Association of arginase 1 gene polymorphisms with the risk of myocardial infarction and common carotid intima-media thickness

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Background: Recently, it was suggested that arginase (ARG)1 plays an important role in atherogenesis. However, because of its complex functions depending on vascular cell type, its impact on atherogenesis remains unclear.

Objective: To evaluate the association between ARG1 polymorphisms and phenotypes related to atherosclerosis.

Methods: Among 10 ARG1 polymorphisms selected from databases, 4 single-nucleotide polymorphisms (rs2781666; rs2781667; rs2781668; rs17599586) were tested for association with myocardial infarction (MI) in a case-control study (350 cases vs 581 controls), and with common carotid artery (CCA) intima-media thickness (CCA-IMT) in an independent sample of 963 subjects (Etude du Vieillissement Artériel (EVA) study).

Results: The genotype distribution of the rs2781666 G/T polymorphism differed significantly between MI cases and controls ($p=0.005$), and the risk of MI was consistently increased for both GT heterozygotes (OR (95% CI) 1.5 (1.1 to 2.0)) and TT homozygotes (OR (95% CI) 2.2 (1.1 to 4.4)). In the EVA study, the rs2781666 polymorphism was also associated with an increase in CCA-IMT ($p=0.010$), a surrogate marker of MI.

Conclusions: The ARG1 rs2781666 polymorphism was consistently associated with MI and an increased CCA-IMT. These findings reinforce the hypothesis of a significant role of ARG1 in vascular pathophysiology.

The aetiology of coronary artery disease (CAD) is complex, and results from interactions between environmental risk factors and individual genetic predispositions. As the overall genetic contribution to CAD has been estimated to range from 20% to 60%,¹ it is essential to establish the genetic basis of CAD to understand the pathophysiology of this disease.

Some very recent studies support a role for arginase (ARG)1 in the initiation, development and complications of CAD.^{2–6} In hepatocytes, as a partner of the urea cycle, ARG1 catalyses the synthesis of L-ornithine and urea from L-arginine, but the ARG1 gene (*ARG1*) has also been shown to be expressed in various cell types. Teupser *et al*² designated *ARG1*, located on 6q23, as a candidate gene that might modulate individual susceptibility to atherosclerosis. In that study, subtractive suppression hybridisation was used to screen for genes that were differentially expressed in macrophages obtained from two strains of rabbits with genetically determined high and low predisposition to atherosclerosis. *ARG1* was shown to be expressed at higher levels in macrophages with low atherosclerotic response than in those with high atherosclerotic response, and the level of expression was correlated with

increased enzymatic activity. These results suggested a protective role for *ARG1* overexpression in the development of atherosclerosis. Consistent with these findings, Thomas *et al*⁷ showed evidence for a decreased *ARG1* gene expression in foam cells compared with non-foamy cells obtained from rabbits. Both studies were of particular interest because *ARG1* was identified as a candidate gene following strategies without any preconceptions about the genes potentially involved in atherogenesis. Nevertheless, other studies suggested that *ARG1* overexpression might be deleterious. Notably, it has been proposed that enhanced ARG expression and/or activity may contribute to endothelial dysfunction in various cardiovascular disorders including atherosclerosis.^{3–6,8} Finally, the role of ARG1 in atherogenesis may result from conflicting actions in the different vascular cell types.

In this context, we attempted to approach the putative role of ARG1 in humans by evaluating its potential implication in clinical manifestations of CAD. First, we searched for associations between *ARG1* polymorphisms and myocardial infarction (MI) in a case-control study (350 cases and 581 controls).⁹ Second, in the longitudinal Etude du Vieillissement Artériel (EVA) study ($n=963$), we examined whether genetic variations in *ARG1* were associated with common carotid artery (CCA) intima-media thickness (CCA-IMT), considered as an indicator of generalised atherosclerosis.^{10–13}

METHODS

Populations

Case-control study of MI

A sample of 350 cases of MI was drawn from the European Action on Secondary Prevention by Intervention to Reduce Events (EUROASPIRE) study.¹⁴ Patients were enrolled from hospitals of the Urban Community of Lille (Northern France) during the two EUROASPIRE surveys conducted in 1995–6 and in 1999–2000, respectively. Both surveys were conducted in two stages. In the first stage, consecutive patients with an established MI were retrospectively identified from hospital admission lists, diagnostic registers, hospital discharge lists or other sources. In the second stage, patients were contacted at least 6 months after their initial hospitalisation to participate in the EUROASPIRE study. About 30% of subjects selected in the first stage did not participate in the second stage, and the reasons for not participating were: refusal to participate or no response (43.5%), dead or not contacted (47.5%) and other

