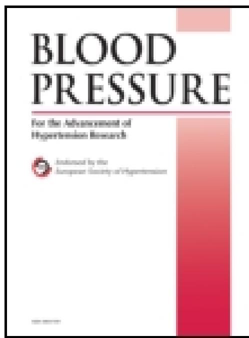

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ORIGINAL ARTICLE

Does the 9p region affect arterial stiffness? Results from a cohort of hypertensive individuals

FRANCESCA CESANA[1,2], STEFANO NAVA[1,2], CRISTINA MENNI[1,3], LUCIA BOFFI[1], MARISA VARRENTI[2], PAOLO MEANI[2], ALESSANDRO MALOBERTI[1], GUIDO GRASSI[1,4], CRISTINA GIANNATTASIO[1,2] & GIUSEPPE MANCIA[5]

[1]*Department of Health Sciences, University of Milano-Bicocca, Milan, Italy,* [2]*Division of Cardiology 4, Cardiothoracovascular Department "De Gasperis", Ospedale Niguarda Cà Granda, Milan, Italy,* [3]*Currently at Department of Twin Research and Genetic Epidemiology, King's College London, UK,* [4]*Istituto di Ricerca a Carattere Scientifico IRCCS Multimedica, Sesto San Giovanni, Milan, Italy, and* [5]*Centro Interuniversitario di Fisiologia Clinica e Ipertensione, Milan, Italy*

Abstract

Objective. Evidence exists that arterial stiffness, i.e. an independent predictor of cardiovascular and all-causes mortality, has a genetic component. The 9p21 region is associated with a greater susceptibility to coronary disease. Whether this can be ascribed to the fact that genes located on chromosome 9p may also regulate arterial stiffness is largely unknown, however. We evaluate the influence of single nucleotide polymorphisms (SNPs) from 9p on carotid–femoral pulse wave velocity (C-F PWV), measured via the Complior method, in a cohort of 821 hypertensive subjects. *Design.* The selected tagSNPs were screened with a custom-designed 384-plex VeraCode GoldenGate Genotyping assay on Illumina BeadXpress Reader platform. Association analysis was done using PLINK considering C-F PWV as a quantitative trait (linear regression assuming an additive model) adjusting for sex, age, systolic blood pressure and body mass index (BMI). We used false discovery rate (FDR) to account for multiple testing. *Results.* Although none of the 384 SNPs was significant after adjusting for multiple testing, probably due to the small sample size of the study population, a trend of association with C-F PWV was observed for rs300622 and rs2381640. *Conclusions.* These data suggest that SNPs located on chromosome 9p may affect arterial stiffness. Further studies are needed to confirm our finding on a larger sample and define the physiopathological link of the present results.

Key Words: *Arterial hypertension, arterial stiffness, arteriosclerosis, atherosclerosis, genetic*

Introduction

Arterial stiffness is well known to be an independent predictor of cardiovascular and all-causes mortality (1), especially when it is measured as carotid–femoral pulse wave velocity (C-F PWV). Moreover, it has been demonstrated an association between the degree of arterial stiffening and the entity of coronary vessels disease (2).

The arterial stiffening process is associated with aging, hypertension, diabetes mellitus and chronic kidney diseases (3,4); nevertheless, a consistent fraction of arterial stiffness variability still remains largely unexplained. Arterial stiffness heritability is estimated to be between 0.13 and 0.54 (5,6), suggesting that arterial stiffness is influenced by genetic status. Candidate genes studies have shown a potential role of genetic polymorphisms located in renin–angiotensin–aldosterone system, NO synthase, G proteins, Elastin, type 1 Collagen, metallopeptidase 3 and 9 genes (7–10). Recently, genome-wide association studies (GWAS) have shown an association between measures of arterial stiffness and loci located in different chromosomes (chromosomes 2, 7, 13, 15), while their physiopathology link with arterial stiffening process remains largely unclear (11–13). In 2007, McPherson et al. (14) identified a 58-kilobase interval on chromosome 9p21 that has been associated with coronary heart disease in six independent

Correspondence: Giuseppe Mancia, Department of Health Sciences, University of Milano-Bicocca, San Gerardo Hospital, Via Pergolesi 33, 20052 Monza (MI), Italy. Tel: + 39 039 233 3357. Fax: + 39 039 322 274. E-mail: giuseppe.mancia@unimib.it

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samples (more than 23,000 participants) from four Caucasian populations. Since then, the 9p21 region was confirmed to be associated with the development of coronary heart disease in many studies, and was also shown to be associated with intracranial and abdominal aneurisms. However, to date, there are few studies that evaluated the association between genes located in this region and cardiovascular intermediate phenotypes (15,16).

