

# Genetic variation in bone morphogenetic proteins family members (BMPs 2 and 4) and hypertension risk in middle-aged men

## The TAMRISK study

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### Abstract

Bone morphogenetic proteins (BMPs) are important regulators of iron metabolism affecting hepcidin expression. We have previously shown that 2 genetic polymorphisms in different genes (histocompatibility complex class I-like transmembrane protein, hemojuvelin) involved in the regulation of hepcidin expression pathways are associated with hypertension. In this study, we analyzed genetic variation sites in BMP2 (rs235756, rs235768) and BMP4 (rs4901474) to get more evidence linking iron metabolism to hypertension risk in the Finnish population.

The study included 321 hypertensive cases and 463 controls from the Tampere Adult Population Cardiovascular Risk study cohort. Genotyping of polymorphisms was done by polymerase chain reaction using DNAs extracted from buccal swabs.

We found that men carrying the GG genotype of BMP2 rs235756 (A>G) polymorphic site had a 4.09-fold risk (confidence interval [CI] 1.61–10.39,  $P = .003$ ) for hypertension at the age of 50 years compared with A-allele carriers. The risk was significant in the age groups of 45 and 40 years as well. In addition, the 15-year follow-up period of the same individuals showed that carriers of the GG-genotype had also significantly higher readings of both systolic ( $P < .001$ ) and diastolic ( $P = .01$ ) blood pressure during the follow-up time. No significant association between BMP2 rs235768 (A>T) and hypertension was found. BMP4 polymorphic site rs4901474 (T>C) also had an effect on hypertension. CC genotype carriers had a 1.48-fold risk (CI 1.03–2.13,  $P = .033$ ) for hypertension at the age of 50 years when compared with T-allele carriers.

In conclusion, BMP2 polymorphic site rs235756 was associated with hypertension in Finnish men. An effect of BMP4 polymorphic site on hypertension was also found.

**Abbreviations:** ANG = angiotensin, ANOVA = analysis of variance, BMI = body mass index, BMP = bone morphogenetic protein, BP = blood pressure, CAD = coronary artery disease, CI = confidence interval, HFE = histocompatibility complex class I-like transmembrane protein, HH = hemochromatosis, HJV = hemojuvelin, OR = odds ratio, PCR = polymerase chain reaction, PHE = periodic health examination, SNP = single-nucleotide polymorphism, TAMRISK = Tampere Adult Population Cardiovascular Risk study.

**Keywords:** BMP, genetic variants, hypertension, iron

## 1. Introduction

Hypertension is a substantial risk factor for cardiovascular diseases, including coronary artery disease (CAD), stroke, left ventricular hypertrophy, congestive heart failure, peripheral vascular disease, renal failure, and aortic aneurysms.<sup>[1]</sup> In consideration of the high prevalence of hypertension, it has also

a huge impact on public health. A better understanding of genetic factors of hypertension will be helpful in the prevention and curing of the disease.

Bone morphogenetic proteins (BMPs) are signaling molecules belonging to transforming growth factor-beta superfamily. Apart from many other functions, they have a key role in iron homeostasis. Affecting the expression of hepcidin, the regulator hormone of iron homeostasis, mutations/genetic variation in BMPs are believed to lead to iron-deficiency and iron-overload syndromes.<sup>[2]</sup> The common single-nucleotide polymorphism (SNP) variants in the BMP genes are rs235756 and rs235768 (BMP2) and rs490141 (BMP4).

We have previously shown the association between the polymorphism of 2 different iron regulator proteins—major histocompatibility complex class I-like transmembrane protein (HFE) and hemojuvelin (HJV) genes and hypertension.<sup>[3,4]</sup> However, the association between BMP2 and BMP4, and hypertension has not been studied earlier. Instead, Milet et al<sup>[5]</sup> tested association of BMP2 polymorphism with serum ferritin levels with conflicting results in 2 different cohort sample of C282Y homozygote hemochromatosis (HH) patients. Recently, Ji et al<sup>[6]</sup> found an association with plasma ferritin levels and rs235756 in the BMP2 gene when they studied Australian blood donors. Association was significant, especially in male donors.

