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SHORT REPORT

Sequence variant on 9p21 is associated with the presence of abdominal aortic aneurysm disease but does not have an impact on aneurysmal expansion

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Abdominal aortic aneurysm (AAA) is among a number of vascular disorders to be recently associated with a common allelic variant situated on chromosome 9p21. To further assess the significance of this region of the genome in AAA development, we genotyped the sequence variation tagged by rs10757278 in two geographically independent cohorts of patients and compared them to matched controls. We also assessed the impact of this variant on AAA growth rate in cohorts with a median surveillance period of 3.2 and 4.5 years. Using meta-analysis to combine the findings of both cohorts, we found a significant association between rs10757278-G and the presence of AAA (OR (95%CI) 1.38 (1.04–1.82) $P=0.03$), an effect size completely consistent with that originally reported. rs10757278 was not significantly associated with altered AAA growth rate in either cohort.
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Introduction

Recently, a sequence variant on 9p21, tagged by rs10757278-G, was reported to be associated with the presence of abdominal aortic aneurysm (AAA; Odds ratio (OR) 1. 31 (1.22–1.42)) and intracranial aneurysm (OR 1.29 (1.16–1.43)) in a large multiple cohort study.[1] Previously, genome-wide association studies had identified an association between this variant and myocardial infarction and coronary artery disease (CAD).[2] Some pathological characteristics and risk factors for these disorders are shared, such as medial wall remodelling, atherosclerosis and hypertension, but there are distinct clinical and histological differences. In identifying this shared association with an as yet undetermined area of the genome, a search has begun to find a biological pathway/system that has a wide-reaching influence on arterial pathophysiology. Interestingly, an association of type 2 diabetes with a different variant in the same area of 9p21 has also been reported.[3–5]

We aimed to assess the association between AAA presence and growth with the 9p21 variant, using rs10757278 in two geographically distinct cohorts of subjects, while taking into account other risk factors for atherosclerotic disease.

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Materials and methods

Patients

Patients were recruited from AAA screening programs carried out in Chichester, UK and Perth, Australia. AAA

was defined by an infrarenal aortic diameter ≥ 3 cm, assessed using ultrasonography. Patients who had AAA were followed up with serial ultrasound scans to assess changes in maximum infrarenal aortic diameter. The Australian cohort was recruited from the Health in Men Study (HIMS) involving over 12 200 participants in a population-based trial of screening for AAAs in the state of Western Australia.[6] Cases were age matched to controls without AAAs and normal aortic diameter (19–22 mm) from the same study. In the UK cohort, cases were matched for age and male gender (using a homogeneous strata within each group[7]) with controls from the Northwick Park Heart Study II (NPHSII), a prospective study of middle aged men recruited from nine UK general practices[8] (for further details of recruitment, see Jones *et al*[8]).

Genotyping

Rs 10757278 was genotyped using TaqMan technology (Applied Biosciences (ABI), Warrington, UK) in the UK cohort, and the homogeneous MassEXTEND (hME; Sequenom) assay in the WA cohort.

Statistical analysis

Cases and controls were compared within cohorts using additive and dominant models. ORs were obtained from logistic regression models for the WA cohort and conditional logistic regression for the UK cohort. rs10757278 and CAD are associated and so are CAD and AAA. A subanalysis was performed in subjects who did not have a history of CAD to assess this potential confounding effect. Age, smoking history, hypertension, diabetes and hypercholesterolaemia (detailed in Table 1) were adjusted for with multiple logistic regression analysis. A random effects meta-analysis was performed to combine cohorts. AAA growth rate was estimated using the flexible hierarchical model described by Brady *et al*.[9]

