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Running headline: 9p21.3 and arterial stiffness

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Abstract

Objective. Genome wide association studies have consistently reported associations between a region on chromosome 9p21.3 and a broad range of vascular diseases such as coronary artery disease (CAD), aortic and intracranial aneurysms and type-2 diabetes (T2D). However, clear associations with intermediate phenotypes have not been described so far. To shed light on a possible influence of this chromosomal region on arterial wall integrity, we analyzed associations between single nucleotide polymorphisms (SNPs) and degree of stiffness of the abdominal aorta in elderly individuals.

Methods and Results. A total of 400 subjects, 212 men and 188 women, aged 70-88 years were included. Arterial stiffness was examined at the midpoint between the renal arteries and the aortic bifurcation. Two CAD- and aneurysm-associated SNPs (rs10757274 and rs2891168) and one T2D-associated SNP (rs1081161) within the 9p21.3 region were genotyped. Aortic compliance and distensibility coefficients were higher in carriers of the rs10757274G and rs2891168G alleles in men reflecting a decrease in aortic stiffness. Adjustment for age and mean arterial pressure had no effect on these associations. The two SNPs were not associated with intima-media thickness or lumen diameter of the abdominal aorta. There were no associations between the rs10811661 SNP and any measure of aortic stiffness.

Conclusions. Impaired mechanical properties of the arterial wall may explain the association between chromosome 9p21.3 polymorphisms and vascular disease.

Keywords: Arterial stiffness, polymorphism, vascular disease

Introduction

Coronary artery disease (CAD) is the most common cause of death worldwide. In the past year, several genome wide association studies (GWAS) have reported associations between a region on chromosome 9p21.3 and risk of CAD [1-4]. This is currently the strongest and most robust susceptibility locus identified for CAD. The same chromosomal region has also been associated with type 2 diabetes (T2D) in some GWAS [5-7]. However, in the PROCARDIS and deCODE studies, simultaneous tests of the CAD- and T2D-associated single nucleotide polymorphisms (SNPs) showed that the susceptibility regions for CAD and T2D are close but clearly distinct [8, 9].

The CAD-associated SNPs appear to be located within a chromosomal region lacking any coding genes. Originally, focus was placed on genes located within a LD block covering a larger chromosomal region. This region contains three candidate genes, all suggested to be tumor suppressor genes; *p15/CDKN2B*, *p16/CDKN2A* and *p14/ARF*. A large germ-line deletion of this region has been detected in a melanoma-neural system tumor syndrome family [10]. Fine-mapping of this deletion resulted in discovery of a large non-coding RNA named *ANRIL* (Genebank accession no. DQ485453), with a first exon located in the promoter of *ARF* and overlapping the two exons of *CDKN2B* [11]. Recently, fine-mapping of the chromosomal region associated with CAD identified ANRIL as the prime candidate gene in the chromosome 9p21.3 CAD locus [8].

The molecular mechanism underlying the associations with CAD has not been identified. Also, no clear associations between the CAD-associated SNPs and intermediate phenotypes have been described so far. However, the findings that the CAD-associated SNPs also are associated with risk for abdominal aortic aneurysm and intracranial aneurysm [9] suggest that the locus is not directly involved in the development of myocardial infarction (MI) but instead may influence the properties of

the vessel leading to susceptibility to a broad range of vascular diseases. In order to shed light on the possible influence of the SNPs on arterial wall integrity, we investigated the associations between the CAD SNPs within the chromosome 9p21.3 locus and stiffness of the abdominal aorta in elderly individuals.

Methods

Study population

The study included 400 subjects, 212 men and 188 women, aged 70-88 years, participating in a longitudinal study involving all inhabitants in Kinda municipality aged 66-82 years. Of 1130 individuals originally invited, 876 accepted to participate. In years 2003-2005, a follow-up study on 675 individuals was performed. A total of 123 individuals had died before the follow-up study, thus resulting in a participation rate of 90%. Examination comprised physical examination, blood sampling, electrocardiography, chest x-ray and Doppler echocardiography. All participants in the follow-up investigation (n=675) were also asked to take part in the present study of abdominal aortic properties. A total of 223 subjects declined. Of the remaining, 407 individuals had successful investigations of the abdominal aorta, 7 of whom were excluded due to infection or genotyping failures. The final study group thus included 400 subjects (Table 1).

