ORIGINAL ARTICLE

VEGF polymorphisms and severity of atherosclerosis

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Introduction: Vascular endothelial growth factor (VEGF) is a potent angiogenic factor, and neovascularisation has been shown to be important in atherosclerotic plaque development. There is some disagreement as to whether VEGF acts as a pro-atherosclerotic or anti-atherosclerotic factor. In the present study we have sought to clarify this by determining genotypes and haplotypes for three reportedly functional *VEGF* SNPs in a large series of well documented coronary atherosclerosis patients.
Methods: *VEGF* −2578, −1154, and −634 single nucleotide polymorphisms were genotyped in 984 subjects from the Southampton Atherosclerosis Study, using the 5′ nuclease assay for allelic discrimination (*Taq*Man).
Results: *VEGF* −2578 genotypes showed a significantly different distribution in patients without myocardial infarction when stratified according to number of diseased arteries. *VEGF* −2578 was also associated with mean number of stenotic segments in the same patient group. The AA genotype was a risk factor and CC was protective. These associations were significant before and after adjustment for classic risk factors, and were reflected in associations between *VEGF* haplotypes and the number of diseased arteries and stenotic segments. As *VEGF* −2578 CC has been provisionally shown to be associated with higher VEGF expression than the AA genotype, these results are consistent with a protective effect for VEGF in atherosclerosis development. Some changes in *VEGF* −1154 genotype frequencies were also detected, but no significant associations were detected for any one particular genotype.
Conclusions: This study provides preliminary evidence that *VEGF* polymorphism is associated with development of atherosclerosis, possibly via regulation of VEGF expression, supporting a protective effect for VEGF in atherosclerosis. These results require replication in an independent study group, combined with study of additional candidate polymorphisms in the *VEGF* gene.

Atherosclerosis, the underlying pathology of coronary heart disease, is a common multifactorial disorder with both genetic and environmental risk factors. Its heritability is estimated to be 50–60%, but its genetic basis remains incompletely understood,[1] although a number of genes involved in inflammation, lipid metabolism, coagulation, and the regulation of vascular tone have been associated with various atherosclerosis related phenotypes. Functional polymorphisms in candidate genes regulating other biological processes relevant to atherosclerotic plaque development and progression require similar investigation.

Vascular endothelial growth factor (VEGF), a mitogen that promotes vascular endothelial cell proliferation and angiogenesis, is a 45 kDa glycoprotein secreted in the vascular wall by endothelial and smooth muscle cells. Although VEGF has been considered relatively specific for endothelial cells,[2] it also influences monocyte activation and migration, and vascular smooth muscle cell migration.[3–5] In atherosclerotic plaques, monocytes/macrophages are significant producers of VEGF, along with other growth factors and cytokines that have a central role in atherogenesis. Despite this, there remains debate in the literature as to whether VEGF is a pro-atherosclerotic or anti-atherosclerotic factor. For example, it has been reported that administration of recombinant human (rh) VEGF to APOE deficient cholesterol fed mice and rabbits enhances atherosclerotic plaque progression,[6] while VEGF has been shown to be necessary for the development of atherosclerosis and enhances cardiac allograft atheroclerosis in rat models.[7 8] Conversely, a number of studies have shown that administration of transgenic or recombinant VEGF to injured arteries leads to accelerated re-endothelialisation, which in turn leads to a marked reduction of intimal thickening and/or mural thrombus formation.[9–13] These findings are consistent with VEGF functioning as an endogenous regulator of endothelial integrity in the arterial wall.[14]