Abbreviations: ARG, arginase; CAD, coronary artery disease; CCA, common carotid artery; CCA-IMT, CCA intima-media thickness; DBP, diastolic blood pressure; EUROASPIRE, European Action on Secondary Prevention by Intervention to Reduce Events; EVA, Etude du Vieillissement Artériel; LD, linkage disequilibrium; MI, myocardial infarction; NO, nitric oxide; NOS, NO synthase; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism; VSMC, vascular smooth muscle cells

reasons (9.0%). There was no significant difference by gender between participants and non-participants (79.5% vs 75.7% males, respectively). The non-participants were slightly older than the participants (mean (SD) 60.5 (9.5) vs 58.2 (9.7) years, respectively). Of the 350 selected patients with MI, 18.6% had had a coronary bypass graft and 36.3% had had a percutaneous transluminal coronary angioplasty. For all subjects, the major cardiovascular risk factors were collected.

The control group comprised subjects recruited within the framework of the World Health Organization MONICA (Multinational monitoring of trends and determinants of cardiovascular disease) project. Subjects were randomly sampled from the electoral rolls of the Urban Community of Lille between 1995 and 1997.⁹ The overall response rate was 73.4%. No significant difference by gender (50.3% vs 50.7% males, respectively) and age (mean (SD) 51.3 (8.4) vs 52.2 (8.7) years, respectively) could be detected between participants and non-participants. Information about personal habits, medical history and drug intake was obtained using a standardised questionnaire. Subjects with a history or symptoms of CAD were excluded from this population-based sample, which was representative of the general population, and 585 controls, matched by age and gender to the MI cases from the EUROASPIRE study, were selected.

For both cases and controls, hypertension was defined by a systolic blood pressure (SBP) ≥ 140 mm Hg and/or a diastolic blood pressure (DBP) ≥ 90 mm Hg and/or by use of anti-hypertensive drugs. SBP and DBP were measured twice, with an interval of at least 5 min, in a sitting position, after resting for at least 5–10 min. A mercury sphygmomanometer was used for controls, and digital electronic tensiometers were used for cases. About 40% of cases and 23% of controls were under antihypertensive treatment. Subjects were considered as diabetic or hypercholesterolaemic if they used antidiabetic or hypolipemiant drugs, respectively, and/or if the disease(s) was reported during the survey. Only subjects with fully available phenotypic and genetic data were considered for further analyses (350 cases and 581 controls).

The EVA study

The EVA study¹⁵ is a longitudinal study on vascular ageing and cognitive decline, composed of subjects aged from 59 to 71 years who were recruited from the city of Nantes (Western France), starting from the electoral rolls. Subjects were contacted both through the mail and, to a lesser extent, through information campaigns. When a subject was recruited, his or her spouse was systematically asked to participate in the study if he or she was in the required age range. The EVA cohort is composed of volunteers who differ slightly from the general population aged 60–70 years in several respects. In all, 14% of the EVA participants have had ≥ 12 years of schooling, as compared with 7% of the general population; they also have a higher socioeconomic status. Overall, the proportion of men (41.1%) is similar to that in the general population aged 60–70 years (39.9%). During the baseline visit (January 1992–July 1993), subjects had high-resolution ultrasound examinations of the CCAs, including measurements of CCA-IMT at sites free of plaques.¹⁵ The mean of four right and left CCA-IMT measurements was used in the analysis. In the EVA study, hypertension was defined by an SBP ≥ 140 mm Hg and/or a DBP ≥ 90 mm Hg, and/or by antihypertensive treatment. SBP and DBP were measured twice, with an interval of at least 5 min, in a sitting position, after at least 5–10 min of rest, using a digital electronic tensiometer. In the EVA study, about 25% of subjects took antihypertensive drugs. Diabetes was defined by the use of antidiabetic drugs and/or a fasting plasma glucose level ≥ 7 mmol/l; hypercholesterolaemia was defined by the use of

hypolipemiant drugs and/or a fasting plasma cholesterol level ≥ 6.2 mmol/l. Subjects with missing data were excluded from the statistical analyses. The population sample was composed of 963 subjects.