Results

Case–control series from the United Kingdom and Western Australia were examined in this study, resulting in comparison of a total of 741 cases and 1366 controls (Table 1). rs10757278G allele variation was not significantly associated with AAA in either case–control series when analysed separately (in the United Kingdom, OR = 1.21 (0.81–1.82))and in the Western Australia, OR = 1.40 (0.96–2.05; Table 2), but in the combined cohorts (excluding AAA subjects with known CAD), G-allele carriers had higher risk of AAA disease, than AA subjects (OR 1.38 (95% CI; 1.04–1.82) $P = 0.03$, data adjusted for potential CHD confounders). In both cohorts, excluding those with CAD, rs10757278 was not associated with altered AAA expansion (in the United Kingdom, 0.21 mm (−0.33 to 0.76) $P = 0.45$, in the Western Australia, 0.30 mm (−0.11 to 0.72) $P = 0.15$). Combining expansion data for both the cohorts did not reach statistical significance ($P = 0.11$).

Discussion

Individually the UK and not the WA case *vs* control cohort were sufficiently powered to assess an association between AAA and rs10757278 (in the United Kingdom, 88.7% and in WA, 73.1% power to detect a difference at 5% significance, based on the findings of Helgadottir *et al*[1]). However, the reported association between rs10757278-G and AAA,[1]was only confirmed statistically ($P > 0.03$) in the combined group with an overall effect of 1.38 (1.04–1.82),

Table 1 Demographics of the UK and WA cohorts

	UK men			*WA men*		
	Controls (N = 985)	*AAA cases excluding IHD (N = 269)*	*AAA cases including IHD (N = 386)*	*Controls (N = 381)*	*AAA cases excluding IHD (N = 205)*	*AAA cases including IHD (N = 355)*
Age, mean (SD)	72.0 (2.7)	70.3 (4.0)	70.4 (3.9)	73.4 (4.5)	73.4 (4.5)	73.4 (4.4)
Hypertension %	53.8	68.2**	62.2**	44.9	49.8*	55.1**
Diabetes %	7.7	7.9	8.9	7.7	10.9	12.0
Hypercholesterolaemia %	20.7	56.1**	51.2**	27.7	32.2	44.0*
Smoking history %						
Ever %	66.1	91.2**	90**	65.5	82.9**	87.7**
History of CAD, % (N)	27.3	0	30.5	19.0	0	39.3**
Median initial AAA diameter (IQR)		35 (30–43)	35 (30–43)		34 (31–38)	34 (31–39)
Median final AAA diameter (IQR)		51 (37–57)	51 (38–57)		41 (35–47)	41 (35–47)
Median surveillance period (IQR)		3.0 (1.2–6.4)	3.2 (1.4–6.9)		4 (2–5.5)	4.5 (2–5.5)
Median growth rate (95% reference range)		2.1 (−1.0 to 5.3)	2.2 (−1.5 to 5.9)		1.25 (−1.5 to 4.0)	1.27 (−1.8–4.3)

*$P < 0.01$ and **$P < 0.001$ when compared with control group.

Table 2 Genotype distributions, allele frequencies and odds ratios (ORs) of rs10757278A>G case–control comparisons in the two cohorts

	Genotype	*Controls excluding IHD (UK age/sex stratified)*	*AAA cases excluding IHD*	*OR (95% CI)*[a]	*Controls including IHD (UK age/sex stratified)*	*AAA Cases including IHD*	*OR (95% CI)*[a]
UK	AA	200 (28.3)	64 (24.3)	1.00	256 (26.0)	96 (25.7)	1.00
	AG	352 (49.9)	139 (52.9)	1.24 (0.86–1.79)	510 (51.8)	190 (50.8)	1.20 (0.78–1.85)
	GG	154 (21.8)	60 (22.8)	1.30 (0.83–2.01)	219 (22.2)	88 (23.5)	1.24 (0.75–2.04)
				$P=0.43$			$P=0.65$
	G allele freq. (95% CI)	0.467 (0.441–0.494)	0.492 (0.449–0.536)	GG+GA *vs* AA, 1.26 (0.88–1.79) $P=0.20$	0.481 (0.459–0.504)	0.489 (0.453–0.526)	GG+GA *vs* AA, 1.21 (0.81–1.82) $P=0.36$
WA	AA	71 (25.6)	36 (18.3)	1.00	100 (25.6)	75 (21.6)	1.00
	AG	135 (48.7)	110 (55.8)	1.67 (1.04–2.70)	186 (47.6)	189 (54.3)	1.49 (1.00–2.22)
	GG	71 (25.6)	51 (25.9)	1.45 (0.84–2.49)	105 (26.9)	84 (24.1)	1.23 (0.78–1.94)
				$P=0.10$			$P=0.08$
	G allele freq. (95% CI)	0.500 (0.458–0.542)	0.538 (0.487–0.588)	AG+GG *vs* AA, 1.59 (1.01–2.52) $P=0.05$	0.506 (0.477–0.542)	0.513 (0.475–0.551)	AG+GG *vs* AA, 1.40 (0.96–2.05) $P=0.08$