Several single nucleotide polymorphisms (SNPs) have been described in the *VEGF* gene, some of which have been reported to be associated with differential expression of VEGF in vitro.[15–18] Two of these SNPs (positions −2578 and −1154) are located in the *VEGF* promoter,[15 18] and one SNP (position −634) is located in the 5′ untranslated region of the gene (position +405 after transcription initiation site).[16 19] In order to elucidate the role of VEGF in the development of atherosclerosis, we investigated whether these SNPs correlate with severity of coronary atherosclerosis in a large cohort of patients diagnosed with coronary angiography. The analyses were carried out firstly in the sample as a whole, and then after excluding those patients who had had a myocardial infarction (MI). This approach was followed because MI is commonly caused by thrombus formation, and the subsequent incorporation of the thrombus into the atherosclerotic lesion can make a significant contribution to atherosclerotic lesion growth,[20] which might or might not (depending upon the magnitude of any VEGF genotypic effect) confound the effect of VEGF on lesion development and progression.

Abbreviations: MI, myocardial infarction; rh, recombinant human; SNP, single nucleotide polymorphisms; VEGF, vascular endothelial growth factor

Table 1 Characteristics of the subjects

Age (years)	63.29 (9.967)
Male sex	76.6%
Current and ex-smokers	74.5%
Body mass index (kg/m^2)	27.6 (4.25)
Plasma cholesterol (mmol/l)	5.11 (1.02)
Plasma triglyceride (mmol/l)	1.82 (1.26)
Hyperlipidaemia	81.8%
Hypertension	44.8%
Type I diabetes	3.2%
Type II diabetes	10.2%
Family history of CAD	48.4%

Results are mean (SD) or percentage.

SUBJECTS AND METHODS

Subjects

From a cohort of 1178 individuals with coronary artery disease, documented angiographically as having >50% diameter stenosis in at least one major epicardial coronary artery and participating in the Southampton Atherosclerosis Study (SAS),[21] 1000 were included in the present investigation. All were white, and were recruited from consecutive patients undertaking diagnostic and interventional coronary arteriography in the Wessex Cardiothoracic Unit, Southampton General Hospital, during the period May 1999 to March 2002. The study was approved by the local research ethics committee, and all subjects gave written consent.

Demographic and clinical data were recorded, including age, sex, weight, height, occupation, smoking habit, and number of cigarettes consumed per day by each smoker, current medications, particularly the use of lipid lowering drugs, and the presence or absence of hyperlipidaemia (defined as cholesterol >5.2 mmol/l and/or triglyceride >3 mmol/l), hypertension (defined as diastolic blood pressure >95 mmHg and/or systolic blood pressure >160 mmHg), type I or type II diabetes, previous MI, and coronary heart disease in first degree relatives <65 years of age. Total cholesterol and triglyceride levels were measured by the clinical chemistry department of Southampton General Hospital, using standard quality controlled enzymatic methods. The characteristics of these patients are summarised in table 1.

Coronary angiograms were analysed by a consultant cardiologist. The coronary arteries, divided into 16 segments, were examined for the presence of stenosis. Of the 1178 patients, 479 had >50% stenosis in one coronary artery (one vessel disease), 397 had >50% stenosis in two coronary arteries (two vessel disease), and 302 had >50% stenosis in three coronary arteries (three vessel disease). Of the 1178 patients, 639 had suffered from an MI diagnosed according to standard clinical criteria with electrocardiographic and enzymatic changes.

Table 2 *VEGF* genotyping 5′ nuclease (*Taq*Man) primer and probe sequences

Primer/probe	Sequence
−2578 forward	5′TCAGTCCATGCCTCCACAGA3′
−2578 reverse	5′GGAACAAAGTTGGGGCTCTGA3′
−2578 A probe	VIC-TATCCACCCAGATCtTGCCAGGGTC-TAMRA
−2578 C probe	FAM-CCACCCAGATCgTGCCAGGGT-TAMRA
−1154 forward	5′CGGGCCAGGCTTCACTG3′
−1154 reverse	5′GGCGGGGACAGGCGA3′
−1154 A probe	VIC-CTCAGCCCtTCCACA-MGB-NFQ
−1154 G probe	FAM-CTCAGCCCcTCCAC-MGB-NFQ
−634 forward	5′TCCAGAGAGAAGTCGAGGAAGAGA3′
−634 reverse	5′CCCCAAAAGCAGGTCACTCA3′
−634 C probe	VIC-TGCCCCTGTCgCTTTCGCTG-TAMRA
−634 G probe	FAM-TTGCCCCTGTCcCTTTCGCTG-TAMRA

Positions of polymorphisms are marked in lower case. MGB, minor groove binding probe; TAMRA, fluorescence quencher labelled probe. Forward and reverse sequences are primers; A, C, and G are the probe alleles.