Blood samples were drawn following overnight fasting from subjects in the sitting position after 30 min of rest. The samples were collected in pre-chilled plastic tubes containing EDTA (Terumo EDTA K-3) and stored at -70°C until analyses were performed. Body mass index (BMI) was calculated from weight and height measurements. Body surface area (BSA) was estimated according to the formula of Du Bois [12]:

$$BSA\ (cm^2) = weight^{0.425} \times height^{0.725} \times 71.84$$

Diabetes mellitus was defined as ongoing treatment for diabetes or a fasting blood glucose concentration ≥7.0 mmol/L. Informed consent was obtained from each participant; the Regional Ethics Committee for Human Research at the University

Hospital of Linköping approved the study protocol. The study was conducted in accordance with the Declaration of Helsinki.

TABLE 1. Characteristics of study groups

	All	Men	Women
Subjects, n	400	212	188
Mean (SD) age, y	78.2 (3.4)	78.2 (3.1)	78.2 (3.6)
Body mass index, kg/m^2	27.3 (4.2)	26.8 (3.2)	27.9 (5.0)
Blood pressure, mm Hg			
Systolic	149 (23)	145 (22)	152 (24)
Diastolic	75 (10)	76 (10)	75 (10)
Mean arterial pressure	104 (17)	102 (16)	105 (17)
Pulse pressure	73 (19)	69 (18)	78 (19)
History			
Smoker, n (%)	35 (9)	28 (13)	7 (4)
Diabetes, n (%)	92 (23)	50 (24)	42 (22)
Angina, n (%)	77 (19)	42 (20)	35 (19)
Medication			
Beta receptor blocker, n (%)	151 (38)	80 (38)	71 (38)
ACE inhibitor, n (%)	81 (20)	42 (20)	39 (21)
Statins, n (%)	95 (24)	47 (22)	48 (26)

Values are mean (SD) or number of subjects in group (%)

Blood pressure measurements

Non-invasive upper arm blood pressure was recorded by oscillometric technique immediately after measurements of the pulsatile aortic diameter (Dinamap model PRO 200 Monitor, Critikon, Tampa, FL, USA). Blood pressure was measured in both upper arms, taking the highest value as the prevailing pressure. All examinations were performed after at least 15 min of rest with subjects in the supine position. Subjects were requested to refrain from tobacco or beverages containing caffeine for at least 4 hrs prior to examinations. Differences in blood pressure between left and right arm were excluded before the investigation. Comparison between the intra-arterial pressure of the abdominal aorta and the brachial blood pressure obtained non-invasively has shown a slight

systematic underestimation of pulse pressure, which does not affect comparative studies of aortic stiffness between different groups of subjects [13].

Measurement of lumen diameter and intima-media thickness

Lumen diameter (LD) and intima-media thickness (IMT) recordings of the abdominal aorta were performed in diastole. The aorta was examined at the midpoint between the renal arteries and the aortic bifurcation. All examinations were performed after at least 15 min of rest with subjects in the supine position. Measurements were carried out using an ultrasound scanner (Esaote AU5, Esaote Biomedica, Florence, Italy) equipped with a 7.5 MHz linear transducer or a 7.3 MHz curved transducer. The ultrasound system was connected to a PC equipped with the Wall Track System software (WTS2, Pie Medical, Maastricht, The Netherlands). Details of the ultrasound technique have been described elsewhere [14, 15]. In brief, ECG electrodes were connected to the subject, followed by visualization of the abdominal aorta in a longitudinal section. The scanner was then switched to M-mode and radio frequency (RF) signals from both the anterior and the posterior walls were transferred to the PC. A sample volume was automatically positioned on the anterior and the posterior wall. Before data on average diastolic vessel diameter and arterial distension waveforms were calculated, manual adjustment of the sample volume could be made. Two skilled ultrasonographers examined all subjects on one single occasion. Mean data from three consecutive recordings were used for the statistical analysis.

Calculations

The distensibility coefficient (DC) and compliance coefficient (CC) were defined as follows [16]:

$$DC = (2 \times Dd \times \Delta D + \Delta D^2)/(Dd^2 \times \Delta P)$$

The unit for DC is 10^{-3}/kPa where *Dd* is the end-diastolic diameter (mm), *ΔD* is the diameter change ($D_{systolic} - D_{diastolic}$, mm), and *ΔP* is the pressure change ($P_{systolic} - P_{diastolic}$, kPa). The distensibility coefficient is the change in arterial diameter in relation to a given increase in pressure [16]:

$$CC = \pi (2 \times Dd \times \Delta D + \Delta D^2)/(4 \times \Delta P)$$

The unit for CC is mm^2/kPa.