In the present paper, we examined the effects of single nucleotide polymorphisms (SNPs) in the short arm of chromosome 9 on arterial stiffness. We assessed arterial stiffness with the C-F PWV method, in accurately phenotyped hypertensive individuals.

Participants and methods

Ethical considerations

The protocol of this study has been approved by institutional ethics review committees at the relevant organizations involved. The study protocol complies with the Declaration of Helsinki (as revised in 2004) (World Medical Association Declaration of Helsinki, Ethical principles for Medical Research Involving Human Subjects. http://www.wma.net/e/policy/b3.htm) All participants provided informed written consent.

Essential hypertensive cohort

Eight hundred and twenty-seven hypertensive outpatients, aged 18–80 years old, followed by the Hypertension Centre of S. Gerardo Hospital, Monza, Italy, were enrolled from September 2006 until October 2008.

Exclusion criteria were the presence of secondary hypertension, chronic renal disease, chronic pulmonary disease, substance abuse and history of cancer.

A mercury sphygmomanometer was used to measure clinic BP twice with the patient in the sitting position for at least 5 min and with the arm placed at heart level. The first and fifth phase of Korotkoff sounds were taken as systolic blood pressure (SBP) and diastolic blood pressure (DBP) values while the cuff was deflated at a rate of 2 mmHg/s. We then assessed BP twice with a semiautomatic device. The average of the two manual and two semiautomatic BP readings was recorded. Hypertension was defined as SBP $\geq$ 140 mmHg and DBP $\geq$ 90 mmHg, or as the reported use of antihypertensive drugs. Levels of fasting serum glucose, serum total cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol and serum triglycerides were determined from venous blood sample.

Arterial stiffness was evaluated by pulse wave velocity (PWV) between the carotid and the femoral artery of the same side with the patient in the supine position (17). The pressure pulse waveforms were simultaneously obtained from the two arterial sites using an automatic device (Complior System, Colson, Les Lilas, Francia) and their distance calculated by taking the distance between hip and neck via a rigid paediatric tape. Two measurements were obtained in each patient and the average value was used for the analysis.

Single nucleotide polymorphism selection and genotyping

We selected 384 tagSNPs on the short arm of chromosome 9 from HapMap Phase II (http://www.hapmap.org) using the linkage disequilibrium (LD) tagSNP selection approach (18). Selection criteria included: SNPs with validation data, successful predictive genotyping scores for Illumina GoldenGate assays, a minor allele frequency (MAF) $\geq$ 0.05 and a pairwise LD threshold of $r^2 \geq 0.80$ for Caucasians. A further refinement was made selecting SNPs belonging to coding, intronic and 5 and 3 untranslated regions with similar proportions. We also included 7 SNPs belonging to the matrix metallopeptidase 9 (MMP9), elastin (ELN) and endothelin receptor type A (ET-A) genes and to the cyclin-dependent kinase inhibitor 2A (CDKN2A) and cyclin-dependent kinase inhibitor 2B (CDKN2B) regions, which were previously been shown to be associated with arterial stiffness (15,16).

Genomic DNA was extracted from 300 µl of fresh peripheral blood using Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) and then resuspended in 70 µl of pure water. DNA aliquots were stocked at −80°C. Genotyping was performed on the on Illumina BeadXpress Reader platform (Illumina Inc., San Diego, CA, USA). Genotypes were called using GenomeStudio. To validate Illumina results, 100 samples were screened further for six of the 384 SNPs on the ABI Prism 3130 Avant Automatic Sequencer (Applied Biosystems).

