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*Arterioscler Thromb Vasc Biol* 2010;30;2678-2683; originally published online Sep 16, 2010;

DOI: 10.1161/ATVBAHA.110.213785

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75214

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# Coronary Artery Disease–Related Genetic Variant on Chromosome 10q11 Is Associated With Carotid Intima-Media Thickness and Atherosclerosis

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**Objective**—To investigate whether chromosome 10q11.21 influences common carotid intima-media thickness (IMT) and atherosclerosis and whether it is associated with stromal cell–derived factor-1 $\alpha$  (SDF-1 $\alpha$ ) plasma levels.

**Methods and Results**—Variation on chromosome 10q11.21 has been consistently associated with coronary artery disease. The genetic variant lies upstream of the gene encoding SDF-1 $\alpha$ . We genotyped 3 population cohorts (Bruneck [age range, 45 to 94 years; 50.0% men; n=738], Health2000 [age range, 46 to 76 years; 55.4% men; n=1237], and essential hypertension in families collected in the region of Oxford [HTO] [age range, 19 to 88 years; 47.9% men; n=770]) for single-nucleotide polymorphism *rs501120* at the 10q11.21 locus and conducted a meta-analysis in these cohorts to ascertain a relationship between the polymorphism and carotid IMT. The analysis showed that individuals with the *T/T* genotype had a significantly higher carotid IMT than individuals with the *C/T* or *C/C* genotype (pooled weighted mean difference, 23  $\mu\text{m}$  [95% CI, 9 to 37  $\mu\text{m}$ ],  $P=0.0014$  under a fixed-effects model; and 23  $\mu\text{m}$  [95% CI, 6 to 41  $\mu\text{m}$ ],  $P=0.009$  under a random-effects model). In the Bruneck cohort, in which data for carotid atherosclerosis and plasma SDF-1 $\alpha$  levels were available, we observed an association of the *T/T* genotype with a higher burden of atherosclerosis and increased susceptibility to the development of atherosclerosis during a 5-year follow-up (multivariable odds ratio, 1.73 [95% CI, 1.18 to 2.52];  $P=0.005$  for the recessive model) and an association between the *T/T* genotype and lower SDF-1 $\alpha$  levels (2.62 ng/mL for *T/T* versus 2.74 ng/mL for *C/C* or *C/T*;  $P=0.023$ ).

**Conclusion**—The coronary heart disease–related variant at the 10q11.21 locus is associated with carotid IMT and atherosclerosis. (*Arterioscler Thromb Vasc Biol.* 2010;30:2678-2683.)

**Key Words:** chromosome 10q11 ■ coronary artery disease ■ carotid intima-media thickness ■ carotid atherosclerosis ■ stromal cell–derived factor-1 $\alpha$

Most coronary artery disease (CAD) and stroke cases are caused by advanced atherosclerosis, whose pathogenesis is a long-term process that takes place over many years. These 2 diseases share many genetic and environmental factors. For instance, genomewide association studies (GWASs) have revealed that a DNA sequence variant on chromosome 9p21 increases the risk of both CAD and stroke.<sup>1–5</sup> Information on the effects of the various genetic and environmental factors on the different stages of atherosclerosis development and progression can advance our understanding of the underlying mechanisms.

Carotid intima-media thickness (IMT) is a reliable marker for early atherosclerosis and predicts future cardiovascular events.<sup>6,7</sup> Several genetic variants previously shown to be related to risk of CAD and/or stroke have subsequently been reported to be associated with interindividual variability in carotid IMT,<sup>8,9</sup> suggesting that the influence of these genetic variants on IMT may be a mechanism contributing to altered susceptibility to CAD and/or stroke. However, some other CAD- and stroke-related genetic factors, such as the chromosome 9p21 variant,<sup>10–12</sup> are not associated with carotid IMT, indicating that they do not affect CAD

Received on: January 5, 2010; final version accepted on: August 30, 2010.

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*Arterioscler Thromb Vasc Biol* is available at <http://atvb.ahajournals.org>

DOI: 10.1161/ATVBAHA.110.213785

and stroke risk through a mechanism reflected by changes in carotid IMT.

