Genetic Variant Coding for Iron Regulatory Protein HFE Contributes to Hypertension, the TAMRISK Study

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Abstract: Iron is essential for body homeostasis, but iron overload may lead to metabolic abnormalities and thus increase the risk for atherosclerosis and many other diseases. Major histocompatibility complex class I-like transmembrane protein (HFE) is involved in body iron metabolism. The gene coding for HFE has 3 well-known polymorphic sites of which H63D (c.1799945, C>G) has recently been associated with hypertension in a genome-wide association study (GWAS) study. In the present study, we wanted to clarify whether the genetic variant associates with hypertension in a Finnish cohort consisting of 50-year-old men and women.

The study included 399 hypertensive cases and 751 controls from the Tampere adult population cardiovascular risk study (TAMRISK) cohort. Genotyping of polymorphisms was done by polymerase chain reaction using DNAs extracted from buccal swabs.

We found that individuals with the mutated form of the H63D polymorphic site (G-allele) had a 1.4-fold risk (P = 0.037, 95% confidence interval [CI] 1.02–1.89) for hypertension at the age of 50 years compared with the CC genotype carriers. When obese subjects (body mass index > 30 kg/m²) were analyzed in their own group, the risk for hypertension was even stronger (odds ratio 4.15, P < 0.001, 95% CI 1.98–8.68). We also noticed that the blood pressure (BP) readings were higher in those with the minor G-allele when compared to ones having a normal genotype already at the age of 35 years. Means of systolic/diastolic BPs were 127/81 mm Hg for CC and 131/83 mm Hg for CG + GG groups (P < 0.001 for systolic and P = 0.005 for diastolic pressure).

In conclusion, HFE genetic variant H63D was associated with essential hypertension in Finnish subjects from the TAMRISK cohort confirming a previous GWAS study. The effect of this SNP on BP was also confirmed from a longitudinal study.

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Abbreviations: BMI = body mass index, CI = confidence interval, HFE = histocompatibility complex class I-like transmembrane protein, PHE = periodic health examination.

INTRODUCTION

Hypertension has a substantial impact on public health because of its high prevalence and associated complications. Efforts for the discovery of genes affecting hypertension have been conducted, but the vast majority of genetic contribution to blood pressure (BP) remains still unexplained. The major lifestyle contributors to hypertension in Western countries are overweight, physical inactivity, high salt intake, and low potassium intake. However, less attention has been allocated to iron metabolism and especially of the effects of mild iron overload on hypertension.

Iron is essential for mammalian homeostasis because of its presence in hemoglobin for oxygen transport, and in many biochemical reactions. Iron uptake occurs in duodenum and jejunum and it is mediated by transport proteins, such as ferroportin. Iron is stored in hepatocytes and macrophages within polymers of ferritin. Excess iron in the body leads to iron accumulation in the liver and other organs and may cause, among others, diabetes, liver cirrhosis, bone and joint disease, heart disease, and hepatocellular carcinoma.

HFE is a major histocompatibility complexity class I-like transmembrane protein, which associates with class I light-chain β2-microglobulin. HFE mediates cell uptake of transferrin-bound iron. HFE also modulates expression of the key regulator of plasma iron, hepcidin.

Two most common mutations in the HFE gene are C282Y (rs1800562) and H63D (rs1799945), of which the C282Y allele has been found as the major mutation behind significant iron overload. The carrier frequency of the H63D mutation leads to dysfunctional HFE protein, which could result in mild iron overload. The carrier frequency of the H63D mutation is 8.1% worldwide and 14% to 22% in European populations. There are only a few previous studies that have addressed the association of HFE-mediated dysfunctional iron regulation with hypertension. Ellervik et al showed that men homozygous for the C282Y allele had increased prevalence of antihypertensive medication >55 years of age. A recent genome-wide association study (GWAS) has given evidence that H63D mutation in the HFE gene is associated with BP.

In the present study, we wanted to study a possible association between H63D polymorphism and hypertension in the Finnish Tampere adult population cardiovascular risk study (TAMRISK) cohort.