Statistical analysis

Quality checking included manual review of all cluster plots, genotype frequency, call rate and deviation from Hardy–Weinberg equilibrium. For Hardy–Weinberg equilibrium, a p-value threshold of 5×10^{-4} was used. Analysis of variance and the χ^2 test were used to compare continuous and categorical variables, respectively, between gender. Association analysis was done using PLINK (19). We performed linear regression using the additive model and adjusting for covariates determined by stepwise regression. The covariates used for multivariate adjustments were age, sex, SBP and body mass index (BMI), which are known to be major determinant of the arterial stiffening process. Because testing multiple SNPs could lead to false-positive associations, we applied the false discovery rate (FDR) (20).

Table I. Demographic characteristics of the cohort overall and by gender.

Variable	All	Males	Females	p-value
(%)	821	464 (57)	357 (43)	
Age, years	53.92 (13.72)	52.98 (13.46)	55.14 (13.98)	0.0248
BMI, kg/m^2	26.76 (4.19)	27.38 (3.63)	25.94 (4.70)	<0.0001
SBP, mm Hg	142.40 (18.34)	142.93 (18.07)	141.7 (18.68)	0.345
DBP, mm Hg	86.78 (10.55)	87.64 (10.68)	85.65 (10.28)	0.0077
CF ECG bpm	66.42 (10.74)	65.28 (10.93)	67.90 (10.32)	0.0006
Total cholesterol, mg/dL	197.03 (35.19)	192.43 (33.84)	202.98 (36.05)	<0.0001
HDL cholesterol, mg/dL	53.46 (13.62)	48.97 (11.6)	59.31 (13.86)	<0.0001
LDL cholesterol, mg/dL	118.72 (31.87)	117.16 (31.47)	120.74 (32.32)	0.1521
Triglycerides, mg/dL	121.66 (77.96)	129.51 (81.75)	111.39 (71.54)	0.0015
Glucose mg/dL	89.99 (23.27)	92.66 (25.99)	86.52 (18.63)	0.0004
Creatinine mg/dL	0.86 (0.22)	0.96 (0.21)	0.75 (0.16)	<0.0001
LVMI g/m^2	111.52 (32.71)	119.91 (32.25)	100.58 (30.01)	<0.0001

BMI, body mass index; SBP, systolic blood pressure; DBP, Diastolic Blood Pressure; CF ECG, cardiac frequency electrocardiogram; LVMI, left ventricular mass index.

Results

As shown in Table I, 57% of the total cohort was represented by males, they were younger than females, had higher BMI, DBP, total cholesterol, triglycerides, glucose, creatinine and left ventricular mass index (LVMI). They had significantly lower heart rate (cardiac frequency electrocardiogram, CF ECG), HDL- and LDL-cholesterol. Eighteen per cent of men and 16% of females were current smokers, and 81% men and 78% females were on anti-hypertensive treatment. Thirty-one of the 384 autosomal SNPs selected, after quality control, were excluded because of poor clustering, two were excluded because of MAF less than 0.01, three were excluded because of deviation from Hardy–Weinberg equilibrium and 348 SNPs were included in the final analysis. Genotyping was successful in 99.5% of individuals.

Single marker association analysis showed nominal significance for rs300622 (beta = 0.017, SE = 0.006, p-value = 0.003) and rs2381640 (beta = 0.013, SE = 0.004, p-value = 0.003) as shown in Table I and in the regional association plots (Figures 1 and 2). The allelic distribution of the two SNPs is also reported in Table II. The major allele for rs300622 is A (frequency = 0.87) while the minor is C (frequency = 0.13); the major allele for rs2381640 is C (frequency = 0.7) and the minor is T (frequency = 0.3). SNP rs300622 is located in flanking 3′ untranslated region of the TMEM215 gene on the short arm of chromosome 9 (9p21.1) Rs2381640 is located in flanking 5′ untranslated region of the TRIM5 pseudogene (positive strand) and on the RPL4P5 pseudogene (negative strand) of chromosome 9p24.1. These two SNPs lost statistical significance after multiple testing correction.