One of the key loci for CAD, recently identified by a GWAS, is chromosome 10q11.21.<sup>1,4</sup> This locus lies upstream of the *CXCL12* gene, which encodes stromal cell–derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), a chemokine that has a protective effect against atherogenesis.<sup>13</sup> Whether this CAD locus has an influence on carotid IMT is unknown. In this study, we genotyped 3 population cohorts (Bruneck, Health2000, and essential hypertension in families collected in the region of Oxford [HTO]) for single-nucleotide polymorphism (SNP) *rs501120* (located at the 10q11.21 locus), which was associated with risk of CAD in a GWAS<sup>1</sup> and in a subsequent large-scale replication study.<sup>14</sup> We then conducted a meta-analysis using data from these cohorts to ascertain whether the SNP was associated with carotid IMT. In addition, in the Bruneck cohort, in which data for carotid atherosclerosis were available, we tested for a potential relationship of *rs501120* with the development, severity, and progression of atherosclerosis. Because the *CXCL12* gene is located most proximal to SNP *rs501120* and its gene product SDF-1 $\alpha$  may influence atherosclerosis development, we investigated whether there was an association between *rs501120* and plasma SDF-1 $\alpha$  level in the Bruneck cohort, for which data for plasma SDF-1 $\alpha$  levels were available.

## Methods

### Subjects

Our study was performed in 3 population cohorts: Bruneck (age range, 45 to 94 years; 50.0% men; n=738),<sup>11,15–18</sup> Health2000 (age range, 46 to 76 years; 55.4% men; n=1237),<sup>10</sup> and HTO (age range, 19 to 88 years; 47.9% men; n=770).<sup>19,20</sup> Carotid IMT was determined by ultrasonographic scanning of common carotid arteries, and the mean carotid IMT in each subject was used in this analysis.<sup>10,15–17,20</sup> In the Bruneck Study, carotid atherosclerosis was also determined as follows. Atherosclerotic lesions were defined according to 2 ultrasonographic criteria: (1) wall surface (protrusion or roughness of the arterial boundary) and (2) wall texture (echogenicity). The maximum axial diameter of plaques (in millimeters) was assessed on the near and far walls at each of 8 vessel segments. The atherosclerosis score, indicative of atherosclerosis severity, was calculated by summing all diameters at 8 well-defined segments of the common and internal carotid arteries.<sup>15–17</sup> Incident atherosclerotic plaque was defined by development of plaques in segments previously free of atherosclerosis. Atherosclerosis progression was defined as the change in the atherosclerosis score over time. The supplemental data (available online at <http://atvb.ahajournals.org>) provide further details of the Bruneck, Health2000, and HTO cohorts.

### Determination of Genotypes

Genotypes for SNP *rs501120* were determined using an SNP genotyping method (TaqMan) with a predeveloped assay (Assay ID C\_1033658\_10). The nearby SNP *rs1746048* was typed in the Bruneck cohort to test linkage disequilibrium (LD), with the use of the KASPar method (Competitive Allele Specific PCR SNP genotyping system). Random duplicates or random repeats were used as a quality control.

### Measurements of Plasma SDF-1 $\alpha$ Levels

Plasma SDF-1 $\alpha$  levels in the Bruneck cohort were determined. In brief, blood samples were drawn after an overnight fast and 12-hour abstinence from smoking. Plasma from blood samples drawn in 2000 was prepared by centrifuging the blood samples for 15 minutes at 2510 relative centrifugal force. Plasma from blood samples drawn in

2005 was obtained during isolation of mononuclear cells by density-gradient centrifugation (Lymphoprep); plasma was obtained from the upper phase after centrifugation. The plasma samples were stored at  $-80^{\circ}\text{C}$  and immediately used after a single thawing cycle. Plasma SDF-1 $\alpha$  levels in samples obtained during the 2000 and 2005 examinations were measured using commercially available ELISA kits. The mean minimum detectable concentration by the kit is 18 pg/mL, the standard curve ranges from 0 to 10 ng/mL, and intra-assay and interassay coefficients of variation are approximately 3.5% and 10%, respectively.

### Statistical Analysis

The meta-analysis of SNP *rs501120* in relation to sex-/age-adjusted common carotid IMT was conducted using a computer program (StatsDirect), which computes pooled effect size (weighted mean difference) and 95% CI, under the fixed-effects (Mulrow-Oxman) model and the random-effects (DerSimonian-Laird) model, respectively. Also, heterogeneity between cohorts was tested (providing Cochran Q and  $I^2$  statistics). Recessive models best fit the data and were used throughout the study.