DC is the change in arterial diameter in relation to a given increase in pressure; the lower the DC, the higher is the arterial stiffness. CC is the absolute increase in cross-sectional area for a given increase in arterial pressure, assuming that the vessel length is constant during the pulse wave; a lower CC indicates a reduced buffering capacity for the heart [16]. Distensibility characteristics of the abdominal aorta depend on the extent to which it is expanded, being very distensible at low pressures and small diameters and getting gradually stiffer (less compliant) with increasing pressure and diameter. Thus, there is a nonlinear pressure-diameter relationship of the aortic wall. This means that the mentioned indices are somewhat dependent on blood pressure. However, the index stiffness (β) seems to be less sensitive to pressure changes [17]. The index stiffness (β) was calculated as follows [18]:

$$\text{Stiffness } (\beta) = \ln(P_{systolic}/P_{diastolic})/(D_{systolic} - D_{diastolic})/D_{diastolic}$$

where $P_{systolic}$ and $P_{diastolic}$ are systolic and diastolic blood pressures (mmHg), and $D_{systolic}$ and $D_{diastolic}$ are systolic and diastolic vessel diameters of aorta (mm).

Circumferential wall stress (dyne/cm^2) was calculated from the Laplace´s equation: Wall stress = DP × (LD/2)/IMT, where DP is the diastolic pressure (dyne/cm^2), LD is lumen diameter (cm), and IMT is intima-media thickness (cm). Diastolic blood pressure

was used as IMT and LD measurements were performed in diastole; 1 mmHg equals 1333 dyne/cm^2.

DNA preparation and genotyping

Genomic DNA was isolated from peripheral blood leukocytes using the QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany). Genotyping was performed by TaqMan Allelic discrimination (Perkin Elmer Biosystems, Foster city, CA, USA). Primers and probes were obtained from Applied Biosystems (PE Applied Biosystems) (C_26505812, C_1754680 and C_31288917). A total of 20 ng DNA was amplified in 15 µl aliquots. All SNPs were amplified with initial denaturation of 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Post-PCR allelic discrimination was carried out measuring allele-specific fluorescence on the ABI Prism® 7000 Sequence Detection System (Applied Biosystems) using the Sequence Detection System software version 1.01. Genotyping was successful in 93, 98 and 98% of the samples for the rs10757274, rs2891168 and rs10811661 polymorphisms, respectively.

Statistical analyses

Men and women were analyzed separately. All data are presented as mean values ± standard deviation (SD). Hardy-Weinberg equilibrium was evaluated for each SNP by use of χ^2 test, allele-frequencies were calculated by gene counting. Differences in baseline characteristics and arterial properties between groups were tested using independent-samples t-test. Skewed data were log-transformed to a gaussian distribution before comparisons were made. Categorical data was evaluated using chi-squared test. A *P* value <0.05 was considered statistically significant. Statistical analyses were carried out using SPSS 15.0 for Windows software (SPSS, Chicago, IL, USA).

Results

As there is very strong linkage disequilibrium within the CAD-associated region on chr 9p21.3, we only genotyped two of the previously described CAD-associated SNPs, i.e. rs10757274 [2] and rs2891168 [8]. Furthermore, we also included rs10811661 since this SNP has previously been associated with T2D but not with CAD [6-8]. The frequencies of the minor rs10757274G, rs2891168G and rs10811661C alleles were 0.44 (males 0.47, females 0.41), 0.45 (males 0.48, females 0.42) and 0.14 (males 0.13, females 0.15), respectively, which is in accordance with previous reports [2, 6-8]. Of note, the frequencies of the minor alleles of the CAD-associated SNPs were lower in women than in men. Whereas the genotype distributions of all tested SNPs were found to be in Hardy-Weinberg equilibrium in men, the CAD-associated SNPs were not in Hardy-Weinberg equilibrium in women (p=0.002 for both rs10757274 and rs2891168).