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The aim of this study was to evaluate whether polymorphic sites in BMP2 and BMP4 genes are associated with hypertension in the Finnish (Tampere Adult Population Cardiovascular Risk study [TAMRISK]) cohort.

## 2. Methods

### 2.1. Participants

The TAMRISK is a prospective, longitudinal population-based health survey study in Tampere, a city in southern Finland, with a population of 230,000. The data for the TAMRISK was collected from the periodic health examinations (PHEs) done for 50-year-old men and women living in Tampere. The PHE included one 60-minute session with a public health nurse at the center's health examination unit as previously described.<sup>[3,7]</sup> TAMRISK data include information of risk factors for hypertension: blood pressure (BP), weight, lipid values and smoking, alcohol consumption, diabetes, and family history of cardiovascular diseases. Current and previous diseases were identified based on self-report of diagnosis by a physician, including hypertension. Cases in this study were the subjects who had hypertension and/or CAD at the age of 50 years as diagnosed by a physician by normal healthcare procedures. For most patients, physicians diagnose hypertension when BP readings are consistently 140/90 mm Hg or above. For each case, at least 1 normotensive control with the same sex and similar smoking habits were chosen from a PHE cohort (n=6000). Smoking status was evaluated based on self-reporting. Alcohol use was documented by individual interviews using a structured questionnaire. Weekly alcohol consumption was assessed distinguishing the amounts of beer, wine, and liquor consumed, and was then calculated as unit/wk. One unit corresponds to 12 g of ethanol.

Buccal swabs for DNA extraction and a permission form to use PHE data were collected by mail separately of the physical examination during years 2006 to 2010. Informed consent was obtained from all participants. The Ethics Committees of the Tampere University Hospital and the City of Tampere approved the study.

### 2.2. DNA genotyping

DNA was extracted from buccal swabs using a commercial kit (Qiagen Inc., Valencia, CA). Polymerase chain reaction (PCR) was performed in a final volume of 5  $\mu$ L containing 10 ng of sample DNA, 0.05  $\mu$ L of custom SNP-specific Assay mix, and 2.18  $\mu$ L of Taqman Universal PCR Master Mix. The Assay mixes used were C\_\_2513516\_10 (rs235756), C\_\_2244893\_10 (rs235768), and C\_\_7844917\_10 (rs4901417). Amplification proceeded for 40 cycles of 15 seconds at 95°C and 60 seconds at 60°C. Genotyping followed the Applied Biosystems (Pleasanton, CA) protocol. Automatic genotype call was performed after PCR, by scanning plates on the 7900 HT Fast Real-Time PCR, which provides the SDS2.3 software (Applied Biosystems).

### 2.3. Statistical analysis

Hardy–Weinberg equilibrium of the genotypes was calculated using online encyclopedia calculator for genetic epidemiology studies.<sup>[8]</sup> T test and 1-way analysis of variance (ANOVA) for continuous variables (clinical characteristics), and chi-square test for categorical variables (hypertension, CAD) were applied for the comparison of BMP genotype groups. Associations of the genotyped BMP2 and BMP4 gene variants with hypertension/coronary artery disease with risk factors (BMI, sex, smoking

[years], and alcohol consumption [unit/wk]) were analyzed using logistic regression analysis. The ANOVA was used to assess the differences in mean BPs between genotypes at the age of 35, 40, 45, and 50 years. The model included the main effects of group factor and time, and their interaction. Statistical analyses were assessed using IBM SPSS Statistics 23, and the statistical significance was set to 0.05.

## 3. Results

Clinical characteristics of the middle-aged (50  $\pm$  0 years) study participants are listed in Table 1. Briefly, case group comprised 321 hypertensive cases and control group 463 normotensive subjects with the same sex distribution and similar smoking habits. A total of 22 subjects were found to have CAD at the age of 50 years.