In the Bruneck Study, relationships of *rs501120* genotypes with carotid atherosclerosis, plasma SDF-1 $\alpha$ , and other cardiovascular risk factors were tested using computer software packages (SPSS 15.0 and BMDP). Variables with a skewed distribution were  $\log_e$  transformed to satisfy the assumption of normality and constant variance of the residuals. With the exception of the power calculation, all probability values were 2-sided. The associations of *rs501120* genotypes with presence of carotid atherosclerosis, incident atherosclerotic plaques, atherosclerosis score, and atherosclerosis progression were tested by logistic and linear regression analyses. Base models were adjusted for age and sex. Multivariable models were adjusted for presence or absence of hypertension, smoking status, diabetes mellitus, level of alcohol consumption, levels of high- and low-density lipoprotein, body mass index, waist:hip ratio, and high-sensitivity C-reactive protein and lipoprotein(a) levels. SDF-1 $\alpha$  showed a moderate deviation from a gaussian distribution. Accordingly,  $\log_e$ -transformed SDF-1 $\alpha$  levels, which approximate a gaussian distribution (Kolmogorov-Smirnov statistics, 0.052 [ $P=0.052$ ] and 0.036 [ $P=0.088$ ]) were used in all analyses. The association between plasma SDF-1 $\alpha$  level and *rs501120* genotype was analyzed by general linear models adjusted for age and sex, with  $\log_e$ -transformed SDF-1 $\alpha$  level used as the dependent variable and the genotype coded as 1 (*T/T*) or 2 (*C/T* or *C/C*). LD between SNPs *rs501120* and *rs1746048* in the Bruneck cohort was assessed using a computer program (HaploView). The relationships of demographic variables, clinical characteristics, and cardiovascular risk factors with *rs501120* genotypes were assessed using general linear and logistic regression models.

## Results

### Association Between the 10q11 Locus and Carotid IMT

The genotype distributions (Table 1) in the Bruneck, Health2000, and HTO cohorts were all consistent with the Hardy-Weinberg equilibrium, and the allele frequencies were similar to those previously reported in other population samples of European ancestry.<sup>1,14</sup>

Age- and sex-adjusted common carotid IMT values according to the *T/T*, *C/T*, and *C/C* genotypes of the *rs501120* SNP in the Bruneck, Health2000, and HTO studies are shown in Table 1. Individuals with the *T/T* risk genotype had higher IMT values than those with the *C/T* or *C/C* genotype (42, 17, and 11  $\mu\text{m}$ , respectively). A meta-analysis using data from the 3 studies showed a statistically significant difference in IMT value between genotypes in a recessive model (*T/T* versus *C/T* and *C/C*): pooled weighted mean difference, 23  $\mu\text{m}$  (95% CI, 9 to 37  $\mu\text{m}$ ;  $P=0.0014$ ) under a fixed-effects

**Table 1. Common Carotid IMT Values by SNP *rs501120* Genotypes\***

Study	Genotype		
	<i>T/T</i>	<i>C/T</i>	<i>C/C</i>
Bruneck	0.986±0.172 (n=506)	0.944±0.171 (n=212)	0.950±0.169 (n=20)
Health2000	0.813±0.151 (n=876)	0.798±0.166 (n=325)	0.779±0.159 (n=36)
HTO	0.803±0.195 (n=575)	0.791±0.196 (n=182)	0.808±0.196 (n=13)

\*Data are given as mean±SD values (in millimeters) of age- and sex-adjusted carotid IMT. The number of subjects is given in parentheses.

model; and 23  $\mu\text{m}$  (95% CI, 6 to 41  $\mu\text{m}$ ;  $P=0.009$ ) under a random-effects model (Figure 1). There was no significant heterogeneity among the data from the 3 individual cohorts ( $P=0.240$ ).

### Association Between the 10q11 Locus and Carotid Atherosclerosis

In the Bruneck cohort, in which data for carotid atherosclerosis were available, we observed consistent significant relationships between the *rs501120* SNP *T/T* genotype and increased presence, severity, and progression of carotid atherosclerotic plaques. Details are depicted in Table 2.

### Association Between the 10q11 Locus and Plasma SDF-1 $\alpha$ Level

Because the nearest annotated gene in relation to SNP *rs501120* on chromosome 10q11 is *CXCL12*, which encodes SDF-1 $\alpha$ , we ascertain a relationship between *rs501120* and plasma SDF-1 $\alpha$  level. Data for plasma SDF-1 $\alpha$  levels were available in the Bruneck cohort but not in the Health2000 and HTO cohorts. In the Bruneck cohort, we observed an asso-

ciation between the *rs501120* *T/T* genotype and lower plasma SDF-1 $\alpha$  level measured in blood samples drawn in 2000 (2.62 ng/mL for the *T/T* genotype versus 2.74 ng/mL for the *C/C* or *C/T* genotype;  $P=0.023$ ) (Figure 2) and 2005 (2.56 ng/mL for the *T/T* genotype versus 2.63 ng/mL for the *C/C* or *C/T* genotype;  $P=0.039$ ). When using means of the 2000 and 2005 data, the results were similar (2.58 and 2.68 ng/mL, respectively;  $P=0.004$ ).

