disrupt highly predictable transcription factor binding sites, such as USF2 and TATA.

Luminescence assays

Holo-transferrin and ferric citrate

IL-6 is able to increase the promoter activity, while cobalt chloride, mimicking hypoxia, represses it.

Interleukin-6 and cobalt chloride

Both holo-transferrin and ferric citrate stimuli partially inhibit the 1.3kb HAMP promoter activity.

GDF-15 stimulus seems not to affect the HAMP promoter activity neither at physiological nor pathological (beta-thalassemia) concentrations.

No differences in the luciferase activity were observed in the polymorphic promoter when compared to the normal under the different analysed stimuli.

In conclusion, c.-1010 C>T and c.-582 A>G polymorphisms seem to be a risk factor to iron overload development in individuals that by their H63D or C282Y background are already prone to develop this phenotype.

References


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