Genotyping was successful in 756 subjects for the BMP2 rs235756 (A>G), in 784 subjects for the rs235768 (A>T), and in 766 subjects for the BMP4 rs4901474 (T>C). In the study population, genotype distribution of the rs235756 in the BMP2 gene was 37% AA, 48% AG, and 15% GG, and of the rs235768 it was 33% TT, 48% TA, and 19% AA. Genotype distribution for BMP4 rs4901474 was 31% TT, 50% TC, and 19% CC. The measured genotype frequencies were not significantly different from the expectations of Hardy–Weinberg equilibrium ( $\chi^2=0.613$  for rs235756,  $\chi^2=0.432$  for rs235768, and  $\chi^2=0.204$  for rs4901474).

In the whole study population, at the age of 50 years, the BMP2 SNP rs235756 (A>G) associated significantly with hypertension ( $P=.035$ ). More detailed analysis showed that individuals carrying the GG genotype had more often hypertension (47%) compared with the AG or AA genotype carriers (36.9% and 45.1%, respectively). Statistically significant difference was found only between GG and AG genotype carriers ( $P=.047$ ), and when A-allele carriers were combined, only a trend for hypertension risk was found ( $P=.152$ ). When men and women were analyzed separately, we found that the risk for hypertension was significantly higher only among men ( $P=.035$  for genotype effect and  $P=.016$  for GG vs A-allele). In the latter analysis,

**Table 1**  
Clinical characteristics of cases and controls of the study population.

Clinical characteristics	Cases (n=321)	Controls (n=463)	P
Age, y	50 $\pm$ 0	50 $\pm$ 0	
BMI, kg/m <sup>2</sup>	28.4 $\pm$ 5.0	25.2 $\pm$ 3.5	<.001
Hemoglobin	146.4 $\pm$ 13.2	143.8 $\pm$ 12.8	.020
Cholesterol, mmol/L	5.39 $\pm$ 0.95	5.40 $\pm$ 0.88	.769
Triglycerides, mmol/L	1.49 $\pm$ 1.15	1.17 $\pm$ 0.69	<.001
HDL cholesterol, mmol/L	1.58 $\pm$ 0.45	1.69 $\pm$ 0.44	.002
LDL cholesterol, mmol/L	3.17 $\pm$ 0.88	3.19 $\pm$ 0.82	.710
Glucose, mmol/L	5.21 $\pm$ 1.44	4.83 $\pm$ 0.53	<.001
Systolic blood pressure, mm Hg	143.0 $\pm$ 16.8	129.0 $\pm$ 15.3	<.001
Diastolic blood pressure, mm Hg	92.7 $\pm$ 9.0	84.3 $\pm$ 9.6	<.001
Hypertension, %	100	0	<.001
Diabetes, %	13.4	0	<.001
Daily smokers, %	27.1	22.7	.154
Alcohol consumption, unit/wk	6.8	5.3	.007
Lipid-lowering drugs, %	14.7	3.2	<.001
Family history of hypertension, %	73.5	41.1	<.001
Sex (male), %	56.1	53.1	.416

Data are presented as mean  $\pm$  standard deviation.

BMI=body mass index, HDL=high-density lipoprotein, LDL=low-density lipoprotein.

55.9% of males carrying the GG genotype had hypertension compared with 37.8% of A-allele carriers. We also found a higher susceptibility to hypertension incidence already when the same men were 10 years younger. At the age of 40 years, 19% of the GG genotype carriers had hypertension compared with 8.1% of the A-allele carriers ( $P=.019$ , for genotype effect  $P=.006$ ). Multivariate logistic regression with A-allele as reference (adjusted with BMI, smoking history [years] and alcohol consumption [unit/wk]) showed that at the age of 50 years, the risk for hypertension was 4.09-fold in GG-genotype carriers (confidence interval [CI] 1.61–10.39,  $P=.003$ ), at the age of 45 years it was 3.14 (CI 1.03–9.55,  $P=.043$ ), and at the age of 40 years it was 2.55 (CI 1.13–5.72,  $P=.024$ ). As expected, no statistically significant association was found between genotypes and hypertension at the age of 35 years due to small amount of hypertension cases. In women, no statistically significant association with hypertension at any age group was found.