DNA extraction and storage

DNA was extracted from peripheral blood samples using the ''salting out'' method, and stored in 96 well plates at the concentration of 7 ng/μl.

VEGF SNP genotyping

VEGF −2578 (A/C), −1154 (G/A), and −634 (G/C) SNPs were genotyped using the 5′ nuclease assay for allelic discrimination. Primers and *Taq*Man probes were designed using Primer Express software (version 2.0; sequences shown in table 2) and synthesised and supplied by Applied Biosystems UK. The reporter dyes chosen were 6-FAM and VIC. Using the Applied Biosystems Allelic Discrimination PCR protocol, 5 μl PCR reactions containing 14 ng of DNA, 0.9 μmol primers, and 0.2 μmol probes (final concentrations) were performed in 384 well plates and run on the Applied Biosystems 7900 HT sequence detection system. PCR annealing temperatures were 62℃ for *VEGF* −2578 and −634, and 60℃ for *VEGF* −1154. Each genotyping plate contained no DNA template (water) controls and randomly selected duplicate samples. SDS version 2.0 software was used to analyse real time and endpoint fluorescence.

Statistical analysis

The χ^2 test and ordinal logistic regression analysis were used to examine differences in genotype frequency between patient groups with single, double, or triple vessel disease. Analyses of variance were performed to test differences in the number of stenotic segments in the coronary arteries, among different genotype groups. This was carried out firstly without, and then with, adjustment for classic coronary heart disease risk factors including age, sex, body mass index, smoking, hypercholesterolaemia, hypertension, and diabetes. The above analyses were carried out using SPSS software (version 11.5). Linkage disequilibrium between the SNPs, haplotype frequencies, and haplotypic effects on the number of diseased vessels, and the number of stenotic segments, were determined using the Thesias program (http://genecanvas.idf.inserm.fr/). Because the polymorphisms studied (which are in linkage disequilibrium) are not independent, nor are the phenotypes (number of diseased coronary arteries and number of stenotic segments), a Bonferroni correction for multiple tests is conservative and was not applied. Nevertheless, some of the associations reported (see Results) remained significant at the level implied by such a correction.

RESULTS

Genotypes were determined in 941 subjects for the *VEGF* −2578 SNP, 953 subjects for −1154, and 984 subjects for −634. In the SAS sample as a whole, the frequencies of *VEGF* genotypes were: −2578 AA 29.0%, AC 49.1%, CC 21.9%; −1154 GG 42.9%, AG 44.7%, AA 12.4%; and −634 GG 42.0%, CG 41.2%, CC 16.9%. *VEGF* −2578, −1154, and −634 genotypes were distributed in accordance with a close fit to Hardy-Weinberg equilibrium.

VEGF genotype distributions in patients with one, two, and three vessel disease are shown in table 3. There were no significant difference in distribution of *VEGF* −2578 genotypes between all patients with one, two, or three vessel disease; however, genotypes showed a significantly different distribution after excluding patients with MI ($p = 0.030$). In particular, the frequency of the AA genotype increased stepwise with number of involved vessels, compared with

Table 3 *VEGF* genotype frequencies in patients with one, two, and three vessel disease

