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**Original Scientific Paper**

# *HMGCR* gene polymorphism is associated with stroke risk in the EPIC-Norfolk study

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***Background*** **Earlier, a G/T single nucleotide polymorphism (SNP) in the *HMGCR* gene was shown to significantly reduce the overall serum lipids response to pravastatin. This study aimed to investigate the relationship of the rs17238540 SNP with coronary heart disease, stroke and cardiovascular disease risk.**

***Design*** **Cross-sectional study from the European Prospective Investigation into Cancer and Nutrition-Norfolk cohort.**

***Methods*** **Genotype was determined by pyrosequencing 23 011 participants, for whom clinical and biochemical data were available. Baseline risk factors according to genotype were evaluated, and the risk for fatal and nonfatal stroke, ischaemic heart disease and all types of cardiovascular diseases were assessed by logistic regression after approximately 11 years of follow-up.**

***Results*** **The G allele carriers presented 1.4 mmHg higher systolic blood pressure and 0.8 mmHg higher diastolic blood pressure than those who were TT carriers. They also presented higher risk of prevalent total (odds ratio: 1.44, 95% confidence interval: 1.05–1.97, $P=0.025$) and nonfatal (odds ratio: 1.56, 95% confidence interval: 1.12–2.17, $P=0.009$) stroke events compared with the TT individuals in the multivariate models.**

***Conclusion*** **An association between the rs17238540 SNP and stroke risk was observed, independent of the effect of the SNP on the blood pressure. The possible mechanisms involved, besides the effect on blood pressure, might be related to pleiotropic functions of the *HMGCR*, and remain to be explored. *Eur J Cardiovasc Prev Rehabil* 17:89–93 © 2010 The European Society of Cardiology**

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## Introduction

Cardiovascular diseases (CVDs), mainly coronary heart disease and stroke, are the main causes of death worldwide. In 2004, stroke caused 9.7% of all deaths around the world and was listed, just after coronary heart disease, as the second cause of death worldwide [1]. Epidemiologic studies have clearly shown that modifiable factors, such as smoking, hypercholesterolaemia and hypertension, are the most common risk factors for CVD in developed and in developing countries [2–4].

Numerous trials have shown that the 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCR) inhibitors (statins) decrease coronary events in primary and secondary prevention of coronary heart disease, and also stroke risk [5,6]. Besides the well-known effects on serum lipids, statins have pleiotropic effects [6–9], which have been proposed as potentially contributing to the observed reduction in major cardiac events [5–7].

Several polymorphisms were identified in the *HMGCR* gene locus [8–11], and they have been studied for associations with lipids levels. However, most of these studies did not address the effects of these polymorphisms in the risk for CVD [12–15], apart from one recent

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publication [16]. Two tightly linked single nucleotide polymorphisms (SNPs) were found to be significantly associated with a difference in the change in the serum lipids response to pravastatin treatment, but not with differences in the basal serum lipids concentration [8]. A significant reduction in the overall efficacy of pravastatin of 22.3% was observed for SNP rs17238540 [8].

The aim of this study was to investigate the influence of the SNP rs17238540 of the *HMGCR* gene in coronary heart disease, stroke and CVD risk in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Norfolk cohort study.

## Materials and methods

### Study protocol

EPIC-Norfolk is a prospective population study of White men and women recruited at the age of 45–75 years from general practice age–sex register in Norfolk, UK from 1993 to 1997. Approximately, 25 000 people participating in the baseline survey, who had filled a detailed health and lifestyle questionnaire, attended a health checkup wherein blood and urine samples, and data on height, weight, waist circumference and blood pressure were collected as described earlier [17]. Medical history, blood lipids, smoking status and habitual physical activity were assessed as described elsewhere [18–20]. Alcohol intake was assessed by a Food Frequency Questionnaire filled by the participants before the first health checkup [21].

Participants were followed up for health end points. Data on fatal and nonfatal events were identified from death certificates or hospital discharge codes for stroke (ICD-9, 430–438), ischaemic heart disease (ICD-9, 410–414) and CVDs (ICD-9, 401–448). This study reports follow-up data until 29 February 2008. The EPIC-Norfolk Study was approved by the Norfolk Health District Ethics Committee.

### Genotype determination

Genomic DNA was obtained from blood samples collected by health examinations occasion [19]. *HMGCR* G/T SNP (rs17238540) was assessed using pyrosequencing, detailed procedure of which has been published earlier [22]. The genetic analysis were repeated in separated experiments for a total of 1322 samples of 23 011 successfully genotyped participants to check the reproducibility of the method, and these analysis were 99.9% concordant.

### Statistical analysis

Data on the *HMGCR* SNP was available for 23 011 though only 20 805 had complete data on all variables for the full multiple adjusted analyses. Allele frequencies were obtained by gene counting and differences between the observed and the expected genotype frequencies were tested by $\chi^2$ test.