In addition, 15-year follow-up period (35, 40, 45, and 50 years) of the same individuals showed that carriers of the GG genotype had also significantly higher readings of both systolic ( $P<.001$ ) and diastolic ( $P=.01$ ) BP during the follow-up time (Table 2, Fig. 1). Finally, we also analyzed association of this polymorphism with diagnosed CAD in men. Although there were only 22 men who had CAD at the age of 50 years, those carrying the GG genotype tended to have more often CAD than A-allele carriers (11.5% vs 4.1%, respectively;  $P=.027$ ).

For the other polymorphic site of the BMP2 gene (rs235768), no statistically significant association with hypertension or CAD was found (data not shown).

No significant association between BMP4 rs4901474 (T>C) variants and hypertension was found ( $P=.092$ ) in the whole study population. However, after combining the T-allele carriers, we found that 52.1% of the rare genotype CC carriers had hypertension at the age of 50 years compared with 42.3% of the T-allele carriers (odds ratio [OR] 1.48, CI 1.03–2.13,  $P=.033$ ). When BMI, smoking history (years), and alcohol consumption (unit/wk) were included into analyses, risk for hypertension among CC carriers was even higher (OR 2.00, CI 1.21–3.32,  $P=.007$ ). At the age of 45 years, risk for hypertension was still significant (OR 1.98, CI 1.02–3.82,  $P=.043$ ), but disappeared at the age of 40 and 35 years.

No statistically significant association with CAD was found for BMP4 gene rs4901474.

#### 4. Discussion

In the present study, we found a significant association between hypertension and genetic variation of the BMP2 gene (rs235756). Furthermore, a slight effect of BMP4 polymorphic site (rs4901474) on hypertension was found. The risk for hypertension was significantly higher within men in age groups of 40, 45, and 50 years for GG genotype of SNP rs235756. Both systolic and diastolic BPs were also significantly higher among GG genotype carriers already at the age of 35.

Bone morphogenetic proteins have earlier been found to have role in human development and 2 vascular disorders—pulmonary arterial hypertension and hereditary hemorrhagic telangiectasia.<sup>[9]</sup> Moreover, a number of BMPs are up-regulated at sites of vascular injury and atherosclerotic plaques, suggesting that BMPs could play a role in regulating normal vascular homeostasis and disease-associated vascular pathology. In addition, different studies have revealed major role for the BMP family of ligands and receptors in iron homeostasis. BMP signaling in vascular development and cardiovascular diseases have recently been excellently reviewed by Morrel et al,<sup>[10]</sup> and Lowery and deCaestecker.<sup>[11]</sup>