		The sample as a whole					After excluding patients with MI				
SNP	Geno type	One vessel disease	Two vessel disease	Three vessel disease	OR (95% CI)	*OR (95% CI)	One vessel disease	Two vessel disease	Three vessel disease	OR (95% CI)	*OR (95% CI)
−2578	AA	107 (27.2%)	90 (27.7%)	76 (34.1%)	1.28 (0.92 to 1.79) p=0.149	1.29 (0.91 to 1.82) p=0.149	54 (25.8%)	42 (32.8%)	36 (38.3%)	2.27 (1.38 to 3.74) p=0.001	2.25 (1.35 to 3.77) p=0.002
	AC	194 (49.4%)	168 (51.7%)	100 (44.8%)	1.05 (0.77 to 1.42) p=0.755	1.07 (0.78 to 1.46) p=0.695	92 (44.0%)	61 (47.7%)	43 (45.7%)	1.75 (1.11 to 2.79) p=0.019	1.73 (1.07 to 2.82) p=0.026
	CC	92 (23.4%)	67 (20.6%)	47 (21.1%)	Ref.	Ref.	63 (30.1%)	25 (19.5%)	15 (16.0%)	Ref.	Ref.
	Total	393 (100%)	325 (100%)	223 (100%)			209 (100%)	128 (100%)	94 (100%)		
	χ^2 test		p=0.331					p=0.030			
−1154	GG	184 (45.9%)	131 (40.4%)	94 (41.2%)	0.82 (0.56 to 1.20) p=0.302	0.87 (0.60 to 1.31) p=0.544	107 (50.5%)	53 (40.8%)	33 (34.4%)	0.55 (0.32 to 0.94) p=0.029	0.65 (0.37 to 1.12) p=0.121
	AG	165 (41.1%)	164 (50.6%)	97 (42.5%)	0.96 (0.66 to 1.40) p=0.832	1.02 (0.69 to 1.50) p=0.926	77 (36.3%)	62 (47.7%)	43 (44.8%)	0.90 (0.53 to 1.52) p=0.686	0.99 (0.57 to 1.71) p=0.975
	AA	52 (13.0%)	29 (9.0%)	37 (16.2%)	Ref.	Ref.	28 (13.2%)	15 (11.5%)	20 (20.8%)	Ref.	Ref.
	Total	401 (100%)	324 (100%)	228 (100%)			212 (100%)	130 (100%)	96 (100%)		
	χ^2 test		p=0.024					p=0.028			
−634	GG	169 (44.0%)	144 (44.2%)	100 (46.3%)	1.14 (0.77 to 1.69) p=0.516	1.24 (0.83 to 1.86) p=0.303	82 (41.0%)	60 (45.8%)	44 (48.9%)	1.09 (0.61 to 1.92) p=0.776	1.18 (0.65 to 2.13) p=0.580
	CG	165 (43.0%)	151 (46.3%)	89 (41.2%)	1.09 (0.74 to 1.63) p=0.654	1.14 (0.76 to 1.72) p=0.515	92 (46.0%)	58 (44.3%)	32 (35.6%)	0.81 (0.46 to 1.44) p=0.480	0.89 (0.49 to 1.60) p=0.687
	CC	50 (13.0%)	31 (9.5%)	27 (12.5%)	Ref.	Ref.	26 (13.0%)	13 (9.9%)	14 (15.6%)	Ref.	Ref.
	Total	384 (100%)	326 (100.0%)	216 (100%)			200 (100%)	131 (100.0%)	90 (100%)		
	χ^2 test		p=0.544					p=0.406			

*Adjusted for age, sex, body mass index, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes. Ref., reference.

the CC genotype as reference. This association was significant both before (p = 0.001; odds ratio (OR) 2.27; 95% CI 1.38 to 3.74)) and after (p = 0.002; OR 2.25 (1.35 to 3.77)) adjustment for age, sex, BMI, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes. In these same patients, the CC genotype showed a stepwise decrease in frequency compared with the number of involved vessels. *VEGF* −1154 genotypes were also differently distributed among all atherosclerosis patients (p = 0.024) and in patients without MI (p = 0.028). Considering individual −1154 genotypes, none showed a significantly different distribution in the full patient series, but the GG genotype showed a stepwise decrease in frequency with number of involved vessels (p = 0.029; OR 0.55 (0.32 to 0.94) after excluding patients with MI. However, this association did not retain significance after adjusting for covariates. *VEGF* −634 genotypes were not differently distributed either when considering all patients or after excluding those with MI.