Characteristics of people with different genotypes were compared. Differences in means were tested using analysis of variance and differences in the frequency of the categorical variables and of cardiovascular events (stroke, ischaemic heart disease and all types of CVDs) between the genotype groups were examined using $\chi^2$. Logistic regression models were used to ascertain the odds ratio (OR) for the occurrence of total, nonfatal and fatal cardiovascular events according to the genotype group. Continuous [age, systolic blood pressure (SBP), diastolic blood pressure (DBP), waist circumference, BMI, alcohol intake, triglycerides and LDL-cholesterol] and categorical (sex, smoking status, physical activity and use of antihypertensive and lipid-lowering drugs) variables were included as covariates in the models. Covariates were measured at baseline. The results are presented as ORs and 95% confidence intervals. All *P* values are two-tailed and a *P* value of less than 0.05 was considered significant. The data were analysed using SPSS for Windows, version 16.0 (SPSS Inc., Chicago, Illinois, USA).

## Results

Genotype frequencies of 23 011 participants for whom the genetic data were available were, TT 95.65%, TG 4.29% and GG 0.06%. The genotype frequencies were in the Hardy–Weinberg equilibrium ($\chi^2 = 0.068$) and did not differ between men and women. The baseline characteristics and distribution of selected cardiovascular risk factors according to genotype are presented in Table 1. Individuals carrying the minor allele (G) were pooled in the same group, because of the low number of homozygous for this allele. Individuals carrying the G allele were older and had higher SBP and DBP than TT individuals. No other difference was observed in the variables analysed between the genotype groups, apart from a significantly higher proportion of history of stroke in the G allele carriers group. Individuals using lipid-lowering drugs were very few at the baseline ($n = 340$), and we do not have information on the nature of drug (whether statins or not) or how many would have started using statins during follow-up.

Nonfatal and total (nonfatal and fatal) stroke occurred more frequently in the G allele carriers, whereas the frequency of fatal stroke did not differ between the genotypes (Table 2). Otherwise, the frequencies of ischaemic heart and all types of CVDs (fatal, nonfatal and total) were not different between the genotype groups.

To further investigate the odds of CVD related to the genotype, we performed logistic regression using different models, the results of which are shown in Table 3. Allele G carriers showed 48% higher odds for stroke (nonfatal and fatal) than the TT individuals after adjusting for age and sex (Model 1). Further adjustments by SBP, DBP, waist circumference, LDL-cholesterol, triglycerides, smoking status, physical activity and use

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**Table 1 Baseline characteristics and some cardiovascular risk factors distribution according to SNP rs17238540 on *HMGCR* in EPIC-Norfolk**

| | TT | | TG + GG | | |
|---|---|---|---|---|---|
| Variables | *n* | Mean ± SD | *n* | Mean ± SD | *P** |
| Age (years) | 22 010 | 58.7 ± 9.3 | 1001 | 59.3 ± 9.2 | 0.03 |
| BMI (kg/m$^2$) | 21 395 | 26.3 ± 3.9 | 977 | 26.4 ± 4.0 | 0.67 |
| Waist circumference (cm) | 21 412 | 88.0 ± 12.4 | 977 | 88.3 ± 12.3 | 0.36 |
| SBP (mmHg) | 21 389 | 135.3 ± 18.4 | 974 | 136.7 ± 18.4 | 0.02 |
| DBP (mmHg) | 21 389 | 82.4 ± 11.2 | 974 | 83.2 ± 11.4 | 0.03 |
| Total cholesterol (mmol/l) | 20 816 | 6.19 ± 1.17 | 949 | 6.19 ± 1.18 | 0.88 |
| LDL-cholesterol (mmol/l) | 20 150 | 3.97 ± 1.04 | 913 | 3.99 ± 1.06 | 0.56 |
| HDL-cholesterol | 20 149 | 1.43 ± 0.43 | 913 | 1.42 ± 0.41 | 0.77 |
| Triglycerides (mmol/l) | 20 814 | 1.81 ± 1.10 | 949 | 1.78 ± 1.04 | 0.44 |
| | *n* | (%) | *n* | (%) | |
| All | 22 010 | 95.6 | 1001 | 4.4 | 0.79** |
| Men | 9512 | 95.7 | 428 | 4.3 | |
| Women | 12 498 | 95.6 | 573 | 4.4 | |
| Current smokers | 2514 | 11.5 | 126 | 12.7 | 0.17 |
| Physically active (moderately active and active) | 9003 | 40.9 | 392 | 39.2 | 0.27 |
| Lipid-lowering drug users | 328 | 1.5 | 12 | 1.2 | 0.46 |
| Antihypertensive drug users | 3995 | 18.2 | 192 | 19.2 | 0.40 |
| History of myocardial infarction | 634 | 2.9 | 36 | 3.6 | 0.19 |
| History of stroke | 286 | 1.3 | 23 | 2.3 | 0.007 |

DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; SD, standard deviation; SNP, single nucleotide polymorphism. **P* value for one-way analysis of variance tests (continuous variables) or Pearson's $\chi^2$ tests (categorical variables) between genotypes groups: TT and TG + GG. ***P* value for Pearson's $\chi^2$ test for differences in the genotype distribution between men and women.