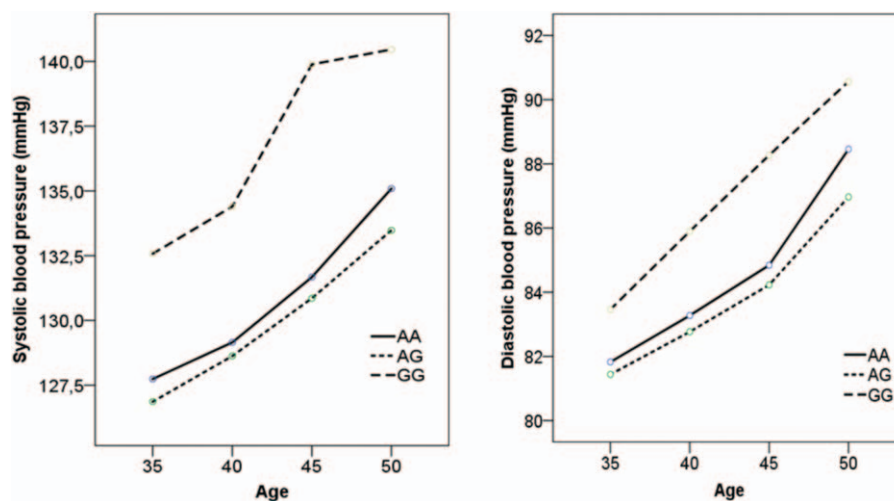
Bone morphogenetic proteins are also involved in iron homeostasis regulation, and in liver, they act as coreceptors of HJV. Activation of the HJV-BMP signaling pathway leads to transcription of hepcidin, which is the major known regulator of systemic iron homeostasis. By reducing dietary iron uptake and release from macrophages, hepatocytes, and other cell types, it affects the amount of systemic iron.<sup>[12]</sup> In 2003, hepcidin deficiency was revealed as a cause of iron overload. HH patients with HJV mutations also had low levels of serum hepcidin<sup>[13]</sup> and HH patients with HFE mutations had lower expression of hepcidin in the liver compared with controls.<sup>[14]</sup> In addition of HJV-BMP pathway, there are other pathways affecting hepcidin expression as well. We have previously found an association between hypertension and 2 iron metabolism regulator proteins, HFE and HJV.<sup>[3,4]</sup> The present study is consistent with the earlier findings.

**Table 2**

**Means of systolic and diastolic blood pressures at different ages according genetic variants.**

	Syst	Diast	Syst	Diast	Syst	Diast	Syst	Diast
<b>rs235756</b>	<b>AA</b>		<b>AG</b>		<b>GG</b>		<b>P</b>	
Age 50	135.1 ± 17.5	88.4 ± 10.0	133.5 ± 16.2	86.8 ± 10.0	139.1 ± 18.8	89.8 ± 10.2	.009	.011
Age 45	132.5 ± 13.6	85.3 ± 9.2	131.3 ± 14.7	84.2 ± 9.5	137.4 ± 20.0	86.5 ± 10.6	.002	.084
Age 40	128.9 ± 13.1	83.1 ± 9.9	129.3 ± 12.4	82.9 ± 9.6	132.8 ± 15.0	85.5 ± 10.9	.037	.063
Age 35	127.7 ± 12.6	81.5 ± 10.1	127.5 ± 12.8	81.7 ± 9.3	132.9 ± 13.9	83.7 ± 10.3	.002	.191
<b>rs235768</b>	<b>AA</b>		<b>AT</b>		<b>TT</b>		<b>P</b>	
Age 50	134.6 ± 18.1	87.9 ± 10.9	134.4 ± 16.8	87.5 ± 9.7	135.5 ± 17.3	88.0 ± 10.5	.696	.747
Age 45	132.7 ± 16.4	84.7 ± 10.4	133.0 ± 14.9	85.2 ± 9.3	131.7 ± 14.8	84.4 ± 9.5	.587	.647
Age 40	129.8 ± 13.6	83.5 ± 10.9	129.7 ± 13.0	83.4 ± 10.0	129.1 ± 12.5	82.9 ± 9.1	.804	.786
Age 35	127.9 ± 12.7	80.7 ± 10.5	128.3 ± 12.8	82.4 ± 9.6	128.8 ± 13.5	81.9 ± 9.3	.829	.275
<b>rs4901474</b>	<b>TT</b>		<b>TC</b>		<b>CC</b>		<b>P</b>	
Age 50	134.4 ± 16.3	87.6 ± 9.8	135.2 ± 17.1	87.8 ± 9.8	136.5 ± 16.3	89.2 ± 9.6	.481	.260
Age 45	133.1 ± 15.0	84.7 ± 9.3	132.7 ± 14.5	85.4 ± 9.3	131.9 ± 13.4	86.1 ± 9.5	.756	.415
Age 40	131.1 ± 13.3	84.1 ± 9.1	130.5 ± 12.5	83.7 ± 9.6	131.0 ± 13.4	84.5 ± 13.0	.872	.728
Age 35	129.1 ± 13.6	82.0 ± 8.5	128.8 ± 12.6	82.1 ± 9.5	129.7 ± 12.9	84.0 ± 10.0	.839	.181

Data are presented as mean ± standard deviation.