Association between *VEGF* genotypes and number of stenotic segments was then investigated and results are presented in Table 4. For the *VEGF* −2578 SNP in the total patient group, the mean number of stenotic segments differed according to genotype, with the highest number seen in patients with AA genotype (2.64) and the lowest in patients with CC (2.34). This difference was significant only after adjusting for age, sex, BMI, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes (p = 0.021). The same pattern of association was seen after excluding patients with MI, although in this case significance was achieved both before (p = 0.003) and after (p = 0.004) adjustment for the above variables. No associations between *VEGF* −1154 and −634 and the number of stenotic segments was demonstrated, with or without adjustment for the above risk variables.

VEGF−2578, −1154, and −634 haplotype frequencies are presented in table 5, and analysis of the relationship between *VEGF* haplotype and number of diseased coronary arteries is given in table 6. Among all patients, the CGG haplotype was associated with the smallest mean number of diseased vessels per haplotype (0.83 (0.76 to 0.92)), but this was only of borderline significance (p = 0.050) and was lost when adjusting for covariates. However, after excluding patients with MI, the CGG haplotype showed a stronger association with the lowest mean number of diseased vessels (0.71 (0.62 to 0.79)), both before (p = 0.001) and after (p = 0.002) adjusting for covariates. The second haplotype, bearing the −2578 C allele (CGC), showed the next lowest mean number of diseased vessels, an association that was significant in patients without MI (mean 0.83 (0.68 to 0.99)), both before (p = 0.014) and after (p = 0.033) adjusting for covariates.

Finally, the relationship between *VEGF* −2578, −1154 and −634 haplotype and number of stenotic segments was investigated. Results are presented in table 7. Among all patients, the CGG haplotype was associated with the lowest mean number of stenotic segments per haplotype (1.12; 0.98 to 1.27), although this association was only significant before adjustment for covariates (p = 0.031). However, among patients without MI, this same association with the lowest number of stenotic segments (0.96; 0.80 to

Table 4 Number of stenotic segments in different genotype groups

		The sample as a whole			After excluding patients with MI		
SNP	Genotype	No. of stenotic segments	p	p*	No. of stenotic segments	p	p*
−2578	AA	2.64 (1.41), n=268	0.072	0.021	2.57 (1.37), n=131	0.003	0.004
	AC	2.62 (1.46), n=449			2.57 (1.51), n=190		
	CC	2.34 (1.26), n=202			2.03 (1.12), n=101		
−1154	GG	2.46 (1.38), n=401	0.193	0.180	2.27 (1.32), n=188	0.070	0.157
	AG	2.64 (1.45), n=417			2.57 (1.46), n=179		
	AA	2.58 (1.45), n=114			2.53 (1.36), n=62		
−634	GG	2.57 (1.40), n=403	0.990	0.669	2.50 (1.32), n=182	0.690	0.737
	CG	2.55 (1.41), n=396			2.39 (1.42), n=179		
	CC	2.51 (1.33), n=106			2.48 (1.41), n=52		

Adjusted for age, sex, body mass index, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes.

1.46) was significant both before (p = 0.008) and after (p = 0.017) adjustment for covariates.

In all of the above comparisons, it should be noted that demonstrated associations in the whole study group or in patients without MI remained significant after excluding diabetic subjects from the analysis.

DISCUSSION

VEGF genotype frequencies in the SAS study group were in broad agreement with previous studies in other healthy white subjects,[15 16 18] including unselected population studies from the same geographical area, performed by our group.[22] Haplotype frequencies were also in broad agreement with those determined in an earlier study.[22] Comparisons of *VEGF* genotype and haplotype frequencies between patients with atherosclerosis and angiographically normal individuals remain to be determined.

