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CLINICAL RESEARCH
Heart failure/cardiomyopathy

A genetic variant on chromosome 9p21 and incident heart failure in the ARIC study

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Aims Recent studies showed that polymorphisms on chromosome 9p21 are associated with coronary heart disease (CHD), but few studies examined the association with heart failure (HF), stroke, or other subclinical atherosclerotic diseases. We tested the association of chromosome 9p21 polymorphisms with non-coronary atherosclerotic diseases.

Methods and results We studied 4018 African-American and 11 085 white participants from the Atherosclerosis Risk in Communities Study, aged 45–64 at baseline (1987–89). We examined associations of rs10757274 and rs2383206 polymorphisms with incident HF through 2005 and ischaemic stroke through 2004, and with prevalent carotid atherosclerosis and peripheral artery disease (PAD) at baseline. The GG genotype of rs10757274 was associated with increased HF risk for whites. This association seemed independent of the established link between rs10757274 and clinical CHD, although an impact of rs10757274 on subclinical CHD leading to HF is not eliminated. Among whites, GG homozygotes were at weakly increased carotid atherosclerosis risk. There seemed to be no associations for ischaemic stroke or PAD. The results were essentially similar for rs2383206.

Conclusion The GG genotype of rs10757274 on chromosome 9p21, which has been shown to increase CHD risk, is also associated with increased HF risk among whites. It is weakly or not associated with several other atherosclerosis outcomes.

Keywords Epidemiology • Cohort study • Genetics • Congestive heart failure • Atherosclerosis

Introduction

Recently, two reports[1,2] independently identified a relatively strong association of coronary heart disease (CHD) in whites with a common allele represented by single-nucleotide polymorphisms (SNPs) (rs2383206, rs10757274, rs10757278, or other nearby SNPs within a genomic region of ~100 kb). This genomic region contains no annotated genes, but is located near the *CDKN2A/B* gene on chromosome 9p21. This locus seems to increase CHD risk independently of traditional coronary risk factors and independently of a nearby SNP (rs10811661) associated with diabetes.[3–5] A recent report showed that this variant may also increase the risk of aortic and cerebral aneurysms,[6] thus implicating altered vascular structural integrity as one causal mechanism. More recently, another study indicated that this area of SNPs influences carotid atherosclerosis development and progression.[7] To further explore the link between this chromosome 9p21 variant and cardiovascular disease, we prospectively examined the associations of the rs10757274 and rs2383206 polymorphisms, which were identified by a previous consortium,[1] including Atherosclerosis Risk in Communities (ARIC), with incident heart failure (HF) and ischaemic stroke, as well as the cross-sectional prevalence of carotid atherosclerosis and peripheral artery disease (PAD) in the ARIC cohort.

Methods

Study cohort

The ARIC study cohort comprised a community-based sample of 15 792 persons from four research field centres: Forsyth County,

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NC; the city of Jackson, MS (African-Americans only); northwestern suburbs of Minneapolis, MN; and Washington County, MD. Participants were 45–64 years of age at the baseline examination (1987–89). The ARIC study protocol was approved by each field centre's institutional review board. After written informed consent was obtained, participants underwent a baseline clinical examination. Only African-Americans and whites were included in the present study.

Participants were genotyped for the rs10757274 and rs2383206 single-nucleotide polymorphism (SNPs) using the TaqMan assay system (Applied Biosystems, Foster City, CA, USA), as reported previously.[1] The agreement between replicate pair was 98.2% for rs10757274 and 94.2% for rs2383206. Methods for measuring other risk factors in the ARIC study have been described elsewhere.[8] For exclusion, we defined prevalent CHD as the presence of a myocardial infarction on the baseline electrocardiogram, or a self-reported history of myocardial infarction or coronary revascularization; stroke as a reported history of physician diagnosed stroke; and HF as reported current medication use for HF, or having manifest HF as defined by Gothenburg criteria[9] stage 3.