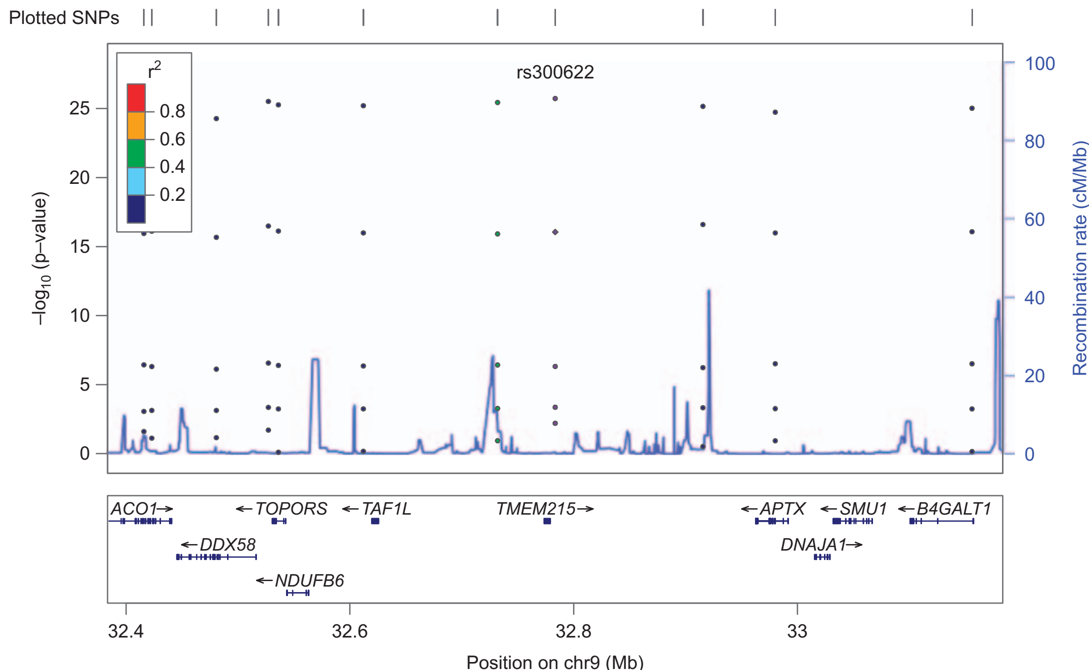

Figure 1. Regional association plot for rs300622.