All study protocols were approved by the local ethics committees, and written informed consent was obtained from all subjects. Table 1 shows the main phenotypic characteristics of the participants of the MI case-control study and the EVA study.

Single-nucleotide polymorphism selection and genotyping

By combining data from the National Center for Biotechnology Information/single-nucleotide polymorphism (SNP; <http://www.ncbi.nlm.nih.gov/SNP>) and the HapMap project (<http://www.hapmap.org>) databases, a total of 10 SNPs spaced throughout the ARG1 gene were chosen on the basis of their location and/or putative functionality (fig 1). Three SNPs, named rs2781659, rs2781665 and rs2781666, were located within the 5' promoter sequence. Interestingly, rs2781665 and rs2781666 were putative binding sequences for transcription factors. A similar argument guided the selection of rs2781667 and rs2781668, located in intron 1. The SNPs rs115444111 and rs1063493 were selected because they encoded non-synonymous substitutions in the peptide sequence (Gly→Glu and Gln→Glu, respectively). Finally, three polymorphisms, rs2297637, rs17599586 and rs1803151, permitted coverage of the 3' region of the ARG1 gene. Moreover, in the HapMap database, rs2781668 and rs17599586 were defined as tag SNPs for other polymorphisms (rs2781668 for rs2246012; rs17599586 for rs3850245 and rs17657829).

The 10 selected SNPs were genotyped by enzymatic digestions after PCR amplifications (supplementary table 1, available at <http://jmg.bmj.com/supplemental>).

Statistical analysis

The Hardy-Weinberg equilibrium was tested separately in controls from the MI core-control study and in the EVA study. In the MI case-control study, genotype distributions between cases and controls were compared using Pearson's χ^2 test. The associations of the different SNPs with the risk of MI were assessed by multiple logistic regression analyses. In the EVA study, CCA-IMT means were compared between genotypes by an analysis of covariance that used a general linear model (General Linear Model procedure, type III test). The tests were performed assuming an allele-dose effect after the goodness of fit of the model had been evaluated by the likelihood ratio test. All the tests were adjusted for age, gender, body mass index, hypercholesterolaemia, hypertension, diabetes and smoking habits. For smoking habits, the subjects were classified as non-smokers and ever smokers. These analyses were carried out with the SAS release V.8.2 software. The pairwise linkage disequilibrium (LD) was estimated using the standard definition of r^2 . The haplotype frequencies were estimated by using a stochastic-EM algorithm implemented in the THESIAS software (freely available from <http://www.genecanvas.org>).¹⁶ The difference between estimated haplotype frequencies in MI cases and controls was evaluated by a likelihood ratio test. In the present work, four polymorphisms were selected for association studies. To take multiple testing into account, the Bonferroni correction was applied and a level of significance of $p < 0.013$ was retained.

RESULTS

Genetic data and selection of relevant SNPs for association studies

The 10 ARG1 SNPs were genotyped in a randomly selected subgroup of 186 subjects participating in the EVA study,

	MI case-control study		EVA study n = 963
	MI n = 350	Controls n = 581	
Age (years)	55.5 (8.1)	55.2 (8.1)	65.1 (3.0)
Men (%)	84.6	81.4	41.4
BMI (kg/m ²)	28.7 (4.9)*	27.1 (4.8)	25.5 (3.8)
Current or former smokers (%)	80.6*	63.7	32.6
Diabetes (%)	22.6*	7.1	6.7
Hypertension (%)	58.0	55.6	44.9
Hypercholesterolaemia (%)	73.7*	31.7	69.2

BMI, body mass index; EVA, Etude du Vieillessement Artériel; MI, myocardial infarction.
*p<0.001, cases vs controls.

allowing to determine allele frequencies (supplementary table 1, available at <http://jmg.bmj.com/supplemental>) and pairwise LD (fig 1). The data obtained enabled the selection of the most relevant *ARG1* SNPs for association studies. As their minor allele frequencies were found to be <5%, rs11544411, rs1063493, rs2297637 and rs1803151 were excluded from the analyses. Among the remaining polymorphisms, rs2781659, rs2781665 and rs2781667, were in complete LD ($r^2=1$), indicating that any one of these three SNPs could reflect the same haplotype block of *ARG1*. Among these three SNPs, we selected rs2781667, because it potentially introduced a binding site for nuclear factor κ B, a feature that would be of importance in CAD. The remaining rs2781666, rs2781668 and rs17599586 polymorphisms were independant ($0.03 \leq r^2 \leq 0.56$ for rs2781666, $0.02 \leq r^2 \leq 0.56$ for rs2781668 and $0.02 \leq r^2 \leq 0.17$

for rs17588586). Hence, the *ARG1* SNPs selected were rs2781666, rs2781667, rs2781668 and rs17599586.