METHODS

Participants

The TAMRISK is a prospective, longitudinal population-based health survey study in Tampere, a city in southern Finland with a population of 210,000. The Tampere city health care center has provided regular periodic health examinations (PHEs) for screening and counseling for the adult population of the city since 1980.
Table 1. Clinical Characteristics of Cases and Controls of the Study Population

| Clinical Characteristic                      | Cases (n = 399) | Controls (n = 751) | P Value |
|---------------------------------------------|----------------|-------------------|---------|
| Age, y                                      | 50.0 ± 0       | 50.0 ± 0          | <0.001  |
| Body mass index, kg/m²                      | 28.5 ± 5.1     | 25.4 ± 3.6        | <0.001  |
| Cholesterol, mmol/L                        | 5.4 ± 1.0      | 5.4 ± 0.9         | 0.9     |
| HDL cholesterol, mmol/L                     | 1.6 ± 0.4      | 1.7 ± 0.4         | <0.001  |
| LDL cholesterol, mmol/L                     | 3.2 ± 0.9      | 3.2 ± 0.8         | 0.6     |
| Triglycerides, mmol/L                       | 1.5 ± 1.2      | 1.2 ± 0.7         | <0.001  |
| Glucose, mmol/L                             | 5.2 ± 1.2      | 4.8 ± 0.6         | <0.001  |
| Systolic blood pressure, mm Hg              | 143.0 ± 16.2   | 129.8 ± 14.7      | <0.001  |
| Diastolic blood pressure, mm Hg             | 93.0 ± 8.9     | 84.4 ± 9.4        | <0.001  |
| Hypertension (%)                            | 100            | 0                 | <0.001  |
| Daily smokers (%)                           | 26.1           | 23.2              | 0.3     |
| Exercise (at least twice a week) (%)        | 66.2           | 71.9              | 0.06    |
| Diabetes (%)                                | 7.1            | 6.0               | <0.001  |
| Lipid-lowering drugs (%)                    | 15.1           | 3.5               | <0.001  |
| HFE allele distribution, rs1799945: C/G     | 0.84/0.16      | 0.87/0.13         | 0.025   |

HDL = high-density lipoprotein, HFE = histocompatibility complex class I-like transmembrane protein, LDL = low-density lipoprotein. Data is presented as mean ± standard deviation.
and 0.02 for GG (n = 8), whereas in the control group they were 0.76 for CC (n = 572), 0.22 for CG (n = 168), and 0.015 for GG (n = 11). Because of low number of GG gene variants, CG and GG were combined for further analysis.

To find the interpretative factors for hypertension, we used logistic regression analysis. When the association of H63D was defined alone, the risk for hypertension among G-allele carriers was 1.4-fold (P = 0.018, 95% confidence interval 1.06–1.82) compared with CC genotype carriers. When H63D variants, family history of hypertension, and BMI were included into logistic regression analysis as factors (forward conditional), adjusted odds ratios (ORs) for hypertension were 1.4 for H63D G-allele carriers, 3.6 for family history of hypertension, and 1.2 for BMI (model 1, Table 2).

The association of HFE gene variants with hypertension was further calculated separately for the participants who were overweight or obese (model 2; BMI > 25 kg/m², n = 663), and finally only for those who were obese (model 3; BMI > 30 kg/m², n = 269). In these subgroup analyses, the risk for hypertension at the age of 50 years was even stronger among G-allele carriers (Table 2).

Next we analyzed BP data from the ages of 35, 40, 45, and 50 years. Systolic and diastolic BPs at the age of 35 years were found to be significantly different between the 2 genetic variant groups. Means of systolic and diastolic pressures were 127/81 mm Hg for the CC group and 131/83 mm Hg for the CG + GG group (P < 0.001 for systolic and P = 0.005 for diastolic pressure).

The difference remained significant also at the age of 40 years for systolic BP (P = 0.027), but disappeared at the ages of 45 and 50 years (Figure 1). By analysis of repeated measures, there was no statistically significant interaction in the change of systolic/diastolic BP between the variants and the follow-up time (Figure 1).

**TABLE 2. Adjusted OR Results Obtained from Logistic Regression Models (Forward Conditional) for Hypertension**

| Model                     | n   | Factor                  | OR  | 95% CI       | P Value |
|---------------------------|-----|-------------------------|-----|--------------|---------|
| Model 1; (all participants) | 1150| BMI                     | 1.19| 1.15–1.23    | <0.001  |
|                           |     | Family history          | 3.60| 2.71–4.78    | <0.001  |
|                           |     | rs1799945 (G-allele)    | 1.39| 1.02–1.89    | 0.037   |
| Model 2; BMI > 25 kg/m²   | 663 | BMI                     | 1.23| 1.16–1.29    | <0.001  |
|                           |     | Family history          | 3.66| 2.58–5.22    | <0.001  |
|                           |     | rs1799945 (G-allele)    | 1.61| 1.10–2.34    | 0.013   |
| Model 3; BMI > 30 kg/m²   | 269 | BMI                     | 1.17| 1.06–1.29    | 0.002   |
|                           |     | Family history          | 2.91| 1.54–5.51    | 0.001   |
|                           |     | rs1799945 (G-allele)    | 4.15| 1.98–8.68    | <0.001  |

BMI = body mass index, CI = confidence interval, OR = odds ratio.

