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Research Article

A *HMGCR* polymorphism is associated with relations between blood pressure and urinary sodium and potassium ratio in the Epic-Norfolk Study

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Abstract

A polymorphism in the *HMGCR* gene (rs17238540) was related to a lower response to pravastatin treatment and we aimed to investigate whether an interaction is present for this polymorphism on blood pressure (BP) and salt intake. Cross-sectional urinary sodium and potassium concentration and the polymorphism were assessed in a large population study. Participants with the mutated allele (G) had significantly higher BP than homozygous TT. There were highly significant positive trends between BP and urinary sodium:potassium ratio across quartiles in men, with less effect in women, especially women carrying the mutated allele, G. Multivariate regression showed a significant positive association between BP and the urinary sodium: potassium ratio that differed in men and women according to genotype. In men carrying the G allele, the regression slopes for diastolic BP and systolic BP were higher than in men TT and the opposite was observed in women. Our results suggest that the SNP rs17238540 in the *HMGCR* is associated with the BP response to urinary sodium: potassium ratio, the magnitude of the association differing according to possession of the G allele. J Am Soc Hypertens 2009;3(4):238–244.

Keywords: 3-Hydroxy-3-Methylglutaryl-coenzyme A reductase; genetic polymorphism; systolic blood pressure; diastolic blood pressure.

Introduction

High blood pressure (BP) is a condition related to higher risk for stroke and myocardial infarction that affects people worldwide. It has been estimated that more than a quarter of the world's adult population (nearly one billion) had hypertension in the year 2000.[1]

Hypertension and hyperlipidemia are conditions that synergistically contribute to cardiovascular risk and the management of both is very often a clinical aim.[2] Clinical management of both conditions includes, besides changes in life style (diet and physical activity), the use of statins (such as simvastatin, pravastatin, and atorvastatin). Statins are allosteric inhibitors of the 3-Hydroxy-3-Methylglutaryl-coenzyme A reductase, an enzyme that participates in a limiting step in the endogenous cholesterol synthesis. They lower serum total cholesterol and low-density lipoprotein (LDL), effectively reducing the cardiovascular risk.[3] Several polymorphisms have been identified in

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the 3-Hydroxy-3-Methylglutaryl-coenzyme A reductase gene (*HMGCR*) *locus*[4–7] and two tightly linked single-nucleotide polymorphisms (SNP) (SNP12 and SNP29, rs17238540) were found to be significantly associated with a difference in the change in the serum lipids response to pravastatin treatment.[7]

In addition to effects on blood cholesterol and LDL levels, some trials have shown an effect of statins in lowering BP. Such effect has been shown to be cholesterol lowering-independent and the mechanisms are not completely clear. HMGCR inhibition by statins has been shown downregulates the angiotensin II type 1 receptor (AT1 receptor) expression and the effect is reversed by mevalonate and also by geranylgeranyl-PP, products of HMGCR activity.[8–10] However, although there are known effects of genetic variation on blood lipids, no study has examined the effect of polymorphisms of *HMGCR* on BP.[11–13] Angiotensin II modulates most of the biological effects of the renin-angiotensin system (RAS) via stimulation of the AT1 receptor. The RAS plays a pivotal role in the regulation of sodium excretion and balance is sensitive to alterations in sodium intake.[14–17] Variants on the angiotensinogen gene (precursor of angiotensin II) were shown to affect the sensitivity of BP to salt intake.[18–20]

Considering the effect of the rs17238540 SNP on the statin response and the effect of the HMGCR inhibition on AT1 receptor, the aim of the present study was to examine whether the referred polymorphism of the *HMGCR* gene is related to BP; and, following our and other previous findings of an interaction with variants of the angiotensinogen gene,[18–20] to investigate whether such an interaction was also present for this polymorphism on BP and salt intake. As salt intake cannot be accurately assessed from food record[20,21] urinary electrolytes were used as biomarkers of salt consumption in a large population study, the European Prospective Investigation into Cancer in Norfolk (EPIC—Norfolk) Cohort Study.