Carotid intima-media thickness (IMT) was measured using B-mode ultrasound during the baseline examination in the bilateral common (optimal angle), bulb, and internal carotid arteries IMT, using a standardized protocol.[10] For this analysis, we used the mean of these six values. Carotid atherosclerosis was defined as IMT >1.0 mm or the presence of carotid plaque or shadowing. Ninety-three per cent of participants had IMT measurement. Ankle–brachial blood pressure index was also measured, using one random leg, in the baseline examination for 96% of participants. An ankle–brachial index of <0.9 and/or the presence of claudication based on the Rose Intermittent Claudication questionnaire[11] was considered PAD.

Participants were followed for incident cardiovascular events, through 2004 for stroke and through 2005 for CHD and HF, via annual telephone contact and surveillance of hospital and death records. Cases of HF, ischaemic stroke, and CHD were identified and classified.[12–14] Briefly, incident HF was defined by the first HF hospitalization (ICD-9 code 428 in any position), or any deaths where the death certificate included an HF code (code 428, ICD-9 or I50, ICD-10, in any position). Ninety-four per cent of cases were identified by hospitalization and 6% by death certificate.[12] Non-hospitalized, non-fatal HF was not captured. Strokes were classified according to published criteria based on the occurrence and duration of neurological signs and symptoms, the results of neuroimaging and other diagnostic procedures, and treatments provided. Strokes secondary to trauma, neoplasm, haematological abnormality, infection, or vasculitis were not counted, and a focal deficit lasting <24 h was not considered a stroke. Incident CHD was defined as a definite or probable hospitalized myocardial infarction, silent myocardial infarction by electrocardiogram, or coronary revascularization.[14]

Statistical analyses

Of the 15 744 African-Americans and whites in ARIC, we excluded persons with missing DNA or no consent for DNA use ($n = 44$ excluded). Persons with missing SNP data were also excluded ($n = 597$ for rs10757274 and $n = 1133$ for rs2383206). Thus, we included 15 103 subjects (4018 African-American and 11 085 whites) in the rs10757274 analysis. Differences in baseline characteristics according to rs10757274 SNPs were tested by analysis of variance, χ^2, or Kruskal–Wallis rank tests. For incident HF and stroke analyses, we further excluded persons who reported a baseline history of CHD, stroke or HF, or missing HF ($n = 1723$ excluded). For incidence analyses involving rs10757274, 13 380 (3499 African-Americans and 9881 whites) subjects were followed-up from baseline in 1987–89 to the first endpoint, death, loss to follow-up, or the end of 2004 or 2005. Age- and sex-adjusted and multivariable-adjusted hazard ratio (HR) and 95% confidence interval (CI) of HF and ischaemic stroke were calculated according to genotypes using race-specific Cox proportional hazard models. The proportional hazards assumption was tested using cross-product terms with survival time and was not violated for any SNPs. A few covariates in the multivariable models violated the proportional hazards assumption, but this did not affect the SNP-phenotype results. Linearity of covariates included in the multivariable Cox models with estimated hazards was confirmed by testing and rejecting quadratic terms for each continuous covariate, as they did not affect the SNP associations. The HR for HF was also calculated after censoring for incident CHD. For prevalent carotid atherosclerosis and PAD, no further exclusions were made (i.e. prevalent CHD, stroke, HF, carotid atherosclerosis, and PAD were included in the analysis), and odds ratio (OR) and 95% CIs were calculated according to genotypes using race-specific logistic regression models. For rs10757274 analyses, 14 009 subjects had IMT data, and 14 544 subjects had PAD information. Differences in mean carotid IMT were tested by analysis of covariance, and means were presented in the mean population profile for all covariates. In the multivariable models, we adjusted for baseline values of age, sex (dichotomous), body mass index, systolic blood pressure, antihypertensive medication use (dichotomous), plasma total and high-density lipoprotein cholesterol, diabetes (dichotomous), smoking status (never, past, or current), cigarette years, ethanol and energy intake, education level (below high school graduation, high school graduation, or above that), and sports index (0.0–1.9, 2.0–2.4, 2.5–2.9, 3.0–5.0). Because the studied SNPs have been additively associated with CHD,[1] we assumed an additive model in the present analyses, and trends in associations were tested using the number of alleles as continuous variables (i.e. 0 for AA, 1 for AG, 2 for GG). Interactions of baseline age (45–54 or 55–64), sex, current smoking, prevalent obesity (defined as body mass index ≥ 30 kg/m^2), hypertension (systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg and/or use of antihypertensive medication), diabetes (fasting glucose ≥ 126 mg/dL or non-fasting glucose ≥ 200 mg/dL, and/or history of diagnosed diabetes and/or use of diabetic medication), hypercholesterolaemia (plasma cholesterol of ≥ 240 mg/dL or use of cholesterol-lowering medication) with rs10757274 genotypes in relation to incident HF were tested using cross-product terms. Free software[15] was used to create forest plots (Supplementary material online, figures). We used SAS version 9.1.3 Service Pack 4 (SAS Institute Inc., Cary, NC, USA) for the analyses. All probability values for statistical tests were two-tailed and values of $P < 0.05$ were regarded as statistically significant except for interaction tests. For interactions, we regarded $P < 0.007$ ($= 0.05/7$) as significant since we stratified by seven effect modifiers without *a priori* hypotheses.