Basic characteristics of the participants are shown in Table 1. In brief, the cohort consisted of 212 men and 188 women with a mean age of 78.2±3.1 and 78.2±3.6 years respectively. As the genotype distribution of the CAD-associated SNPs in the female subset deviated from Hardy-Weinberg equilibrium, men and females were analysed separately. As shown in Table 2, the aortic compliance and distensibility coefficients were higher in male carriers of the CAD-associated rs10757274G-allele. Accordingly, the aortic stiffness was decreased in men who carried the rs10757274G-allele. In contrast, there were no associations between the rs10757274 SNP and measures of vessel wall properties in women (Table 2). In a manner similar to that of rs10757274G-allele, male carriers of the rs2891168G-allele (Table 3), but not female carriers (data not shown), had increased compliance and distensibility coefficients and lower aortic stiffness. Adjustment for age and mean arterial pressure had no or little effect on the association between the G-alleles and arterial stiffness (CC, DC and aortic stiffness). None of the

TABLE 2. Characteristics according to rs10757274A/G

	Men				Women			
	A/A	A/G and G/G	P	P_{Adj}	A/A	A/G and G/G	P	P_{Adj}
N	50	144			51	126		
Age, y	77.8 (3.0)	78.2 (3.2)	0.442		78.4 (3.8)	78.3 (3.6)	0.845	
Body mass index, kg/m^2	26.9 (3.2)	26.7 (3.2)	0.762		27.8 (5.3)	28.0 (4.8)	0.780	
Blood pressure, mm Hg								
Systolic	145 (25)	144 (21)	0.893		154 (24)	153 (24)	0.784	
Diastolic	75 (10)	76 (10)	0.520		77 (11)	75 (10)	0.168	
Mean arterial pressure	101 (16)	103 (16)	0.568		109 (19)	105 (16)	0.196	
Pulse pressure	70 (20)	68 (17)	0.599		77 (21)	78 (19)	0.702	
Arterial wall properties								
Intima-media thickness, mm	0.54 (0.13)	0.54 (0.14)	0.896		0.60 (0.23)	0.61 (0.22)	0.980	
Lumen diameter, mm								
Diastolic	17.6 (2.5)	17.4 (3.9)	0.726	0.894†	14.9 (2.6)	14.7 (2.7)	0.627	0.435†
Systolic	18.1 (2.5)	18.1 (3.4)	0.960		15.5 (2.6)	15.2 (2.7)	0.568	
ΔSysDia	0.48 (0.31)	0.64 (0.37)	0.013		0.61 (0.32)	0.56 (0.32)	0.480	
Compliance coefficient, mm^2/kPa	1.68 (1.27)	2.08 (1.41)	0.048	0.025††	1.49 (0.85)	1.40 (1.01)	0.304	0.148††
Distensibility coefficient, $10^{-3}/kPa$	7.08 (5.47)	9.40 (7.06)	0.023	0.018††	9.13 (6.10)	8.44 (5.94)	0.446	0.261††
Aortic stiffness (β)	35.77 (24.41)	25.96 (18.99)	0.008	0.011†††	23.80 (16.30)	27.67 (20.76)	0.258	0.193†††
Wall stress, $dyne/cm^2$	17.78 (5.17)	17.80 (5.63)	0.987	0.986††††	14.06 (4.95)	13.73 (5.01)	0.719	0.692††††
History								
Smoker, n (%)	6 (12)	22 (15)	0.570		0	7 (6)	0.195	
Diabetes, n (%)	12 (24)	36 (24)	0.965		13 (26)	25 (20)	0.407	
Angina, n (%)	7 (14)	28 (19)	0.388		11 (22)	23 (18)	0.612	

Values are mean (SD) or number of subjects in group (%). †Adjusted for age, body surface area and mean arterial pressure; ††Adjusted for age and mean arterial pressure; ††† Adjusted for age, mean arterial pressure and intima-media thickness; ††††Adjusted for age.

CAD-associated SNPs were associated with either IMT or LD of the abdominal aorta (Tables 2-3). There were no associations between the T2D-associated rs10811661 SNP and measures of aortic stiffness in men (Table 3) or women (data not shown).

