HAMP promoter mutation nc.-153C>T in 785 HEIRS Study participants

Island and colleagues recently described a 37-year old French man with *HFE* p.C282Y homozygosity, severe iron overload (IO), and heterozygosity for the novel hepcidin (*HAMP*) promoter mutation nc.-153C>T.[1] *In vitro* nc.-153C>T decreased transcriptional activity of the promoter altered its interleukin-6 (IL-6) responsiveness and prevented binding of SMAD1/5/8/4 protein complex to the bone morphogenetic protein-responsive element (BMP-RE) of *HAMP*. Thus, nc.-153C>T could decrease hepcidin levels and contribute to IO.[1]

In the HEmochromatosis and IRon Overload Screening (HEIRS) Study of 99,711 primary care participants 25-years of age or older,[2] we used denaturing high-performance liquid chromatography (DHPLC) to analyze the *HAMP* promoter in a subset of 785 participants.[2,3] We selected 75 *HFE* p.C282Y homozygotes with the highest transferrin saturation (TS) and serum ferritin (SF) values (high TS/SF), and 75 p.C282Y homozygotes with the lowest TS and SF (low TS/SF).[3] We randomly selected 76 p.C282Y homozygotes as controls, including 16 high and 19 low TS/SF participants. We also selected 295 participants without p.C282Y homozygosity (74 non-Hispanic whites, 75 Hispanics, 74 blacks, 72 Asians) with the highest percentile for TS or SF of their respective race/ethnicity groups.[3] We selected 299 other participants without regard for TS and SF as controls (75 non-Hispanic whites, 74 Hispanics, 75 blacks, 75 Asians).

DHPLC screening was performed using a Transgenomic WAVE® 3500 HT system and a reverse-phase chromatography column (DNASep® HT). We used these PCR primers: ACATGCCAGACACTCCTGAG (forward) and TTGAGCTTGCTCTGGTGTCT (reverse). All samples that appeared to have a mutation were sequenced in both directions using the same PCR amplicons employed for screening. We numbered intronic mutations from the ATG start of translation.

We did not detect *HAMP* nc.-153C>T in any of 191 *HFE* p.C282Y homozygotes. In race/ethnicity groups, we detected *HAMP* nc.-153C>T (heterozygosity) only in a Hispanic woman in her fifth decade (screening TS/SF 51%/3,180 µg/L). On repeat testing, she had TS/SF 37%/87 µg/L without report of treatment for IO. She also had heterozygosity for *HAMP* -443 nc.C>T, *SLC40A1* c.663T>C (p.V221V), and *FTL* c.163T>C (p.L55L). She did not have *HFE* p.C282Y, p.H63D, or other exon 2 *HFE* mutation detectable by DHPLC. Lee *et al.* did not detect nc.-153C>T in diverse Americans with primary IO.[4] Similarly, nc.-153C>T was not detected in 100 French subjects with normal serum iron and hemoglobin measures.[1] Ascertaining any role of nc.-153C>T in causing the TS/SF phenotype in the HEIRS Study participant or her family members was beyond the scope of our study. Evaluating family members of the French patient was not possible.[1]

Two highly conserved and sequence-identical BMP-RE at positions -84/-79 and -2,255/-2,250 of the *HAMP* promoter are critical for basal hepcidin mRNA expression and hepcidin response to BMP-2 and BMP-6.[5] The former BMP-RE is proximal to a STAT-binding site important for hepcidin response to IL-6.[5] *HAMP* promoter mutations in areas outside BMP-REs or the STAT-binding site may also contribute to the development of IO. For example, 2 Italian patients with beta-thalassemia trait, hepatitis C, and IO were *HAMP* –nc.72C>T heterozygotes.[6] Nonetheless, the frequency of nc.-153C>T is too low in HEIRS Study participants to account for most TS/SF phenotype heterogeneity (regardless of *HFE* genotype or race/ethnicity). We conclude that routine testing to detect *HAMP* nc.-153C>T is not indicated in population-based hemochromatosis and IO screening programs in North America.

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HAMP promoter mutation nc.-153 C>T in 785 HEIRS Study participants: author reply

We thank Barton and collaborators who gave their attention to our recent publication in Haematologica[1] on the –nc.153 C>T mutation found at heterozygous state in the hepcidin promoter of a patient homozygous for the