Results

Distributions of the rs10757274 and rs2383206 genotypes did not deviate from Hardy–Weinberg equilibrium for either African-Americans ($P = 0.11$ for rs10757274 and $P = 0.61$ for rs2383206) or whites ($P = 0.67$ for rs10757274 and $P = 0.12$ for rs2383206). Other than race, few baseline risk factors differed by genotype (*Table 1*). At baseline, 690 participants had PAD (prevalence = 5%) and 4350 had carotid atherosclerosis (prevalence = 31%). During a median of 17 years of follow-up (inter-quartile range = 16–18), 1331 incident HF events (6.4 per

Table 1 Baseline characteristics according to rs10757274 genotypes, ARIC, 1987–89

Number	rs10757274							
	African-Americans				Whites			
	AA 2482	AG 1330	GG 206	*P*-value[a]	AA 2921	AG 5517	GG 2647	*P*-value[a]
Age (year)	53.4 (5.8)	53.6 (5.8)	54.2 (6.0)	0.11	54.3 (5.8)	54.4 (5.7)	54.3 (5.8)	0.86
Male (%)	37.6	38.8	40.3	0.63	47.6	47.1	46.7	0.79
Body mass index (kg/m^2)	29.6 (6.1)	29.7 (6.1)	29.3 (6.1)	0.60	27.2 (5.0)	27.0 (4.8)	26.9 (4.8)	0.01
Systolic blood pressure (mmHg)	129 (21)	129 (22)	128 (23)	0.86	119 (17)	118 (17)	118 (17)	0.39
Diastolic blood pressure (mmHg)	80 (12)	80 (12)	79 (13)	0.24	72 (10)	71 (10)	71 (10)	0.27
Antihypertensive medication (%)	43.8	43.1	40.8	0.68	25.2	25.8	26.7	0.45
Plasma total cholesterol (mg/dL)	215 (45)	214 (46)	215 (46)	0.97	215 (39)	215 (41)	214 (40)	0.39
Plasma HDL cholesterol (mg/dL)	55 (18)	54 (17)	54 (17)	0.30	50 (17)	51 (17)	50 (16)	0.71
Plasma triglyceride (mg/dL)[b]	96 (71–133)	97 (72–135)	98 (72–137)	0.60	116 (82–168)	114 (82–163)	114 (82–164)	0.74
Diabetes (%)	19.5	19.1	23.2	0.39	8.9	9.2	9.1	0.88
Sports index (range 1–5)[b]	2.0 (1.8–2.5)	2.0 (1.8–2.5)	2.0 (1.8–2.5)	0.96	2.5 (2.0–3.0)	2.5 (2.0–3.0)	2.5 (2.0–3.0)	0.04
Education (>high school graduate, %)	37.0	38.3	35.1	0.60	46.9	46.8	45.8	0.65
Current smoking (%)	29.4	30.4	31.4	0.71	24.9	24.9	24.4	0.88
Cigarette-years[b]	10 (0–345)	56 (0–400)	0 (0–375)	0.11	120 (0–600)	150 (0–620)	163 (0–600)	0.27
Alcohol intake (g/week)[b]	0.0 (0.0–13.2)	0.0 (0.0–0.0)	0.0 (0.0–13.2)	0.51	0.0 (0.0–51.8)	0.0 (0.0–54.2)	0.0 (0.0–51.8)	0.39
Total energy intake (Kcal/day)	1593 (622)	1571 (618)	1594 (647)	0.59	1651 (608)	1635 (600)	1626 (608)	0.30

Values are means (standard deviations) or percentages, except for variables indicated with 'b'.
[a]*P*-values for overall difference.
[b]Median (inter-quartile range) presented.