Association of ARG1 SNPs with the risk of MI

The four selected *ARG1* SNPs were characterised in the 350 MI cases and in the 581 controls. Genotype distributions fulfilled the Hardy-Weinberg equilibrium. Associations between the *ARG1* SNPs and MI were assessed by comparing the genotype distributions between MI cases and controls (table 2). No significant difference in genotype distributions could be observed for rs2781667, rs2781668 and rs17599586. Conversely, genotypes carrying the minor rs2781666 T allele were statistically more common in MI cases than in controls ($p = 0.005$). Assuming an allele-dose effect, the logistic regression analysis showed that the rs2781666 T allele was associated with a

Figure 1 Single-nucleotide polymorphism (SNP) location and pairwise linkage disequilibrium (LD) between polymorphisms in *ARG1*. Pairwise LD was calculated for polymorphisms with minor allele frequencies above 5%. These polymorphisms are listed below the diagram. Shading of the diamonds represents the magnitude and significance of the pairwise r^2 between the two SNPs, defined by the top left and the top right sides of the diamond, with black reflecting high r^2 ($r^2 > 0.8$) and white reflecting low r^2 ($r^2 < 0.2$). The eight exons are indicated by black boxes. (A) Polymorphisms with a minor allele frequency <5%, (B) The rs2246012 T/C SNP was tagged by the rs2781668 C/T SNP according to the HapMap database. (C) The rs3850245 C/T and the rs17657829 T/C SNPs were tagged by the rs17599586 T/C SNP according to the HapMap database.

Table 2 Comparison of genotype distributions of the *ARG1* single-nucleotide polymorphisms between cases of myocardial infarction and controls

	Controls	Cases	p Value*	p Value†	Adjusted OR (95% CI)‡
rs2781666					
GG	385 (66.3)	198 (56.6)	0.005	0.001	1
GT	180 (31.0)	133 (38.0)			1.5 (1.1 to 2.0)
TT	16 (2.7)	19 (5.4)			2.2 (1.1 to 4.4)
rs2781667					
CC	274 (47.1)	146 (41.7)	0.160	0.058	1
CT	263 (45.3)	168 (48.0)			1.2 (0.9 to 1.7)
TT	44 (7.6)	36 (10.3)			1.3 (0.7 to 2.3)
rs2781668					
CC	404 (69.5)	240 (68.6)	0.597	0.991	1
CT	160 (27.5)	103 (29.4)			1.0 (0.7 to 1.4)
TT	17 (2.9)	7 (2.0)			0.6 (0.2 to 1.7)
rs17599586					
CC	435 (74.9)	281 (80.3)	0.059	0.142	1
CT	139 (23.9)	62 (17.7)			0.8 (0.5 to 1.1)
TT	7 (1.2)	7 (2.0)			1.3 (0.4 to 4.9)

Values are n (%) unless otherwise specified.

*Pearson's χ^2 test.

†Logistic regression analysis performed assuming an allele-dose effect.

‡Adjusted for age, gender, body mass index, hypercholesterolaemia, hypertension, diabetes and smoking habits.

significantly increased risk of MI (OR (95% CI) 1.5 (1.2 to 1.9); $p = 0.001$). The increase in the risk of MI could be observed both in rs2781666 GT heterozygotes (OR (95% CI) 1.5 (1.1 to 2.0)) and in rs2781666 TT homozygotes (OR (95% CI) 2.2 (1.1 to 4.4)). Our results suggested that significant associations of *ARG1* polymorphisms with MI were restricted to rs2781666. However, taking LD into account, the impact of rs2781666 could be, at least partly, modulated by the three other SNPs. In order to define the independent effect of each polymorphism on MI, we performed a haplotype analysis (table 3).