**Table 2 Nonfatal, fatal and total cardiovascular events according to SNP rs17238540 on *HMGCR* in EPIC-Norfolk**

| | TT (*n* = 22 010) | | TT + TG (*n* = 1001) | | |
|---|---|---|---|---|---|
| Cardiovascular events | *n* | % | *n* | % | *P** |
| Stroke | | | | | |
| Total | 778 | 3.5 | 49 | 4.9 | 0.024 |
| Nonfatal | 660 | 3.0 | 44 | 4.4 | 0.012 |
| Fatal | 277 | 1.3 | 16 | 1.6 | 0.31 |
| Ischaemic heart disease | | | | | |
| Total | 2703 | 12.3 | 114 | 11.4 | 0.40 |
| Nonfatal | 2360 | 10.7 | 100 | 10.0 | 0.46 |
| Fatal | 591 | 2.7 | 27 | 2.7 | 0.98 |
| All cardiovascular disease | | | | | |
| Total | 5988 | 27.2 | 290 | 29.0 | 0.22 |
| Nonfatal | 5531 | 25.1 | 270 | 27.0 | 0.19 |
| Fatal | 1103 | 5.0 | 57 | 5.7 | 0.33 |

SNP, single nucleotide polymorphism. **P* value for Pearson's $\chi^2$ tests between genotypes groups.

of antihypertensive and lipid-lowering drugs (Model 2) did not materially alter the results. Alcohol intake and BMI were also tested as covariates, but the results did not change and they were not included in the fully adjusted model. The odds for nonfatal stroke was also significantly more than 50% higher for the G allele group in both models tested. The G allele carriers showed only a nonsignificant 16% higher odds for fatal stroke in the fully adjusted model. Otherwise, the OR for ischaemic heart disease and all types of CVDs did not show relationship with the polymorphism.

**Table 3 Association between SNP rs17238540 on *HMGCR* and risk of cardiovascular disease events risk in EPIC-Norfolk[a]**

| Cardiovascular events | TT, *n* = 19 930, OR (95% CI) | TG + GG, *n* = 905, OR (95% CI) | *P* |
|---|---|---|---|
| Stroke | | | |
| Total | | | |
| Model 1 | 1.00 (Ref) | 1.48 (1.08–2.02) | 0.015 |
| Model 2 | | 1.44 (1.05–1.97) | 0.024 |
| Nonfatal | | | |
| Model 1 | 1.00 (Ref) | 1.60 (1.15–2.22) | 0.005 |
| Model 2 | | 1.56 (1.12–2.17) | 0.009 |
| Fatal | | | |
| Model 1 | 1.00 (Ref) | 1.21 (0.70–2.09) | 0.51 |
| Model 2 | | 1.16 (0.67–2.01) | 0.61 |
| Ischaemic heart disease | | | |
| Total | | | |
| Model 1 | 1.00 (Ref) | 0.82 (0.66–1.03) | 0.09 |
| Model 2 | | 0.82 (0.65–1.04) | 0.10 |
| Nonfatal | | | |
| Model 1 | 1.00 (Ref) | 0.86 (0.68–1.09) | 0.21 |
| Model 2 | | 0.87 (0.68–1.10) | 0.25 |
| Fatal | | | |
| Model 1 | 1.00 (Ref) | 0.88 (0.57–1.36) | 0.56 |
| Model 2 | | 0.87 (0.56–1.35) | 0.54 |
| All cardiovascular diseases | | | |
| Total | | | |
| Model 1 | 1.00 (Ref) | 1.06 (0.91–1.25) | 0.45 |
| Model 2 | | 1.03 (0.88–1.22) | 0.69 |
| Nonfatal | | | |
| Model 1 | 1.00 (Ref) | 1.08 (0.92–1.27) | 0.35 |
| Model 2 | | 1.06 (0.90–1.25) | 0.51 |
| Fatal | | | |
| Model 1 | 1.00 (Ref) | 1.09 (0.80–1.47) | 0.60 |
| Model 2 | | 1.06 (0.78–1.44) | 0.72 |

Model 1: Adjusted by age and sex. Model 2: Adjusted by age, sex, systolic blood pressure, diastolic blood pressure, waist circumference, low-density lipoprotein-cholesterol, triglycerides, smoking status, physical activity and use of antihypertensive and lipid-lowering drugs. CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism. [a]Analysis restricted to subset with data for all variables available.