Recently, it was reported that plasma SDF-1 $\alpha$  level was associated with SNP *rs1746048*,<sup>21</sup> another SNP located at the 10q11 locus and associated with CAD.<sup>4</sup> In addition, we genotyped the Bruneck cohort for SNP *rs1746048* and found that the *rs501120* *T* allele associated with lower SDF-1 $\alpha$  levels in our study was in LD ( $r^2=0.95$ ) with the *rs1746048* *C* allele associated with lower plasma SDF-1 $\alpha$  levels in the previous study.<sup>21</sup>

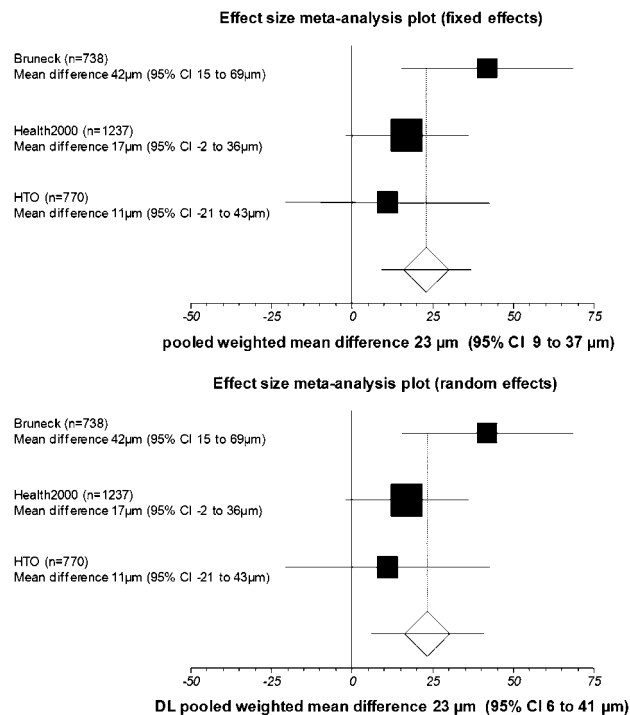
### 10q11 Locus, Circulating Endothelial Progenitor Cell Numbers, and Classic Cardiovascular Risk Factors

Because SDF-1 $\alpha$  is thought to be involved in endothelial progenitor cell (EPC) mobilization, we tested whether there was a relationship between SNP *rs501120* and circulating EPC number in the Bruneck cohort, for which such data were available. We observed that circulating EPC numbers were lower in subjects with the *T/T* genotype than in those with the *C/C* or *C/T* genotype (mean EPC number, 428.8 for *T/T* and 475.3 for *C/C* or *C/T*), although the difference did not reach statistical significance ( $P=0.34$ ).

No significant association was found between the *rs501120* genotype and any of the following classic cardiovascular risk factors in the Bruneck Study: smoking status, hypertension, body mass index, diabetes, Framingham risk score, and levels of low- and high-density lipoprotein cholesterol, lipoprotein(a), and C-reactive protein.

### Discussion

GWASs have identified chromosome 10q11.21 as a key locus that influences CAD risk.<sup>1,4</sup> A GWAS by Samani et al<sup>1</sup> showed that the *T* allele of SNP *rs501120*, located on chromosome 10q11.21, is associated with increased risk of CAD; this association was replicated in a subsequent large-scale study.<sup>14</sup> Kathiresan et al<sup>4</sup> undertook a further GWAS of CAD and reported an association of the *C* allele of SNP *rs1746048* on chromosome 10q11.21 with increased CAD risk. Based on an analysis of data from the Bruneck cohort and from the HapMap project, we observed that the *T* allele



**Figure 1.** Meta-analysis of *rs501120* in relation to carotid IMT. *T/T* vs *C/T* or *C/C* genotype in relation to age- and sex-adjusted carotid IMT. Top, fixed-effects model; and bottom, random-effects model. DL indicates DerSimonian-Laird.

**Table 2. Association of the *rs501120* T/T Genotype With IMT and Carotid Atherosclerosis in the Bruneck Study**

Carotid Artery Phenotypes	Age- and Sex-Adjusted Model		Multivariable Model	
	Value (95% CI)*	P Value	Value (95% CI)*	P Value
Common carotid IMT†				
2000	0.042 (0.016 to 0.068)	0.002	0.048 (0.022 to 0.074)	<0.001
2005	0.040 (0.008 to 0.072)	0.014	0.045 (0.013 to 0.077)	0.007
Atherosclerosis severity by score‡				
2000	0.41 (−0.04 to 0.86)	0.073	0.34 (−0.12 to 0.79)	0.146
2005	0.55 (0.17 to 0.92)	0.005	0.52 (0.13 to 0.91)	0.009
Atherosclerosis progression/incidence, 2000–2005§				
Atherosclerosis progression	0.40 (0.05 to 0.75)	0.020	0.38 (0.09 to 0.67)	0.009
Incident atherosclerotic plaques	1.53 (1.07 to 2.20)	0.021	1.73 (1.18 to 2.52)	0.005