1000 person-years) occurred. For ischaemic stroke, 524 events (2.6 per 1000 person-years) occurred during a median of 16 years of follow-up (inter-quartile range = 15–17).

As *Table 2* shows, compared with the AA genotype, the risk of HF was greater among carriers of the GG genotype of rs10757274 among whites [multivariable HR = 1.30 (1.07–1.58)]. A similar tendency was observed for African-Americans, but was not statistically significant. The association observed in whites was slightly attenuated, but still statistically significant, when CHD events were censored at their occurrence. Ischaemic stroke and PAD were not associated with rs10757274 genotype in either African-Americans or whites. A weak association was observed for carotid atherosclerosis among whites [OR for GG genotype = 1.14 (1.00–1.29)]. For rs2383206, the associations were similar to those for rs10757274 (Supplementary material online, *Table*). These two SNPs were highly linked with each other among whites ($r^2 = 0.86$) and modestly among African-Americans ($r^2 = 0.36$).

Since associations were primarily observed between rs10757274 and HF, we further tested the interactions of genotype with age, sex, current smoking, obesity, hypertension, diabetes, and hypercholesterolaemia (Supplementary material online, *Figures S1* and *S2*). In African-Americans, an association between rs10757274 and HF was observed among men, but not for women, although the interaction ($P = 0.02$) was not quite statistically significant by our $P < 0.007$ criterion. Among African-American men, the multivariable-adjusted HRs (95% CIs) for HF were 1.18 (0.84–1.65) for the AG genotype and 2.15 (1.23–3.76) for the GG genotype compared with the AA genotype. The respective HRs among African-American women were 0.81 (0.62–1.06) and 1.08 (0.60–1.96). No other tested interaction reached statistical significance.

Discussion

We report here that the GG genotype of rs10757274, which increases susceptibility to CHD,[1] is also associated with increased risk of HF among whites in ARIC. This SNP was weakly associated with prevalent carotid atherosclerosis among whites. No such association was observed for incident ischaemic stroke or prevalent PAD. The results were similar for rs2383206 due to a high correlation with rs10757274 among whites. To our knowledge, this is the first report to examine an association between this area of chromosome 9p21 and incident HF.

The association between the rs10757274 polymorphism and CHD was first discovered in a genome-wide association study which included ARIC data.[1] This association, or that involving a highly linked nearby SNP such as rs10757278,[2] has been consistently replicated.[6,7,16–23] Our finding for HF seems concordant with these results for CHD, because HF is often a consequence of CHD. Surprisingly, though, when we censored for incident cases of CHD in addition to excluding prevalent CHD at baseline, the association did not attenuate greatly. Thus, it can be speculated that the GG genotype of rs10757274 may contribute to HF independently of clinical CHD events. Yet, this should be interpreted cautiously, because it is likely that unmeasured or unreported subclinical ischaemia contributed to many ARIC HF events even after censoring reported CHD occurrence. The relationship between rs10757274 and HF should be confirmed by further studies, along with a search for plausible mechanisms, such as possible effect on myocardial remodelling.

Similar, but non-significant, associations were observed for HF among African-Americans. Non-significant results could be explained by smaller sample sizes, since only 32 HF events occurred among African-Americans with the GG genotype. Indeed, the HR of HF among African-Americans, namely 1.39 (0.94–2.08), was somewhat higher than in whites, although not statistically significant ($P = 0.10$). On the other hand, it is also possible that rs10757274 does not associate with CHD or HF in African-Americans. The relatively low correlation ($r^2 = 0.36$) between rs10757274 and rs2383206 in African-Americans suggests that these SNPs are not tag-SNPs for the culprit genetic variant responsible for CHD. The fact that these SNPs have not linked to incident CHD in previous ARIC and Dallas Heart Study analyses among African American,[1] nor to the other atherosclerotic diseases presented here, may support this explanation. Unfortunately, our study could not resolve this issue because of a very low frequency of risk genotypes among African-Americans. A larger study of African-Americans is needed to confirm our observation.