## Discussion

Here, we observed the association between the SNP rs17238 540 in the *HMGCR* gene and the OR for stroke in a large prospective population study. To the best of our knowledge, this polymorphism has not been studied in relation to any end point for CVD before, although it has been reported to reduce the efficacy of pravastatin in lowering serum lipids [8]. In our study, we did not find any association of the SNP rs17238540 with the baseline serum lipids, in concordance with other studies [8,14,23].

In the past years, there has been an increasing interest in the genetic epidemiology of stroke [24–27]. Although very much attention is being paid to the search for 'stroke genes', [28,29], the pathogenesis of sporadic strokes is multifactorial as are most complex disorders, and mediated by several dynamic processes, which may be

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genetically determined. Recently, several studies have addressed genes involved in such processes [19,30–32], which would partially influence the phenotype.

Hypertension is one of the most powerful risk factors for stroke [33,34]. The relationship of stroke mortality to usual blood pressure is observed at all ages, with no evidence of a threshold in the range of usual SBP above 115 mmHg or of usual DBP above 75 mmHg [35]. We observed higher SBP and DBP in the G allele carriers. Individuals carrying the G allele had a 1.4 mmHg higher SBP and 0.8 mmHg higher DBP than those who were TT carriers. However, when we included established risk factors, including SBP and DBP (Model 2), in the regression model, only a minor change in the OR was observed (approximately 3%) for both total and nonfatal stroke. Thus, the magnitude of the differences in stroke risk between the genotypes did not seem to be totally explained by SBP or DBP differences. Consistent with lack of effect of the polymorphism on blood lipids, we did not find any effect of the genotype in the OR for ischaemic heart disease.

Our results indicate that the effect of the *HMGCR* SNP rs17238540 on the odds for stroke is independent and apparently only partially explained by its effect on the blood pressure.

The mechanisms underlying our observation are speculative as the biological effect of this SNP is uncertain. As the polymorphism was found to reduce lipid changes in response to pravastatin [8], 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 pleiotropic effects of statins, such as improved endothelial function, decreased platelet aggregability and reduced vascular inflammation [4,9,36], which has been associated with significant reduction of the stroke risk and has been proposed to prevent either primary or secondary cerebrovascular events [5]. 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 reflect this.

The strengths of our study are the large population and the fair number of strokes; in addition, the lost to follow-up is negligible because mortality certification in the UK is virtually complete. Our stroke end points were ascertained through routine record linkage of all participants with death certification and hospital admissions all over the UK National Health Service hospitals. A validation study carried out showed high accuracy of stroke ascertainment through the death certification and hospital record linkage methods used in the EPIC-Norfolk study [37]. All the variables were measured at baseline with end points assessed during 11 years follow-up. The strength of this approach is that the exposures are measured before the outcome, and therefore do not suffer from recall biases that are a problem in case–control studies. We have shown earlier that this polymorphism is associated with the blood pressure response to urinary sodium/potassium ratio [22]. This and the observed lower effect of the polymorphism on the pravastatin response were the basis for the a priori hypothesis of this study. As it is not a whole genome association study examining thousands of genes, independent confirmatory studies are not essential. However, as with all other research, even for well-accepted associations, further studies are always helpful but other studies will need to obtain substantial additional data to do this research.

This study has some limitations. We did not distinguish between different stroke subtypes, and our results may therefore mask differences between these subtypes and the association found. Sporadic stroke is a complex disorder that can be divided into two major categories, haemorrhagic and ischaemic stroke, each having several subcategories, which can be identified as distinct phenotypes with their own aetiologies. Whether the association found in this study is related to a specific subtype deserves further investigation. However, the end points included (fatal and nonfatal stroke) are clinically relevant in terms of population impact. Another issue is that the end points were identified through mortality data and hospital records; therefore, it is possible that some mild stroke cases that were not hospitalized have been included in the 'comparison/control' group. Nevertheless, stroke misclassifications are likely only to attenuate any associations observed. The single measure of variables at baseline does not take into account changes during the period of follow up. However, the random measurement error resulting from this is likely only to attenuate any association observed, not producing spurious associations. Finally, we cannot rule out the possibility that the association found is because of chance, although these analyses examining the relationship of the candidate genotype to CVD were based on a priori hypothesis.

In summary, in this large prospective study, an independent association between stroke and the *HMGCR* rs17238540 SNP was found. Although the polymorphism was also associated with higher blood pressure, the magnitude of its effect on the OR for stroke cannot be explained by its effect on blood pressure. The mechanisms involved are possibly related to pleiotropic roles for *HMGCR* gene beyond lipids metabolism, which remain to be explored.

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Conflicts of interest: none declared.

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