\*Data are given as regression coefficient (95% CI), except for incident atherosclerosis plaques, for which data are given as odds ratio (95% CI). The T/T genotype was compared with the C/T or C/C genotype (recessive model), derived from logistic and linear regression analyses. Multivariable analyses were adjusted for age, sex, smoking status, hypertension, diabetes mellitus, alcohol consumption, high- and low-density lipoprotein cholesterol levels, body mass index, waist:hip ratio, and log<sub>e</sub>-transformed concentrations of high-sensitivity C-reactive protein and lipoprotein(a).

†IMT was assessed in plaque-free sections of the left and right common carotid arteries.

‡Atherosclerosis scores, indicative of atherosclerosis severity, were calculated by summing the maximum diameter of atherosclerotic plaques at 8 well-defined segments of common and internal carotid arteries. Log<sub>e</sub>-transformed atherosclerosis scores were used in the analysis.

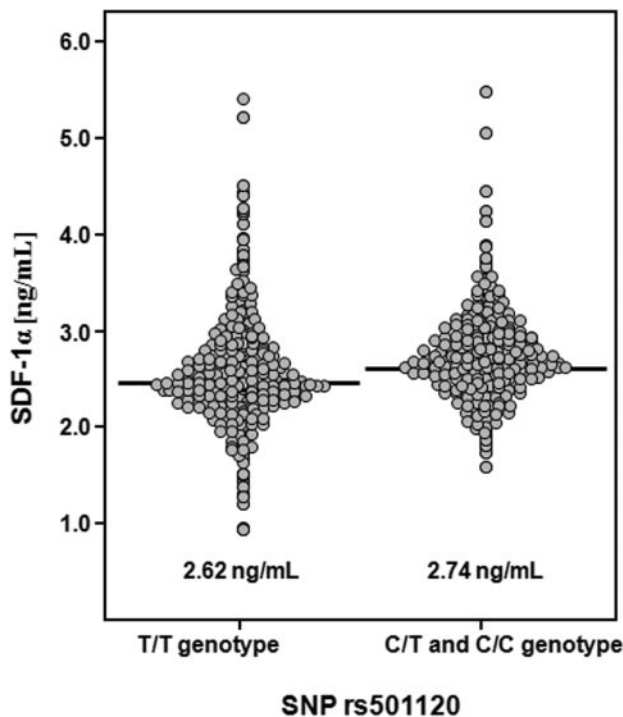
§Atherosclerosis progression was defined as the change in the atherosclerosis score between 2000 and 2005. Incident atherosclerotic plaques were defined by the development of plaques in segments previously free of atherosclerosis.

of SNP *rs501120* is in almost complete LD with the C allele of SNP *rs1746048*.

Our study shows that the 10q11.21 locus influences common carotid IMT, an early marker for atherosclerosis and a

predictor of risk of cardiovascular diseases, including CAD and stroke.<sup>6,7</sup> This finding indicates that the effect of the 10q11.21 locus on CAD risk may involve pathological remodeling of blood vessel walls, reflected by changes in carotid IMT. This is in contrast to the 9p21 locus that, although associated with CAD risk,<sup>1–3</sup> has no discernable effect on carotid IMT.<sup>10–12</sup> Our findings for the 10q11.21 locus extend to definite carotid atherosclerosis, with significant associations detected for presence, severity, and progression of plaques in the Bruneck cohort.

The CAD-related SNPs on chromosome 10q11.21 reside upstream of the *CXCL12* gene, which encodes the chemokine SDF-1 $\alpha$ . In the present study, we found an association between the *rs501120* T/T genotype and lower SDF-1 $\alpha$  level. Recently, another study<sup>21</sup> showed that plasma SDF-1 $\alpha$  levels are associated with SNP *rs1746048*, which, as previously mentioned, is also located at the 10q11 locus and associated with CAD.<sup>4</sup> We found that the *rs501120* risk allele (T allele) associated with lower plasma SDF-1 $\alpha$  levels in our study was in near-complete LD with the *rs1746048* risk allele (C allele) associated with lower plasma SDF-1 $\alpha$  levels in the previous study,<sup>21</sup> suggesting that these 2 SNPs likely represent the same genetic influence on SDF-1 $\alpha$  levels. The mechanisms by which these SNPs correlate with SDF-1 $\alpha$  levels remain unknown. Although these SNPs are approximately 100 kb upstream of the *CXCL12* gene, it is possible that they might influence *CXCL12* gene expression because gene expression can be influenced by regulatory SNPs located several hundred kilobases upstream or downstream.<sup>22</sup> In relation to this point, the SNPExpress database has recorded an association between SNP *rs1746048* and *CXCL12* gene mRNA levels (detected by a probe with identification No. 3286608) in peripheral blood mononuclear cells ( $P=0.0016$ ) (available at: <http://people.genome.duke.edu/~dg48/SNPExpress/index.php>).