TABLE 3. Characteristics according to rs2891168A/G and rs10811661T/C in men

	rs2891168				rs10811661			
	A/A	A/G and G/G	P	P_{Adj}	T/C and C/C	T/T	P	P_{Adj}
N	52	156			55	153		
Age, y	78.0 (3.1)	78.2 (3.1)	0.785		78.2 (3.1)	78.0 (3.1)	0.657	
Body mass index, kg/m^2	26.8 (3.2)	26.7 (3.2)	0.863		26.8 (3.0)	26.7 (3.3)	0.866	
Blood pressure, mm Hg								
Systolic	147 (25)	145 (21)	0.627		146 (22)	145 (22)	0.822	
Diastolic	76 (10)	76 (10)	0.900		76 (10)	76 (10)	0.761	
Mean arterial pressure	102 (16)	103 (16)	0.714		102 (17)	102 (16)	0.992	
Pulse pressure	71 (20)	69 (17)	0.502		69 (18)	69 (18)	0.916	
Arterial wall properties								
Intima-media thickness, mm	0.56 (0.14)	0.55 (0.30)	0.688		0.53 (0.12)	0.55 (0.14)	0.421	
Lumen diameter, mm								
Diastolic	17.7 (2.4)	17.3 (3.3)	0.511	0.876†	17.1 (2.4)	17.5 (3.4)	0.398	0.426†
Systolic	18.2 (2.5)	18.0 (3.3)	0.716		17.7 (2.4)	18.1 (3.4)	0.325	
ΔSysDia	0.50 (0.31)	0.65 (0.38)	0.017		0.59 (0.34)	0.62 (0.38)	0.650	
Compliance coefficient, mm^2/kPa	1.69 (1.24)	2.07 (1.40)	0.066	0.029††	1.87 (1.22)	2.01 (1.44)	0.750	0.726††
Distensibility coefficient, $10^{-3}/kPa$	7.12 (5.35)	9.51 (7.09)	0.024	0.015††	8.74 (6.13)	8.98 (7.07)	0.895	0.928††
Aortic stiffness (β)	34.76 (24.11)	25.87 (19.16)	0.011	0.008†††	27.84 (20.30)	28.49 (21.21)	0.993	0.831†††
Wall stress, $dyne/cm^2$	17.51 (5.13)	17.60 (5.49)	0.921	0.924††††	17.27 (4.78)	17.80 (5.61)	0.556	0.556††††
History								
Smoker, n (%)	5 (10)	22 (14)	0.404		7 (13)	21 (14)	0.852	
Diabetes, n (%)	13 (25)	36 (23)	0.777		12 (22)	37 (24)	0.723	
Angina, n (%)	8 (15)	32 (21)	0.416		10 (18)	29 (19)	0.900	

Values are mean (SD) or number of subjects in group (%). †Adjusted for age, body surface area and mean arterial pressure; ††Adjusted for age and mean arterial pressure; ††† Adjusted for age, mean arterial pressure and intima-media thickness; ††††Adjusted for age.

Discussion

The present study shows, for the first time, that genetic variation within the chromosome 9p21.3 locus is associated with abnormal functional characteristics of the abdominal aortic wall when subjected to pulsatile blood flow. The genetically derived impairment of the intrinsic properties of the arterial wall could explain the association between SNPs and a broad range of arterial wall diseases. Originally, the locus was associated with risk of MI, but subsequent studies indicated that the SNPs are not influencing a susceptibility gene for plaque rupture [9]. We have recently shown that CAD patients without a history of MI tend to harbour the CAD-associated SNPs at a higher frequency than MI patients [8]. Also, a population-based prospective study indicated that the 9p21.3 locus influences atherosclerosis development and progression [19]. However, recent findings of associations between the 9p21.3 locus and intracranial aneurysms [9] imply that the susceptibility gene influences basic properties of the arterial wall that are not directly linked to atherothrombosis. Whereas the chromosome 9p21.3 SNPs in the study by deCODE were associated with susceptibility to abdominal aortic aneurysm formation, there was no evidence of an association with aneurysm growth rate or risk of rupture [9].

The mechanical properties of the aortic wall are determined by the content and composition of the extracellular matrix, mainly by elastin and collagens, although calcification also may contribute [20]. The distensible elastin is load-bearing at low pressures while the much stiffer collagen is load-bearing at high pressures and it is clear that the collagen-to-elastin ratio is the principal determinant of wall mechanics [17, 21]. However, as only a minor part of the collagen is load-bearing in the physiological pressure range, the amount and function of elastin will probably have the largest impact on the mechanics of the aortic wall. Accordingly, fibrillin-1-rich elastic fibres seem to have an impact on aortic stiffness in patients with genetically defective fibrillin-1 as in

Marfan syndrome [22]. However, as the integrity of the aortic wall is dependent on the combined effect of the elastin-collagen network, it may be speculated that the SNPs affect wall integrity either through an increased effect of the elastin fraction or a reduced effect of the collagens.