Data obtained in this study show for the first time that *VEGF* polymorphism may regulate progression of atherosclerosis, particularly in subjects without a previous MI. In this context, results obtained for the *VEGF* −2578 SNP in patients without MI, both before and after adjusting for age, BMI, smoking status, lipid lowering treatment, hypertension, and diabetes, are of particular interest. Among these patients, the AA genotype increased in frequency with increasing number of involved vessels, while the CC genotype showed a similar decrease. In agreement with this, the CC genotype was associated with a smaller number of stenotic segments than AA or AC genotypes. This effect was seen not only in patients without MI, but also in the whole patient series after adjusting for the above covariates, although the association was more significant when patients with MI were excluded. In general, no associations were seen between the −1154 and −634 genotypes and number of involved vessels or stenotic segments, except for the −1154 GG genotype, which decreased in frequency with increasing number of involved arteries in patients without MI, but only before adjusting for covariates.

Table 5 *VEGF* haplotype frequencies in study group

Haplotype	Frequency
AAG	0.337338
AGG	0.169831
AAC	0.009001
AGC	0.013124
CAG	0.003936
CGC	0.304360
CGG	0.162410

These results indicating a possible role for the *VEGF* −2578 SNP (and/or polymorphisms in linkage disequilibrium with this SNP) are supported by our findings with respect to the −2578, −1154, and −634 haplotypes. For example, haplotypes carrying −2578 C (CGG and CGC) were associated with a lower mean number of diseased vessels (most significantly among patients without MI, both before and after adjusting for covariates) and stenotic segments (again, most significantly among patients without MI, both before and after adjusting for covariates). Although a Bonferroni correction of probability values was judged to be problematic and overly conservative when considering SNPs in linkage disequilibrium and linked phenotypes, it is of note that the associations between the −2578 SNP and CGG haplotype with the numbers of diseased vessels (p = 0.002) and stenotic segments (p = 0.004 and 0.017) in patients without MI were of such a magnitude that they would remain significant after Bonferroni correction.

It is also of note that most of the above associations were only observed in patients without a previous MI. This suggests that any *VEGF* genetic determination of severity of atherosclerosis is likely to be modest and masked by larger, independent risk factors, such as thrombus formation as a result of a MI. In agreement with this, the VEGF −2578 AA genotype was associated with an odds ratio for more involved vessels in patients without MI of 2.27 (or 2.25 after adjusting for covariates), compared with the −2578 CC genotype as reference. The corresponding odds ratios for the sample as a whole were 1.28 and 1.29 respectively, although the latter were not statistically significant. Additionally, in patients without MI, the −2578 SNP explained 2.0% of the variance of number of diseased vessels and 2.5% of the variance of

Table 6 *VEGF* haplotypes and number of diseased coronary arteries

Haplotypes			The sample as a whole			After excluding patients with MI		
−2578	−1154	−634	Mean (95% CI)	p	*p	Mean (95% CI)	p	*p
A	A	G	0.95 (0.90 to 1.00)	Reference	Reference	0.99 (0.91 to 1.06)	Reference	Reference
A	G	G	0.93 (0.87 to 0.99)	0.684	0.905	0.88 (0.75 to 1.02)	0.211	0.453
C	G	C	0.90 (0.79 to 1.01)	0.274	0.237	0.83 (0.68 to 0.99)	0.014	0.033
C	G	G	0.83 (0.76 to 0.92)	0.050	0.089	0.71 (0.62 to 0.79)	0.001	0.002

*Adjusted for age, sex, body mass index, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes.