**Figure 2.** Plasma SDF-1 $\alpha$  levels according to *rs501120* genotype in the Bruneck cohort. Plasma SDF-1 $\alpha$  levels assessed in samples drawn in 2000 were lower in subjects with the T/T *rs501120* genotype ( $P=0.023$ ). The probability value given was derived from a general linear model adjusted for age and sex, with log<sub>e</sub>-transformed SDF-1 $\alpha$  level used as the dependent variable and the *rs501120* genotype coded as 1 (T/T) or 2 (C/T or C/C).

SNP *rs501120* is not included in this database. Whether these SNPs influence SDF-1 $\alpha$  levels by affecting *CXCL12* mRNA expression or by other mechanisms warrants further studies.

There is evidence suggesting that SDF-1 $\alpha$  is involved in EPC homing, mobilization, and differentiation<sup>23–25</sup>; and that the SDF-1 $\alpha$  level correlates with circulating EPC number.<sup>26</sup> In a previous study,<sup>27</sup> we showed a significant association of an SNP (*rs2297630*) that is not in LD with *rs501120* ( $r^2=0$ ;  $D'=0.05$ ) located in the *CXCL12* gene with plasma SDF-1 $\alpha$  level and circulating EPC number in the Bruneck cohort. In the present study, we investigated whether SNP *rs501120* was associated with circulating EPC number in the Bruneck cohort and observed that circulating EPC numbers were lower in subjects with the *T/T* genotype than in those with the *C/C* or *C/T* genotype; however, the difference did not reach statistical significance.

Because the *rs501120* *T* allele is associated with increased CAD risk<sup>1,14</sup> and with lower SDF-1 $\alpha$ , and because SDF-1 $\alpha$  has an antiatherogenic effect,<sup>13,28,29</sup> it is conceivable that the effect of the 10q11.21 locus on CAD risk is, to some extent, mediated by an influence on SDF-1 $\alpha$  production. SDF-1 $\alpha$  is thought to exert multiple atheroprotective effects beyond EPC trafficking and homing, such as promoting defense and survival programs that counteract apoptosis and facilitating endothelial and vascular integrity.<sup>13</sup>

Our study has several merits, including the meta-analysis of common carotid IMT in 3 independent studies and the availability of multiple ultrasonographic measures of carotid vessel pathological features during the follow-up in the Bruneck Study. The present study also has the following limitations. (1) The study was conducted in individuals of European ancestry, and findings may not necessarily apply to other ethnicities or to white populations from geographical regions with a distinct genetic background. (2) Although the study was adequately powered to assess an association between the *rs501120* genotype and carotid IMT in our meta-analysis, the sample sizes of the individual studies were on the low side for detection of small IMT differences. To facilitate interpretation of the IMT findings, results of a power calculation are shown in the supplemental data. (3) SDF-1 $\alpha$  can bind to its receptor on platelets; therefore, measurement of SDF-1 $\alpha$  in plasma can be affected by contaminations with platelets.<sup>30–32</sup> Moreover, SDF-1 $\alpha$  plasma levels are subject to short- and long-term variations and influenced by a variety of circumstances, such as immunoinflammatory and neoplastic diseases.<sup>28,33</sup>

In summary, the results of this study indicate that the CAD-related variation on chromosome 10q11.21 is also associated with carotid IMT, without influencing classic intermediate traits, such as hypercholesterolemia and hypertension. These findings will aid the understanding of the biological mechanisms underlying the effects of this genetic factor.