Methods

Study Protocol

EPIC—Norfolk is a prospective population study of men and women recruited at age 45 to 75 years from general practice age-gender register in Norfolk, United Kingdom from 1993 to 1997. Approximately 25,000 people participating in the baseline survey, who had filled a detailed health and life style questionnaire, attended a first health check when blood and urine samples, and data on height, weight, waist circumference, and BP were collected by trained nurses.[21] BP was measured using an Accutorr noninvasive oscillometric BP monitor (Datascope Medical, Huntingdon, United Kingdom) after the participant had been seated for 5 minutes, by trained nurses. The mean of two readings was used for analysis. Body mass index (BMI) was estimated as weight in kilograms/(height in meters)[2].

A casual urine specimen was requested from each participant. They were frozen without preservative at −20°C. In 1998–2002, the urine samples were thawed and assayed for sodium, potassium, and creatinine concentrations (mmol/L). Urinary sodium:potassium ratio was calculated.

Medical history was ascertained with the question, ''Has your doctor ever told you that you have any of the following?,'' which was followed by a list of conditions including ''high BP (hypertension) requiring treatment with drugs'' and ''high lipid levels requiring treatment with drugs.'' Habitual physical activity assessed both work and leisure time activity during the past year, and individuals were allocated to 4 ordered categories of overall activity.[22] The EPIC—Norfolk Study was approved by the Norfolk Health District Ethics Committee.

HMGCR Genotype Determination

The rs17238540 SNP in *HMGCR* was previously found to be significantly associated with a difference in the serum lipids response to pravastatin treatment, but no study has investigated the effects of the SNP on BP or on the response to salt (sodium) intake.[7]

Deoxyribonucleic acid (DNA) for genotyping was extracted from blood samples collected in ethylenediamine tetraacetic acid (EDTA) or from stored red blood cell samples and buffy coats with a phenol: chloroform procedure after digestion with Proteinase K. *HMGCR* SNP (rs17238540) genotype was assessed using Pyrosequencing. Forward biotin labeled (5' biotin - GCAAGCCTGT TTGCAGGTAT) and reverse (5' - TCAGCCTAAT CCATTGTGTCC) primers were designed using Primer3 to generate an amplicon of 162 bp, flanking the polymorphic region of the SNP in the *HMGCR* gene identified in a previous study.[7] The polymerase chain reaction (PCR) reaction tube (12.5 μL) contained 10 ng of DNA, 1x PCR buffer, 2 mmol/L $MgCl_2$, 0.125 mmol/L of each deoxynucleoside triphosphate (dNTP), 10 pmol of each primer, and 2 units of *AmpliTaq* Gold (Applied Biosystems, Inc, Branchburg, NJ). The annealing temperature was set at 56°C at 44 cycles on the Thermal Cycler (PTC-225; MJ Research, Inc, Watertown, MA). The PCR product was visualized and the size verified on 2% agarose gels. The detailed Pyrosequencing sample preparation procedure has been described elsewhere.[23–25] For technical reasons, the reverse strand was assayed. The Pyrosequencing machine (Pyrosequencing AB, Uppsala, Sweden) was prepared as recommended by the manufacturer and the samples were loaded into the machine. The dispensation order for the machine was: TAACACGAGTG. The genetic

analyses were repeated in separated experiments for a total of 1,322 samples out of 23,011 successfully genotyped to check the reproducibility of the method, and these analyses were 99.9% concordant.

Statistical Analysis

Characteristics of people in the different categories were compared between 21,900 participants for whom complete data were available. Differences in means were tested using analysis of variance. Differences in the frequency of the categorical variables as well as the difference between the observed and the expected genotype frequency distributions were examined using the χ^2 test. The statistical analysis for BP, sodium:potassium ratio and genetic data were conducted in approximately 17,674 participants after excluding those in use of antihypertensive and lipid lowering drugs. We compared means of systolic blood pressure (SBP) and diastolic blood pressure (DBP) between people in different quartiles of urinary sodium:potassium ratio after stratifying the participants by genotype, adjusting by gender, BMI, and age for the whole population. The same comparison was also done separately for men and women. In this case, the analysis were not adjusted by gender and gender specific quartiles of urinary sodium:potassium ratio were used. Regressions between BP and urinary sodium:potassium ratio were adjusted as described above and were done for the whole cohort then stratified by gender and genotype. Regression coefficients (β) and standard error were normalized to show the change in mm Hg of SBP and DBP for every standard deviation (SD) change in the urinary sodium:potassium ratio. The results were expressed as two-tailed test for significance (P value) and the 95% confidence intervals (CI). We also compared the slopes of the regression of BP on urinary sodium:potassium ratio for the different genotype groups. All data were analyzed using SPSS for Windows, version 16.0 (SPSS Inc, Chicago, IL).