A very recent study reported that the rs1333049 SNP, which was highly correlated with rs10757274 ($r^2 = 0.89$), was associated with the prevalence and progression of carotid atherosclerosis.[7] Though the number of cases was relatively small, they also reported non-significant associations with CHD (HR for homozygotes of risk allele = 1.4, $n = 56$) and cerebral/peripheral artery events (HR = 1.4, $n = 63$). On the other hand, another study[6] showed the rs10757278 SNP to be associated not only with CHD but also with abdominal aortic and intracranial aneurysms (OR $\approx$ 1.3). Consistent with our findings, that study also showed little association with large artery atherosclerotic/embolic stroke or PAD. Concordantly, some other case–control studies[24,25] found no or weak associations of rs10757274, rs2383206, or nearby SNPs with ischaemic stroke, though one found several haplotypes of 9p21 SNPs that may increase ischaemic stroke risk.[25] Another Finnish study showed no associations of rs1333049 SNP with carotid IMT or impaired brachial artery flow-mediated dilatation.[26] Taken together, this area of chromosome 9p21 can be speculated to contribute more to vascular remodelling or repair[6] than to cerebral or peripheral atherosclerosis. Our results showing no association of rs10757274 with PAD and ischaemic stroke, as well as a weak association with carotid atherosclerosis, may support this hypothesis. ARIC has quite limited information on aneurysms (i.e. only unvalidated aortic aneurysm hospital discharge diagnoses, validated subarachnoid haemorrhages and date of hospitalization, and few cases especially in African-Americans). In a supplementary analysis (not shown), we found no association between rs10757274 and aneurysms in this limited data set: multivariable HR = 1.19 (0.85–1.69) for AG and 1.16 (0.77–1.75) for GG genotypes among whites.

Some limitations to our study warrant mentioning. Firstly, the HF diagnosis was based on unvalidated hospitalized or fatal events with HF ICD codes. Yet, previous evidence suggests that most HF cases are eventually hospitalized and the validity of HF ICD codes is high.[27] Secondly, ankle–brachial blood pressure index was assessed on a single leg, meaning the PAD diagnosis

Table 2 Race-specific hazard and odds ratios (95% confidence intervals) of incident heart failure, stroke, and prevalent peripheral artery disease and carotid atherosclerosis for rs10757274 genotypes, ARIC, 1987–2004