### Sources of Funding

This study was supported by the British Heart Foundation (Drs Xu and Ye). This work forms part of the research themes contributing to the translational research portfolio of Barts and the London Cardiovascular Biomedical Research Unit, which is supported and funded by the National Institute of Health Research. The Bruneck Study was

supported by the “Pustertaler Verein zur Prävention von Herz- und Hirngefäusserkrankungen,” “Sanitaetseinheit Ost,” and “Assessorat fuer Gesundheit,” Province of Bolzano, Italy. The Health2000 study was financially supported by the Medical Research Fund of Tampere University Hospital, the Evolution (EVO) fund of Kuopio University Hospital, the Research Council for Health at the Academy of Finland, and the Finnish Foundation for Cardiovascular Research. Dr Samani holds a British Heart Foundation Personal Chair and is supported by the Leicester National Institute of Health Research Biomedical Research Unit in Cardiovascular Disease. Dr Keavney is supported by a BHF Personal Chair, and his group also received support from the NIHR Biomedical Research Centre in Ageing and Age-Related Disease awarded to the Newcastle Hospitals National Health Service Trust.

### Disclosures

None.

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## Supplement Material

**Bruneck cohort** The subjects of this study were residents of the Bruneck area in the Bolzano Province of Italy, who participated in the Bruneck Study, details of which have been described previously<sup>1-5</sup>. The study protocol was approved by the appropriate ethics committees, and all study subjects gave written informed consent before entering the study. DNA samples were available for 787 subjects, and of which 738 were successfully genotyped for the rs501120 SNP and 751 for the rs1746048 SNP. The characteristics of these individuals were identical to those of the entire population. Data on smoking status and regular alcohol consumption (grams per day) were recorded. Hypertension was defined as blood pressure (mean of 3 measurements)  $\geq 140/90$  mm Hg or the use of antihypertensive drugs. Diabetes mellitus was defined as fasting glucose levels  $\geq 140$  mg/dL (7.8 mmol/L) and/or 2-hour glucose values in oral glucose tolerance test  $\geq 200$  mg/dL (11.1 mmol/L) (World Health Organization definition). Body mass index was calculated as weight divided by height squared ( $\text{kg}/\text{m}^2$ ). Waist and hip circumferences (to the nearest 0.5 cm) were measured by a plastic tape meter at the level of the umbilicus and of the greater trochanters, respectively, and waist-to-hip ratios calculated. Framingham risk score was calculated as described by Wilson et al<sup>6</sup>.

The ultrasound protocol involves the scanning of the right and left internal (bulbous and distal segments) and common carotid arteries (proximal and distal segments). Scanning was performed with a 10-MHz imaging probe and a 5-MHz Doppler probe<sup>1-3</sup>. The scans used in the current analyses were performed in 2000 and 2005 by the same experienced sonographer, who was unaware of the subjects' characteristics. IMT was measured at plaque-free sections of the far wall of the common carotid arteries (intra-observer coefficient of variation, 7.9%,

n=100) as the distance between the lumen-intima and media-adventitia interfaces. The mean maximum intima-media thickness of the left and right common carotid arteries was used in this analysis. Atherosclerotic lesions were defined according to two ultrasound criteria: (1) wall surface (protrusion or roughness of the arterial boundary) and (2) wall texture (echogenicity). The maximum axial diameter of plaques (in millimeters) was assessed on the near and far walls at each of eight vessel segments. The atherosclerosis score, indicative of atherosclerosis severity, was calculated by summing all diameters at 8 well-defined segments of the common and internal carotid arteries [intra-observer coefficient of variation, 13.5% and 14.0% (n=100)]<sup>1-3</sup>. Incident atherosclerotic plaque was defined by development of plaques in segments previously free of atherosclerosis. Atherosclerosis progression was defined as the change in the atherosclerosis score over time.

**Health2000 cohort** The Health2000 Survey was a large Finnish cross-sectional health examination survey carried out in 2000 to 2001. The overall study cohort was a 2-stage stratified cluster sample (8028 persons) representing the entire Finnish population age 30 years and above. To study cardiovascular disease risk factors and diabetes more thoroughly, a supplemental study was carried out (sample size n=1867 and participation rate 82%). The subjects in the supplemental study were 45 years and older, and the study was executed in the catchments areas of the 5 Finnish University Hospitals. Carotid ultrasound examination was part of this supplemental study. There were 1242 subjects (556 men and 686 women; mean age, 58 years; range, 46 to 76 years) with available carotid ultrasound data and DNA samples. Genotyping was successful in 99.5% (n=1237) of these subjects. These subjects formed a study group for the present analysis. The study protocol was approved by the Ethics Committee of the National Public Health Institute and carried out according to the recommendations of the Declaration of Helsinki.