Results

Baseline characteristics of the studied sample separated according to the *HMGCR* SNP genotype are presented in Table 1. Genotype frequencies and alleles distributions for 23,011 participants for whom the genetic data were available were: TT 95.65%, TG 4.29%, and GG 0.06% (Table 1); T 97.8% and G 2.2%, respectively. The genotype frequencies were in Hardy—Weinberg equilibrium ($\chi^2 = .068$) and did not differ between men and women ($P = .77$). Table 1 shows that participants with the mutated allele (TG + GG genotypes) had significantly higher SBP than those homozygous for the wild allele, T. For SBP, the difference remained significant whether or not the G variants were considered as homozygous alone or combined with heterozygous.

Table 2 shows that there were highly significant positive trends between BP and urinary sodium:potassium ratio across quartiles in men, with less effect in women with the TG + GG genotype in whom associations with SBP and DBP were marginally significant or not significant. It can be noticed that in the highest quartiles of urinary sodium:potassium ratio the difference in the mean BP between the genotypes groups is smaller in women than in men.

In the multivariate regression analysis, men and women showed a significant positive association between SBP and DBP and the urinary sodium:potassium ratio (Table 3), although the regression slope of SBP on urinary sodium:potassium ratio was significantly higher for women than for men overall ($Z = 2.44$; $P = .015$). Despite this, Table 3 shows that there were different effects according to genotype in men and women. In men carrying the G allele, the regression coefficient was approximately double that of TT men for each unit increase in SBP by each SD increase in the urinary sodium:potassium ratio ($Z = 2.51$; $P = .012$, for difference between the slopes). In women carrying the G allele, the association between SBP and DBP and the urinary sodium:potassium ratio was not significant and the magnitude of the regression slope was lower than in TT women.

Discussion

In this, the first major study of *HMGCR* variants in relation to BP, we found a significant different effect of genotype on SBP. Individuals carrying the mutated G allele had a 1.4 mm Hg higher SBP ($P = .02$) and 0.8 mm Hg higher DBP ($P = .03$) than those who were TT. This difference brought about by individual heritability might account for the fact that some but not all clinical trials have found effects of statins on BP.[2,26–30] Besides the effect observed on BP, we have shown here that the effects of salt intake on BP differs according to *HMGCR* genotype between men and women, which might also contribute to lack of consistency in findings of trials often with small numbers of subjects not allowing analysis by gender.

The regression analysis showed a higher responsiveness of the BP to the urinary sodium:potassium ratio in women than in men. This difference can be credited to a difference in the regulatory mechanisms of the BP related to gender. It is well established that while men and women possess the same structural elements of the cardiovascular system, the way that those components function to achieve homeostasis and to respond to the stress differs widely.[31–33] Measurement error was unlikely to have accounted for these effects as BP was collected in a standardized manner, and urine samples are objective measures of sodium intake.[21,34]

Although statins treatment has been widely studied for its ability to alter, besides blood lipids, other mechanisms

Table 1
Baseline clinical, anthropometric and biochemical variables, and *HMGCR* genotype distribution

	TT		TG		GG			
Variables	n	Mean ± SD	n	Mean ± SD	n	Mean ± SD	P^a	P^b
Age (years)	22,010	58.7 ± 9.3	989	59.3 ± 9.2	12	58.6 ± 6.8	.09	.27
BMI (kg/m^2)	21,395	26.3 ± 3.9	965	26.4 ± 4.0	12	26.7 ± 3.8	.88	.67
Waist circumference (cm)	21,412	88.0 ± 12.4	965	88.3 ± 12.3	12	89.1 ± 13.7	.64	.36
SBP (mm Hg)	21,389	135.3 ± 18.4	962	136.7 ± 18.5	12	137.9 ± 10.7	.05	.02
DBP (mm Hg)	21,389	82.4 ± 11.2	962	83.2 ± 11.4	12	81.8 ± 10.5	.08	.03
Urinary sodium (mmol/L)	21,034	82.7 ± 46.9	940	81.1 ± 46.9	12	99.7 ± 52.8	.26	.36
Urinary potassium (mmol/L)	21,034	55.0 ± 32.9	940	53.6 ± 32.6	12	74.2 ± 45.5	.05	.28
Urinary sodium:potassium ratio	21,034	1.8 ± 1.2	940	1.8 ± 1.1	12	1.5 ± 0.5	.59	.97
	n	(%)	n	(%)	n	(%)		
All	22,010	95.65	989	4.29	12	0.06	.77[c]	.77[c]
Men	9,512	95.70	424	4.26	4	0.04		
Women	12,498	95.62	565	4.32	8	0.06		
Current smokers	2,514	11.5	125	12.7	1	8.3	.43[d]	.17[d]
Lipids lowering drugs users	328	1.5	11	1.1	1	8.3	.09[d]	.46[d]
Antihypertension drugs users	3,995	18.2	190	19.2	2	16.7	.69[d]	.41[d]

BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; SD, standard deviation.
[a] *P* value for one-way analysis of variance (ANOVA) tests between genotypes groups: TT, TG, and GG.
[b] *P* value for one-way ANOVA tests between genotypes groups: TT and TG + GG.
[c] *P* value for Pearson chi-square test for differences in the genotype distribution between men and women.
[d] *P* value for Pearson chi-square tests for differences between genotypes groups.

involved in artery coronary diseases such as BP,[2,26–30,35] the effect of genetic polymorphisms in the *HMGCR* gene on statins effectiveness is also a matter of several studies. However, the latter are generally restricted to changes on blood lipids or disease endpoints.[4,5,7,11,13,36–39] To our best knowledge, this is the first time that the effect of a polymorphism in the *HMGCR*

Table 2
Adjusted means[a] of SBP and DBP by quartile of urinary sodium:potassium ratio according to *HMGCR* genotype for the whole cohort and separated by gender

		SBP (mm Hg)				DBP (mm Hg)			
		TT		TG + GG		TT		TG + GG	
Urinary sodium:potassium ratio quartiles		Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*	Mean ± SE	*P*
		n = 16,921		n = 753		n = 16,917		n = 753	
All	< 1.1	131.3 ± 0.3	<.001	131.3 ± 1.1	<.001	80.6 ± 0.2	<.001	80.4 ± 0.7	.006
	1.1 – 1.6	132.7 ± 0.2		134.5 ± 1.1		81.4 ± 0.2		82.4 ± 0.7	
	1.7 – 2.3	133.8 ± 0.2		134.4 ± 1.1		81.8 ± 0.2		81.9 ± 0.7	
	> 2.3	136.4 ± 0.3		138.2 ± 1.1		83.2 ± 0.2		84.1 ± 0.7	
		n = 7,317		n = 319		n = 7,315		n = 319	
Men	< 1.2	133.7 ± 0.4	<.001	135.0 ± 1.6	<.001	82.8 ± 0.2	<.001	83.3 ± 1.1	.02
	1.2 – 1.6	135.7 ± 0.4		136.9 ± 1.6		83.9 ± 0.2		84.3 ± 1.1	
	1.7 – 2.4	136.3 ± 0.4		137.9 ± 1.6		84.1 ± 0.2		84.5 ± 1.1	
	> 2.4	138.0 ± 0.4		145.3 ± 1.7		85.0 ± 0.2		88.3 ± 1.2	
		n = 9,604		n = 434		n = 9,602		n = 434	
Women	< 1.0	129.3 ± 0.3	<.001	128.5 ± 1.6	.05	78.9 ± 0.2	<.001	78.8 ± 1.0	.2
	1.0 – 1.4	130.7 ± 0.3		131.5 ± 1.5		79.8 ± 0.2		79.6 ± 1.0	
	1.5 – 2.1	131.9 ± 0.3		132.0 ± 1.5		80.0 ± 0.2		80.2 ± 1.0	
	> 2.1	135.0 ± 0.3		134.4 ± 1.5		81.6 ± 0.2		81.8 ± 0.9	

DBP, diastolic blood pressure; SBP, systolic blood pressure; SE, standard error.
Results shown as mean, SE, and *P* value for trend in the blood pressure across the quartiles of urinary sodium:potassium ratio (for the whole population and also gender specific quartiles) in each *HMGCR* genotype, both for the whole cohort and also stratified by gender.
[a] Univariate analysis of variance adjusted by gender (only for analysis with the whole cohort), BMI, age excluding antihypertension, and lipid lowering drugs users.