	rs10757274							
	African-Americans				Whites			
	AA	AG	GG	*P*-value for trend[a]	AA	AG	GG	*P*-value for trend[a]
Incidence								
Number at risk (*n*)	2174	1149	176		2628	4913	2340	
HF (*n*)	291	157	32		204	432	215	
At risk (person-years)	32 975	17 250	2581		41 793	77 976	37 021	
Age- and sex-adjusted HR	1.00	1.01 (0.83–1.22)	1.38 (0.96–1.99)	0.26	1.00	1.14 (0.97–1.35)	1.22 (1.01–1.48)	0.04
Multivariable HR[b]	1.00	0.96 (0.78–1.18)	1.39 (0.94–2.08)	0.48	1.00	1.17 (0.98–1.38)	1.30 (1.07–1.58)	0.008
HF censored at incident CHD	228	123	24		136	286	141	
At risk (person-years)	32 059	16 806	2542		40 240	74 267	35 101	
Age- and sex-adjusted HR	1.00	1.00 (0.80–1.25)	1.29 (0.85–1.97)	0.45	1.00	1.16 (0.94–1.42)	1.23 (0.97–1.56)	0.08
Multivariable HR[b]	1.00	0.94 (0.75–1.20)	1.40 (0.90–2.18)	0.56	1.00	1.17 (0.95–1.45)	1.28 (1.00–1.62)	0.046
Ischaemic stroke (*n*)	141	64	13		77	170	59	
At risk (person-years)	31 529	16 607	2466		40 077	74 703	35 601	
Age- and sex-adjusted HR	1.00	0.84 (0.63–1.13)	1.15 (0.65–2.03)	0.63	1.00	1.20 (0.92–1.57)	0.89 (0.63–1.25)	0.60
Multivariable HR[b]	1.00	0.83 (0.60–1.15)	1.12 (0.60–2.09)	0.59	1.00	1.23 (0.94–1.62)	0.93 (0.66–1.31)	0.79
Prevalence								
PAD cases[c]	121	82	10		116	235	126	
Number of participants (*n*)	2374	1274	198		2820	5312	2566	
Age- and sex-adjusted OR	1.00	1.27 (0.95–1.70)	0.94 (0.49–1.83)	0.32	1.00	1.07 (0.86–1.35)	1.20 (0.92–1.55)	0.17
Multivariable OR[b]	1.00	1.17 (0.86–1.60)	0.68 (0.31–1.51)	0.93	1.00	1.01 (0.80–1.28)	1.10 (0.84–1.44)	0.49
Carotid atherosclerosis cases[c]	621	351	58		843	1643	834	
Number of participants (*n*)	2191	1148	184		2757	5212	2517	
Age- and sex-adjusted OR	1.00	1.10 (0.93–1.29)	1.08 (0.78–1.51)	0.29	1.00	1.05 (0.95–1.17)	1.14 (1.01–1.28)	0.04
Multivariable OR[b]	1.00	1.08 (0.91–1.29)	1.03 (0.71–1.48)	0.48	1.00	1.02 (0.92–1.14)	1.14 (1.00–1.29)	0.047
Carotid IMT (mean[d] and 95% CI, mm)								
Age- and sex-adjusted	0.73 (0.72–0.73)	0.73 (0.72–0.74)	0.73 (0.71–0.76)	0.36	0.72 (0.72–0.73)	0.73 (0.72–0.73)	0.72 (0.71–0.73)	0.87
Multivariable[b]	0.73 (0.72–0.73)	0.73 (0.72–0.74)	0.73 (0.70–0.75)	0.62	0.72 (0.72–0.73)	0.72 (0.72–0.73)	0.72 (0.72–0.73)	0.87

HR, hazard ratio; HF, heart failure; CHD, coronary heart disease; PAD, peripheral artery disease; OR, odds ratio; IMT, intima-media thickness; CI, confidence interval.
[a]Trends were tested by additive models (assigning 0 for AA, 1 for AG, and 2 for GG genotypes).
[b]Adjusted for age, sex, body mass index, systolic blood pressure, antihypertensive medication use, plasma total and high-density lipoprotein cholesterols, diabetes, smoking status, cigarette-years, ethanol and energy intake, education level, and sports index. Subjects missing covariates were eliminated.
[c]PAD was defined as presence of claudication or ankle–brachial index <0.9; carotid atherosclerosis was IMT >1.0 mm or the presence of plaque or shadowing.
[d]Means are presented at the mean population profile for all covariates.

would have high specificity but some PAD cases may have been missed. However, since the number of true negatives (no PAD) greatly outnumbers the small number of expected false negatives, this misclassification should not affect the 9p21 SNP association with PAD very much. Thirdly, although larger than for African-Americans, the numbers of ischaemic strokes ($n = 306$) and PAD ($n = 477$) among whites were relatively small. Thus, it is possible that we missed a modest association of 9p21 SNPs with ischaemic stroke or PAD, as the previous study found a weak association (OR = 1.14) for PAD.[6] Fourthly, the genotyping success rates of 92.8–96.2% were not very high, which could potentially cause genotyping bias and might obscure associations. Last, the present study could not separate different aetiologies of HF. Although the observed gene–HF associations seemed to be independent of CHD, this observation is not definitive because we undoubtedly missed some silent or subclinical CHD, as discussed earlier. Replication in other populations is needed to confirm whether our finding of this gene–HF association is truly independent of CHD.

In conclusion, in this prospective study, the GG genotype of rs10757274 on chromosome 9p21 was associated with increased risk of HF among whites independently of its association with CHD. This SNP was not appreciably associated with ischaemic stroke, PAD, or carotid atherosclerosis. Our observation might support the hypothesis that this area of chromosome 9p21 contributes to cardiovascular diseases partly via non-atherosclerotic pathways.

Supplementary material

Supplementary Material is available at *European Heart Journal* Online.

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