Association of *ARG1* haplotypes with MI risk

As a consequence of LD, of the 16 theoretical haplotypes predicted by the 4 SNPs, only 4 could be observed with a frequency above 5%. The most common GCCC haplotype, which was composed of the common rs2781666 G, rs2781667 C, rs2781668 C and rs17599586 C alleles, respectively, was considered as the reference haplotype. The haplotype distribution was statistically different between MI cases and controls ($p = 0.005$). The difference could be mainly explained by the frequency of the TTCC haplotype (9.4% in MI vs 5.5% in controls, $p = 0.001$). As a consequence, compared with subjects carrying the GCCC reference haplotype, the risk of MI in patients with the TTCC haplotype was estimated at 1.8 (95% CI 1.1 to 2.7; $p = 0.008$) after adjustment for covariates. These

results suggested that the effect of rs2781666 on the risk of MI was independent of rs2781668 and rs17599586. Unfortunately, because the TCCC haplotype was not represented in our samples, we were unable to estimate whether the impact of rs2781666 was independent or not of rs2781667.

Association of *ARG1* SNPs with CCA-IMT in the EVA study

In order to support the previous findings in an independent study, we tested the association of the four selected *ARG1* SNPs with CCA-IMT, a surrogate marker of MI.¹² In the EVA study, the genotype distributions of the four SNPs fulfilled the Hardy-Weinberg distribution (table 4).

Regarding the rs2781666 polymorphism, the mean CCA-IMT was significantly different between the three groups of genotypes after adjustment for covariates ($p = 0.010$), and the difference was mainly due to the increased CCA-IMT measured in the rs2781666 TT homozygotes ($p = 0.002$). No significant association of *ARG1* rs2781667 and rs17599586 with CCA-IMT was detected. Moreover, the comparison of the mean CCA-IMT between the rs2781668 genotypes did not reach statistical significance, and the trend could be attributable to the high degree of LD between rs2781666 and rs2781668.

Consistent with its association with the risk of MI, these data suggested that rs2781666 was associated with an increased

Table 3 Comparison of the distributions of the *ARG1* haplotypes between cases of myocardial infarction and controls

Haplotypes (%)	Controls, n = 1162	MI, n = 700	Adjusted OR (95% CI)*
GCCC	63.3	61.2	1
TTTC	12.2	14.3	1.1 (0.8 to 1.5)
GTCT	9.9	8.7	0.9 (0.6 to 1.2)
TTCC	5.5	9.4	1.8 (1.1 to 2.7)†

$p = 0.005$

MI, myocardial infarction.

Order of the SNPs in the haplotypes: rs2781666, rs2781667, rs2781668 and rs17599586; minor alleles are indicated in bold.

*Tests were adjusted for age, gender, BMI, hypercholesterolaemia, diabetes, smoking habits and hypertension.

† $p = 0.008$.

Table 4 Comparison of common carotid artery intima-media thickness by *ARG1* single-nucleotide polymorphisms in the Etude du Vieillissement Artériel study

Mean (SD)	n	CCA-IMT (mm)
rs2781666		
GG	641	0.657 (0.107)
GT	293	0.662 (0.118)
TT	29	0.718 (0.162)
p Value		0.017
Adjusted p value*		0.010
rs2781667		
CC	444	0.653 (0.107)
CT	410	0.664 (0.112)
TT	109	0.675 (0.131)
p Value		0.128
Adjusted p value*		0.075
rs2781668		
CC	703	0.656 (0.108)
CT	236	0.666 (0.119)
TT	24	0.720 (0.156)
p Value		0.016
Adjusted p value*		0.040
rs17599586		
CC	721	0.660 (0.114)
CT	219	0.662 (0.106)
TT	23	0.662 (0.120)
p Value		0.980
Adjusted p value*		0.902

*Adjusted for age, gender, body mass index, hypercholesterolaemia, diabetes, hypertension and smoking habits.

CCA-IMT. Haplotype analysis did not allow us to identify one or more specific *ARG1* haplotypes associated with the phenotype (data not shown).

DISCUSSION

In this study, we present the first evidence that genetic variations in *ARG1* may have an impact on atherosclerosis in humans. In the French population, of the 10 SNPs selected from databases, 4 were considered as relevant for association studies, and one of them, the rs2781666 polymorphism, was unambiguously associated with an allele-dose-dependent increased risk of MI and a consistently increased CCA-IMT. Interestingly, CCA-IMT has been proposed as a reliable marker for both the presence and the degree of atherosclerosis,^{10 17} and prospective studies have shown a positive association between increased CCA-IMT and the risk of MI.^{12 13} Results from two independent epidemiological studies consistently showed that an increase of 0.16 mm in CCA-IMT was associated with a 43% increase in the risk of MI.^{12 18} Hence, results obtained in the EVA study were consistent with those obtained in the MI case-control study and reinforce the possible role for *ARG1* in atherogenesis. As a limitation of the MI case-control study, when comparing genotype frequencies between cases and controls, we could not take into account the ancestry or ethnic origin of the subjects because we could not collect these data in France. However, to avoid a potential bias, MI cases and controls were recruited from the same geographical area—that is, the Urban Community of Lille (Northern France).