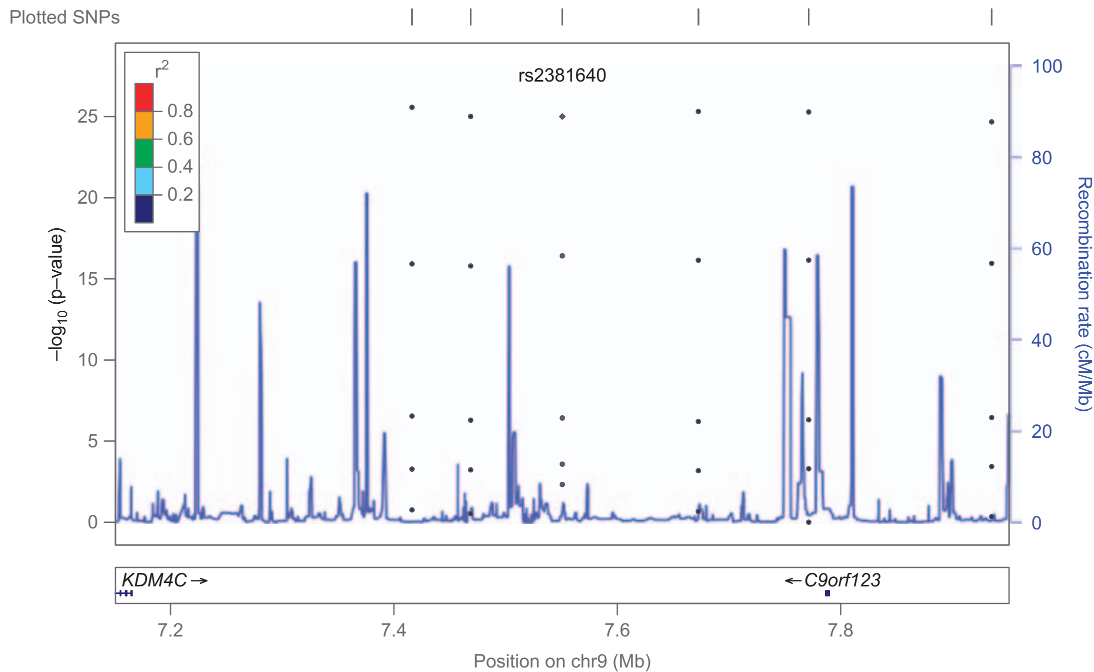

Figure 2. Regional association plot for rs2381640.

Discussion

In an accurately phenotyped cohort of essential hypertensives from Northern Italy, we identified two SNPs on the short arm of chromosome 9 that showed an association trend with C-F PWV.

Rs300622 is located in 9p21.1 on the TMEM215 gene, which encodes for the transmembrane protein 215. Rs2381640 is located in 9p24.1 on the TRIM5 pseudogene (positive strand) and on the RPL4P5 pseudogene (negative strand). TRIM5 encodes for a structural protein while RPL4P5 encodes for a ribosomal protein. The TMEM215 gene and transmembrane protein 215 have been studied in host genetic contribution to vaccine response study (21). The TRIM family proteins share a conserved arrangement of three adjacent domains, which constitutes the tripartite-motif for which the family is named; the TRIM5alpha has a C-terminal B30.2/SPRY domain, which is the major determinant of viral target specificity (22). The RPL4P5 pseudogene function has been evaluated in a study on protein–protein interaction, which suggests the importance of pseudogene in the understanding of protein function and cellular processes (23). Thus, a biologically plausible association of genetic results with the atherosclerotic process is not self-evident and remains unclear; further studies are needed to better dissect the mechanistic basis of these results.

The results of our study deserve some further comments. First, while not surviving to the multiple analysis correction, the results of the present study suggest a role of the short arm of chromosome 9 on arterial stiffness. Since arterial stiffness is one of the most powerful predictors of coronary heart disease (1), one can extrapolate that both the genetic basis of coronary artery disease and arterial stiffness are on the short arm of chromosome 9. An alternative possible interpretation is that, as arterial stiffness is one of the major determinants of the vascular atherogenic process, this physiopathological link might be genetically related. Another observation refers to the pulse wave velocity measurement itself. Indeed, in this study, the measurement was obtained in all enrolled patients, with no exception, and this supports the idea of its feasibility as an alternative, easy and chip target organ damage parameter in hypertension.

Our study has some strengths as well as limitations. The strengths refer to the fact that our discovery cohort is a sample of accurately phenotyped hypertensive patients. This allowed us to account for several potential confounders like SBP, age, gender and BMI. Furthermore, in our study, arterial stiffness was measured as C-F PWV, which is regarded as the gold standard method with a strong prognostic value. Our study also has several limitations, how-

Table II. Allelic distribution of rs300622 and rs2381640.

SNP	Major allele	Minor (effect) allele	Major allele frequency	Minor allele frequency	Beta	SE	*p*-value
rs300622	A	C	0.87	0.13	0.017	0.006	0.003
rs2381640	C	T	0.70	0.30	0.013	0.004	0.003

ever. First, the association results disappear after multiple testing correction. In addition, we were not able to look up our nominally significant results in an independent sample, as rarely do investigators include pulse wave velocity analysis when they phenotype essential hypertensive patients and we could not find a cohort with the same measurement in the same conditions. It is thus possible that with a larger cohort and a replicate, the association we are suggesting in this paper can be confirmed. Moreover, we were not able to replicate our result in normotensive healthy individuals and this will be the objective of future research programmes on the issue.

Conclusions

In conclusion, the 9p region may influence arterial stiffness and the identification of genes that influence intermediate cardiovascular phenotypes may improve early diagnosis and preventive measures. Further studies are necessary to confirm the suggestions and define the physiopathological link of the present results.

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