Increased aortic stiffness leads to premature return of reflected waves increasing systolic pressure and pulse pressure as well as the workload and oxygen consumption of the heart. These effects promote myocardial ischemia, left ventricular hypertrophy and eventual heart failure and render aortic stiffness a predictor of cardiovascular disease [23, 24]. Surprisingly, the CAD-associated SNPs were associated with decreased and not increased aortic stiffness, which might seem counterintuitive. However, stiffness was measured in the abdominal aorta. The impact of local abdominal aortic stiffness on cardiovascular outcome has not been established so far. Instead, aortic pulse wave velocity (PWV) has been a standard method of measuring arterial stiffness. However, aortic PWV is measured over a substantial portion of the arterial tree, including the common carotid artery, thoracic and abdominal aorta, iliac artery, and part of the femoral artery [23]. It is well known that the elastic behaviour of these segments is quantitatively differentiated [25], which means that the two modes of assessing aortic stiffness lead to different results. Because PWV is derived from a longer segment of the central arterial tree, it might be argued that it has a greater impact on cardiac load. Thus, a local change in stiffness of the abdominal aortic wall might reflect unknown mechanisms influencing wall integrity rather than constituting an indication of increased cardiovascular risk.

No association was found between the SNPs and IMT of the abdominal aorta. It has also earlier been shown that the association between the 9p21.3 locus and CAD is uninfluenced by gender, age, smoking, obesity, hypertension and diabetes [8], factors associated with atherosclerosis. Also, considering the finding of a link between the

9p21.3 locus and intracranial aneurysm formation [9], this suggests that the susceptibility gene is not directly involved in the atherosclerotic process, but rather may influence fundamental properties of the vessel wall leading to proneness to a wider range of vascular disease manifestations.

The association between the SNPs and reduced stiffness of the abdominal aorta was only found in men. This contrasts with previous findings of no gender-specific effects on CAD susceptibility conferred by the rs2891168 SNP [8]. A confounding factor might be that the allele frequencies of the rs10757274 and rs2891168 SNPs were not in Hardy-Weinberg equilibrium in the female subgroup. Also, the minor allele frequencies were lower in women than in men. Thus, a selection bias in the recruitment of female participants would explain the observed differences. Further support for a selection bias in the recruitment of women to the study is the fact that more men than women were included although the mean age of the study population was as high as 78 years. This is in apparent contrast with population statistics, which shows that the average life expectancy of women in Sweden is longer compared with men. However, there are also several indications that the aortic wall might be more vulnerable in men than women; wall stress is higher and increases with age in men in contrast to the same in women and wall stiffness increases with age to a higher extent in men. Furthermore, the age-related dilation of the healthy aorta is augmented in men, and a higher prevalence of abdominal aortic aneurysms has been found [26-28].

The functional SNP contained in the 9p21.3 locus neither has been identified hitherto nor has its target gene(s) been identified. The localization of the CAD SNP cluster suggests that the functional SNP could influence the expression or activity of *ANRIL*. However, it should be stressed that the localisation of the SNPs does not exclude an effect on nearby located genes as a result of suppression of a distant enhancer region.

Interestingly, a coordinated transcriptional regulation of *ANRIL* and *ARF* and possibly also of *CDKN2A* and *CDKN2B* has been demonstrated [11]. The 5´ end of the first exon of the *ANRIL* gene is located about 300 bp upstream of the transcription start site of the p14/ARF indicating that the two genes may share a common promoter. Alternatively, the functional polymorphism influences splicing of the ANRIL transcript. The *ANRIL* gene has been shown to contain approximately 19 exons, and two splice forms have so far been described [11]. Hypothetically, a genetically derived change in the splice pattern could result in changes in the expression of downstream targets of ANRIL.

We have recently demonstrated the expression of ANRIL mRNA in abdominal aortic aneurysm and carotid endarterectomy tissues using RT-PCR [8]. Furthermore, ANRIL was expressed in vascular endothelial cells, monocyte-derived macrophages and coronary smooth muscle cells [8]. However, expression of ANRIL, p14/ARF, p16/CDKN2A and p15/CDKN2B mRNA has also been demonstrated in 22 different normal tissues [11], i.e. the expression of ANRIL is not limited to the vessel wall. The measurements of ANRIL mRNA have so far been conducted using RT-PCR, a method which is not fully quantitative. Therefore, a comparison between genotype and expression levels of ANRIL in large collections of vascular tissue is needed to resolve whether there is any genetic effect on the expression level. However, before such analyses can be performed, the various splice forms need to be determined. Thereafter, the potential targets of the different ANRIL antisense mRNAs can be identified using bioinformatic tools.

In conclusion, impaired mechanical properties of the aortic wall may explain the association between chromosome 9p21.3 SNPs and vascular disease. Further studies are needed to disentangle the molecular mechanism(s) of the genotype-phenotype association.

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