[a]Adjusted for covariates (Table 1).

demonstrating the importance of combining cohorts to improve power in candidate gene analysis. When patients with CAD were excluded from the analysis as a potential confounder, the sizes of the effects were essentially unchanged. The size of the association found in our study is consistent with that of Helgadottir *et al*[1](Figure 1), suggesting that the original report did not overestimate the true effect, as has been described for other associations.[10] Combining the published data and ours (3558 cases and 18108 controls), results in an overall is OR = 1.31 (1.22–1.41, $P<0.0001$).

We also assessed the relationship between the 9p21 variant and AAA growth rate using the previously employed multilevel model.[9] Helgadottir *et al*[1] reported a borderline significant association between the rs10757278-G allele and slower AAA growth in the UK small aneurysm trial cohort (AAA growth GG compared with AG −0.46 mm/year, $P=0.05$), and suggested that the sequence variation predisposed to atherosclerosis (important in AAA) formation but subsequently slowed AAA growth. This theory was supported by previous data from the UK small aneurysm trial, which was associated reduced ankle pressure with slower AAA progression.[9] In contrast, in both of our cohorts (combined power >80% to detect 0.46 mm/year decrease in AAA growth rate, at the 5% level), there was no significant association between AAA growth and rs10757278-G allele. The smaller initial mean aortic diameters and longer surveillance periods in our patients than patients in the UK small aneurysm trial may explain the difference between these observations; clearly larger studies will be needed to confirm any genotype effect on AAA growth, which at best appears to be modest. This can only be achieved through the collaboration of AAA surveillance data.

Our confirmation of the association between AAA and the 9p21 variant has added to the urgency to understand

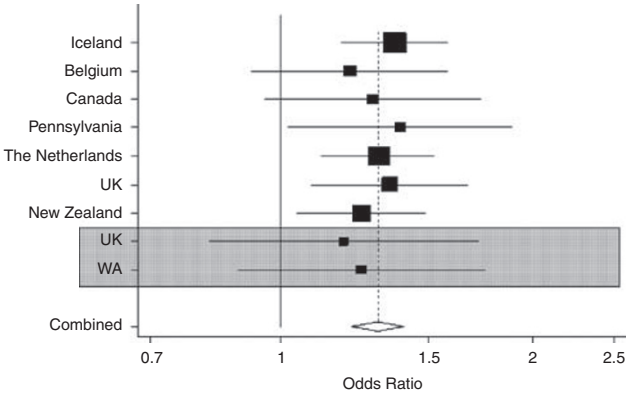

Figure 1 Meta-analysis combining odds ratios for rs10757278-G and AAA association, with current data (highlighted in grey) and previously published data.[1] Combined odds ratio is 1.31 (1.22–1.41) $P<0.0001$. Test for heterogeneity between studies $P=0.99$ (3558 cases and 18108 controls).

this highly significant region of the genome for both occlusive and degenerative vascular disease. It might be expected that different genes would play a role in the progression of disease, which in any case appears to be more complex to assess.

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