**DISCUSSION**

The present study suggests that mutation in the HFE gene is associated significantly with hypertension among a 50-year-old Finnish population. This finding is in line with a recent GWAS study. In addition, we also noticed that subjects with the mutated variant of the gene had higher BPs already at the age of 35 years. Previous studies of the HFE gene have concentrated mainly on hemochromatosis, but we wanted to have a different point of view into the effects of possible mild iron overload on the risk of hypertension.

Genes behind hypertension may cause significantly higher BP already at young age and lead to severe consequences. Hypertension is a significant risk factor for heart disease, stroke, and renal disease. In our study, both systolic and diastolic BPs were significantly higher already at the age of 35 years in the mutated HFE gene group (CG + GG), the difference being 4/2 mm Hg respectively as compared to normal gene carriers. At the ages of 45 and 50 years, no significant difference was found. A possible difference in BP after 45 might be difficult to establish,
since many of the subjects who had hypertension were already on BP medication. It has been shown that a prolonged increase even as small as 2 mm Hg in systolic BP is involved with 10% higher stroke mortality and 7% higher mortality for other vascular causes such as ischemic heart disease in middle age.16

It is known that obesity is a major risk factor to develop hypertension, which was also seen in our study. However, the effect of HFE H63D variant on hypertension was statistically significant even when BMI was taken into account in the analyses. In addition, we found that when overweight and obese subjects were analyzed in their own group, the effect of G-allele in predicting hypertension was even stronger compared with CC-genotype. The highest risk for developing hypertension was obtained among obese subjects (OR 4.2 for carriers of the H63D G-allele), which was greater than the corresponding OR of BMI. Although it is known that ferritin levels are enhanced in obese subjects,17 a mechanism by which obesity could modulate the effect of the HFE polymorphism is not known. However, an obesity-associated effect on hypertension has been shown for SELE 5544T18 and TMEM182,19 which are genes integrating genetic factors and obesity in the development of hypertension.

One possible explanation for higher BP of carriers of the H63D mutation is mild iron overload. Ferrous iron is absorbed into enterocytes in the duodenum and exported into circulation via ferroportin. In the circulation, iron is bound to transferrin.8 The mechanisms of how different H63D variants participate in the control of iron metabolism have not been fully explained. It is assumed that regulation occurs mainly via hepcidin, which is an iron-regulating hormone.7 Hepcidin levels increase due to rising body iron burden and mutations in the HFE gene block hepcidin acting as a feedback inhibitor of iron absorption. Therefore, HFE deficiency may lead to iron accumulation.3,5,20

The impact of H63D mutation on iron overload has been studied with controversial results.3–23 According to a large meta-analysis, homozygosity of the mutated form of H63D was associated with iron overload, but heterozygosity conferred no risk.24 Gochee et al21 suggested that H63D mutation is insufficient in itself to cause significant iron overload and that additional modifying factors are needed. Aguilar-Martinez et al25 searched for genetic modifiers affecting H63D mutation homozygotes, but found no link to potential genetic modifiers within the HFE or other genes. Blood tests for assessing body iron levels reflecting iron overload include serum ferritin and transferrin saturation.24 A limitation of the TAMRISK study population is the lack of these measurements of saturation. However, it has previously been published that men with essential hypertension had greater iron stores than normotensive controls.26 In addition, Cash et al27 observed that BPs were elevated in hemochromatosis patients when compared with controls, mean difference being 10 mm Hg for systolic and 7.9 mm Hg for diastolic BPs. This could suggest a relationship between iron overload and hypertension.

Excess iron in plasma produces reactive oxygen species, which are a possible explanation for organ damage in iron overload.28 Oxidative stress also induces cardiovascular and renal injury, and activates sympathetic nervous system leading to increase in BP. No sole evidence of oxidative stress in the pathogenesis of hypertension exists in humans, but animal studies suggest a causative role.29 In the study by Liu et al30 mutated H63D protein was associated with prolonged plasmatic stress and elevated iron levels in a transgenic mouse model.

Although the mechanism is not yet known, our results suggests that the minor G-allele of the H63D variant (rs1799945) is associated with higher risk for hypertension at the age of 50 years, compared with the CC-genotype carriers. In addition, we found that individuals with the G-allele had also higher BP already at the age of 35 years. Although the difference in systolic and diastolic BP between different genotype carriers was small, the long-term consequences may be remarkable. Further studies should include measurements of plasmatic iron to prove without doubt the relationship between the presence of the allele, mild iron overload, and hypertension. If this mechanism is through iron overload, subjects with mutated H63D should possibly restrict their supply of iron.

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