Table 3
Linear regression of SBP and DBP with the urinary sodium:potassium ratio according to *HMGCR* genotype

		Men			Women		
		All	TT	TG + GG	All	TT	TG + GG
SBP	ß (SE)	1.94 (0.19)[a]	1.85 (0.20)[b]	4.24 (0.93)[b]	2.58 (0.18)[a]	2.64 (0.19)	1.46 (0.83)
	95% CI	1.57 – 2.32	1.46 – 2.23	2.42 – 6.10	2.23 – 2.94	2.27 – 3.00	−0.17 – 3.09
	P	<.001	<.001	<.001	<.001	<.001	.08
DBP	ß (SE)	0.95 (0.13)	0.89 (0.13)	2.06 (0.64)	1.24 (0.11)	1.26 (0.12)	0.84 (0.53)
	95% CI	0.69 – 1.20	0.64 – 1.15	0.80 – 3.33	1.02 – 1.46	1.03 – 1.49	−0.19 – 1.88
	P	<.001	<.001	.001	<.001	<.001	0.11

CI, confidence interval; DBP, diastolic blood pressure; SBP, systolic blood pressure; SE, standard error.
Analysis adjusted by age and body mass index, excluding antihypertension, and lipid lowering drugs users.
Results shown as ß, SE, 95% CI, and *P* for the regression.
Tests for differences in ß between men and women ($^{a}Z = 2.44$; $P = .015$) or between genotypes groups ($^{b}Z = 2.51$; $P = .012$).

gene on BP is reported. The univariate analysis (Table 1) showed a higher SBP and DBP in individuals carrying the mutated allele (G). The rs17238540 SNP in the *HMGCR* gene does not affect the direction of the BP response to urinary sodium:potassium ratio, but the intensity of the response is clearly different in the G allele carriers: men and women presenting an intriguing opposite response. Comparing with the T allele homozygous, men carrying the G allele showed enhancement in SBP and DBP while women carriers of the G allele demonstrate reduced BP in response to an enhancement of the urinary sodium:potassium ratio (Table 3). It seems reasonable to propose that this polymorphism plays a different role in men and women, which deserves further investigation. Besides displaying, as pointed above, different ways to maintain the cardiovascular homeostasis, men and women also present different patterns of high BP, coronary arterial diseases outcomes, and response to treatment.[31,40–43]

This SNP has not previously been studied in a large population from Europe, but the genotype frequencies are in concordance with the frequencies found in a cohort study of largely Whites in the USA (TT 93.23%; TG 6.70% and GG 0.07%).[7] The frequency found for the minor allele (0.022) is also similar to the frequency (0.019) reported for a smaller study comprising participants from Scotland, Ireland, and The Netherlands.[37]

Non-modulation and low-renin hypertension are two dominant mechanisms proposed to lead to sodium sensitivity of hypertension.[44] Non-modulation involves anomalous angiotensin-dependent control of the renal circulation and the adrenal, leading to a disorder in sodium handling and sensitivity of the BP to salt intake,[45] and low-renin essential hypertension being the most common cause of sodium sensitivity of BP.[44] It has long been recognized that genetic factors contribute to the sensitivity of BP to salt intake.[46] The present study presents evidence that genetic variants other than the described polymorphisms of the angiotensinogen gene[18,19] can be involved in this sensitivity of BP to sodium intake.

The mechanisms underlying this observation are speculative as the biological effect of the SNP is uncertain. As the polymorphism was found to reduce lipid changes in response to pravastatin,[7] it might be related to an alteration of the enzyme's expression, activity or drug binding. The *HMGCR* SNP rs17238540 might counteract some of the observed effects of statins, enhancing the AT1 receptor messenger ribonucleic acid stability and expression[47] or altering the angiotensin II vascular response.[48] The *HMGCR* SNP would potentiate the enhancing BP effects of angiotensin II[8] and similarly to angiotensinogen gene variants, its effect on BP can be modulated by salt intake.[18–20] It is also possible that this polymorphism is linked to other genetic changes within functional parts of the *HMGCR* gene and the observed effect in our study might be reflective of this.

Conclusion

Our results suggest that SNP rs17238540 in the *HMGCR* gene is associated with the BP response to urinary sodium: potassium ratio, but the magnitude of the association differs according to possession of the G allele.

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