Pathophysiological hypotheses strongly argue for a significant role of *ARG1* in atherogenesis. Both in animals and humans, the expression of *ARG1* was detected in various cell types in the vascular wall, in addition to the liver, where *ARG1* participates in the urea cycle. In animal models, constitutive *ARG* activity and immunoreactivity were observed in endothelial cells^{8 19} and vascular smooth muscle cells (VSMC).^{20 21} Mori et al²² showed that *ARG1* expression was induced by lipopoly-

saccharide stimulation in rat macrophages. *ARG1* is also expressed and is functionally active in human endothelial cells.²³ However, alterations in the expression of *ARG1* in the vascular wall cells may have complex effects on the atherogenesis according to cell types. It is generally admitted that *ARG* is a key regulator of nitric oxide (NO) signalling, counteracting NO synthase (NOS) activity by limiting their shared substrate. On the one hand, *ARG1* overexpression may be deleterious in endothelial cells, in leading to decreased NO production by the endothelial NOS.^{19 24} Consistent with this, *ARG1* knockdown was shown to enhance NOS activity and restore the endothelial NO signalling in ageing rat vessels.²⁵ On the other hand, *ARG1* overexpression might be atheroprotective in macrophages and VSMC, in causing decreased peroxynitrite production by the inducible NOS.²⁶

Importantly, ornithine, the primary metabolite of the *ARG* pathway, may itself play an important part in atherogenesis. Ornithine feeds into the synthesis of L-proline, and thus is a precursor of collagen biosynthesis.²⁷ In addition, ornithine is involved in the formation of polyamines that play an important role in the mitogenic response of VSMC.^{4 28} Thus, various metabolites of ornithine play an important part in the matrix and cellular composition of the atherosclerotic plaque, a critical feature for plaque vulnerability.^{28 29} Therefore, although the contribution of *ARG* expression to atherosclerosis in each of the vascular cell types is still unknown, quantitative variations in *ARG* expression and/or activity seem to modulate the overall susceptibility to the disease.

Significant and consistent associations of *ARG1* polymorphisms were restricted to the rs2781666 SNP, which was associated with an increased risk of MI and an enhanced CCA-IMT. We propose that these results could be related to the previously described deleterious effect of *ARG1* overexpression, at least in endothelial cells.^{19 24} As the rs2781666 polymorphism is located in the promoter region of the gene, it might modify *ARG1* expression. In silico, an analysis of the putative functionality of this SNP using Genomatix/Matinspector³⁰ suggested that the rs2781666 minor allele could generate a binding site for the X-box binding protein 1, the aryl hydrocarbon receptor nuclear translocator and the tumour suppressor p53. Among these factors, p53 was of particular interest because it is activated in the atherosclerotic plaque. It regulates growth arrest, senescence and apoptosis of VSMC, and promotes apoptosis in macrophages.^{31–33} Thus, binding of p53 might lead to *ARG1* overexpression, potentially promoting both MI and thickening of intima-media. Nevertheless, functional experiments in various cell types will be needed to verify this hypothesis. Moreover, the possibility that the *ARG1* rs2781666 polymorphism may be in LD with another functional polymorphism located in the *ARG1* locus or in another gene nearby cannot be excluded.

Key points

- The arginase (*ARG*)1 gene promoter polymorphism rs2781666 was associated with the risk of myocardial infarction (MI) in a case-control study (350 cases and 581 controls).
- In an independent sample of 963 subjects, the rs2781666 polymorphism was consistently associated with an increased common carotid intima-media thickness, a surrogate marker of MI.
- This study supports recent data suggesting a role for *ARG1* in vascular pathophysiology.

In conclusion, our findings suggest that genetic variations in *ARG1* may predispose to MI and intermediate phenotypes, and reinforce our interest in studies focusing on the role of *ARG1* in vascular pathophysiology.

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Supplementary table 1 is available online at <http://img.bmj.com/supplemental>

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