Carotid ultrasound examination of the right carotid artery was performed according to a standardized protocol using a 7.5 MHz linear array transducer. The examinations were performed by centrally trained and certified sonographers at 5 study locations around Finland. Carotid IMT measurements were performed off-line with the use of automated imaging processing software (PROWIN 23.1). One reader was responsible for reading all ultrasound images. Mean and maximum common carotid IMT values were again calculated, and the mean of the measurements was used in this study. The intrareader reproducibility of the carotid IMT measurements was assessed by calculation of the carotid IMT twice from 571 randomly selected images of 108 study subjects several weeks apart. The mean difference of the 2 measurements was 0.001 mm (SD 0.123), and the intraclass correlation was 0.934 ( $P < 0.001$ )<sup>7</sup>.

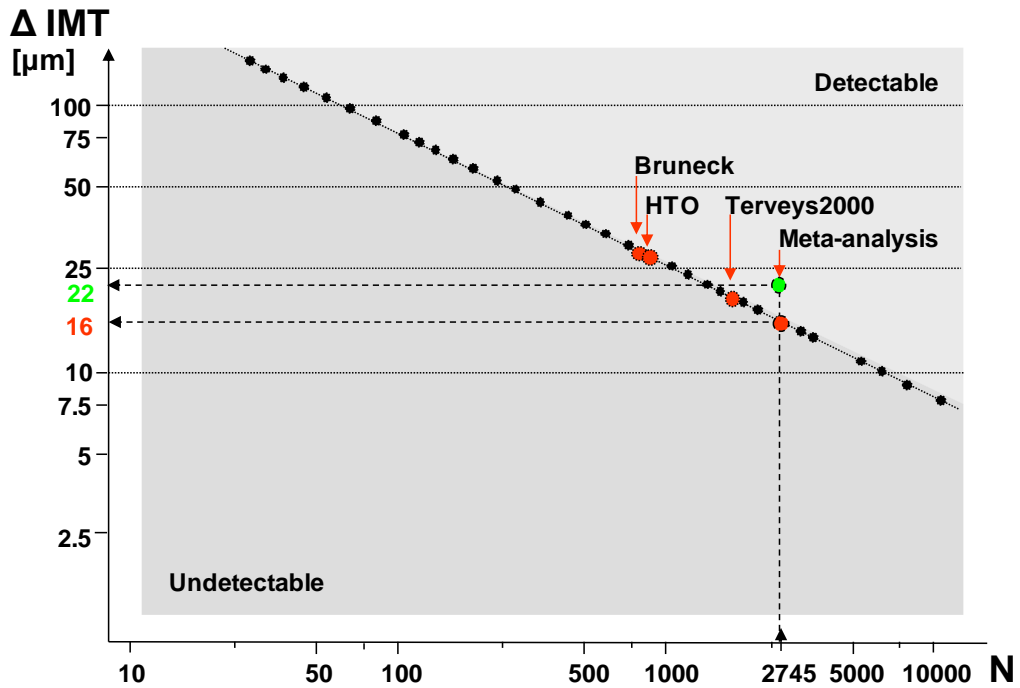
***HTO cohort*** Between 1993 and 1997, British families of European ancestry were ascertained in the Oxford region of the UK through probands with essential hypertension, whose blood pressure was in the top 5-10% of the population distribution. The ascertainment protocol has been described previously<sup>8</sup>. In order to be suitable for the study, families were required to consist of at least three siblings clinically assessable for blood pressure if at least one parent of the sibship was available to give blood for DNA analysis, and to consist of at least four assessable siblings if no parent was available for DNA analysis. Families were extended to include the nuclear families of additional individuals in the sibship who were hypertensive, where applicable. Thus, the majority (65%) of the individuals in the family collection have BP within the normal range, most families consist of single sibships, and the median family size is 5 individuals, with 60% of families comprising between four and six genotyped and phenotyped individuals. A total of 1428 members of 255 families participated

in the initial ascertainment, which included a cardiovascular and general health questionnaire, ambulatory blood pressure monitoring, anthropometry, and blood sampling. Between 1999-2001, families were recalled for additional phenotyping, which included measurement of posterior wall common carotid IMT using methodology previously described in this cohort<sup>9</sup>. Mean and maximum IMT were measured in both carotid arteries. A total of 955 family members attended for the second round of phenotyping, and carotid scans of acceptable quality were available in 854 people from 224 families.

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**Figure I. Minimum difference ( $\Delta$ ) in carotid IMT between rs501120 T/T versus C/T or C/C genotype detectable with a power of 80% ( $\alpha=0.05$  for one-sided hypothesis testing) (y axis) as a function of the number of individuals enrolled. Sample size calculation was based on the following assumptions (rare allele frequency 16%, SD of IMT 0.160 mm). Sample sizes of the individual studies and the meta-analysis are indicated as are the minimum  $\Delta$  IMTs detectable in each of the studies (red dots) and the  $\Delta$  IMT observed in the meta-analysis (green dot).**