**Figure 1.** Differences in mean systolic ( $P < .001$ ) and diastolic ( $P = .01$ ) blood pressure between BMP rs235756 genotypes (A>G) during 15 years of follow-up in men.  $P$  values are from repeated-measures analysis.

Association with BMP2 and BMP4, and hypertension has not been studied earlier. Instead, the straight effects of BMPs on iron metabolism have been under interest. Mutations of BMPs are believed to lead to iron deficiency or overload syndromes.<sup>[2]</sup> Milet et al. found that the polymorphic site of BMP2 (rs235756) is associated with higher serum ferritin levels in HFE p.C282Y homozygous patients,<sup>[5]</sup> but they were not able to replicate the results in a later study.<sup>[15]</sup> They found that the highest serum ferritin levels were among TT (AA) genotypes, whereas our findings suggest that the highest BPs were among GG genotype carriers. Therefore, a link with high ferritin and BP may not be speculated in this context. However, since their studies included only p.C282Y homozygous patients, the results of the studies cannot be fully compared with our present study. In Australian blood donors, BMP2 gene variants were also found to associate with ferritin levels, especially in male donors, but it was not possible to determine the significance of homozygosity and heterozygosity of the variant allele due to insufficient statistical power.<sup>[6]</sup>

Our recent finding suggests the possible role of BMPs in essential hypertension, which may be linked with iron metabolism. Male patients with essential hypertension have been found to have increased serum ferritin levels when compared with normotensive individuals, and a strong association between hepcidin levels and serum ferritin has been observed.<sup>[16]</sup> This underlines the possible effect of altered iron metabolism on hypertension, although other BMP-mediated mechanisms cannot be excluded.

A limitation of the study population is the lack of information about diet, which has an important role in iron metabolism. However, since the average daily iron intake for Finnish people is near the national recommendations<sup>[17]</sup> and our study population is quite large, we believe that there are no significant differences for daily dietary iron among the study population. However, it is known that different genetic polymorphisms affect iron absorption and iron levels in the body. In Finland, markers of body iron stores, serum ferritin, and transferrin saturation are not routinely measured, and therefore a limitation of the TAMRISK study population is the lack of these saturation markers.

The mechanism between iron metabolism and hypertension are currently under study. Although the field of iron metabolism

regulation is far from being understood, hepcidin has been assumed as being the primordial regulator of iron homeostasis. Therefore, our focus is now to investigate the possible role of hepcidin in regulation of BP.<sup>[3,4]</sup> Possible connection of the rennin-angiotensin system and iron metabolism is supported by the observations that angiotensin (ANG) II administration caused 4.7-fold increase in the amount of hepcidin mRNA concentration in the rat kidney, and that this increase could be suppressed by the concomitant administration of losartan.<sup>[18]</sup> An opposite effect was found in the hepatic hepcidin mRNA expression and serum hepcidin concentration in ANG II-induced hypertensive mice.<sup>[19]</sup> However, possible hepcidin-renin-angiotensin system in hypertension warrants further study.

## 5. Conclusions

In conclusion, there was a significant association in men between BMP2 genetic variant (rs235756) and hypertension in the genetically homogeneous Finnish population. Those who carried the rare GG genotype of the gene had higher risk for hypertension already at the age of 40 years. There was also an association between BMP4 (rs4901417) genetic variants and hypertension. Our results suggest that genetic variation in iron metabolism-related genes participating in hepcidin expression may have an effect on hypertension.

The area of investigation is important, as there might be some clinical consequences for the treatment of essential hypertension as well. For example, the iron intake of hypertensive patients would be needed to be assessed more thoroughly to achieve optimal iron balance of these patients. In addition, there might be a chance for some pharmacological applications as well. Still, more investigation will be needed to confirm the connection between iron metabolism and essential hypertension.

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