Table 7 *VEGF* haplotypes and number of stenotic segments

Haplotypes			The sample as a whole			After excluding patients with MI		
−2578	−1154	−634	Mean (95% CI)	p	*p	Mean (95% CI)	p	*p
A	A	G	1.35 (1.25 to 1.45)	Reference	Reference	1.37 (1.23 to 1.51)	Reference	Reference
A	G	G	1.31 (1.21 to 1.42)	0.694	0.893	1.29 (1.03 to 1.55)	0.549	0.893
C	G	C	1.28 (1.07 to 1.48)	0.381	0.265	1.18 (0.89 to 1.46)	0.110	0.154
C	G	G	1.12 (0.98 to 1.27)	0.031	0.066	0.96 (0.80 to 1.46)	0.008	0.017

*Adjusted for age, sex, body mass index, smoking, hypercholesterolaemia, lipid lowering treatment, hypertension, and diabetes.

number of stenotic segments (these values are the R^2 values derived from analyses of variance).

Taken together, the above results indicate that the *VEGF* −2578 SNP and/or polymorphisms in linkage disequilibrium with this SNP are associated with the development of atherosclerosis, with the AA genotype a risk factor and CC protective. Correlations between *VEGF* genotype and expression were not examined in this study. However, in a single study of lipopolysaccharide stimulated peripheral blood mononuclear cells derived from healthy individuals, the CC genotype has been reported to be associated with a significantly higher production of VEGF than the AA genotype.[18] A further study showed that certain VEGF haplotypes, including the −2578 A allele, were associated with lower circulating VEGF levels than other VEGF haplotypes including the −2578 C allele.[23] However, this latter study did not obtain direct evidence for an effect of *VEGF* −2578 on VEGF transcriptional activity, but this was not excluded in the context of contribution to a haplotype mediated effect. While any extrapolation from these reports should be treated with caution, our results are consistent with the *VEGF* −2578 AA genotype acting as a risk factor for atherosclerosis development via association with lower VEGF production, and the CC genotype conferring protection via association with increased VEGF production. Therefore, results from this genetic study may be interpreted in support of a protective role for VEGF in atherosclerotic disease development. This is consistent with VEGF acting as an endogenous regulator of endothelial integrity in the arterial wall,[14] which is supported by a number of studies, including several showing that administration of transgenic or recombinant VEGF to injured arteries leads to accelerated re-endothelialisation and reduction of intimal thickening and/or mural thrombus formation.[9–13] However, it should be noted that plaque neovascularisation (which may be mediated in part by VEGF) promotes the growth of atheromas, as evidenced by studies using angiogenesis inhibitors such as angiostatin.[24] Therefore, the precise relationship between functional polymorphisms in the *VEGF* gene and development of atherosclerosis is likely to be complex. In addition, it is conceivable that patients with MI may have been exposed to genetic and/or environmental factors that not only increase risk of MI but also attenuate the protective effects of VEGF genotype on atherosclerotic lesion growth. This study therefore indicates that further investigation of the role of *VEGF* polymorphism in modulating development of atherosclerosis is required, which should include further SNPs in the *VEGF* gene, including the −460 and +936 SNPs, which have both been reported to be associated with differential VEGF expression. However, results from these studies are variable. The −460 SNP is found in linkage disequilibrium with the −634 SNP (also referred to as +405) and the latter SNP has shown variable associations with VEGF production, both alone[16 19] and in haplotypic association with the −460 SNP.[25] Reported associations between the −936 SNP and VEGF expression are similarly variable.[17 26] These studies have been performed in subjects of differing ethnicity and some of the variability may be attributed to variable linkage disequilibrium with other known and unknown polymorphisms in the *VEGF* gene, underlining the need for further study of *VEGF* polymorphism in atherosclerosis. The importance of examining genetic associations with disease in one ethnic group and confirming such associations in another is highlighted by conflicting reports with respect to VEGF genotypic associations with diabetic retinopathy in white and south Asian (Japanese) study groups.[19 27]

In summary, we report preliminary evidence that *VEGF* polymorphism is associated with development of atherosclerosis, possibly via regulation of VEGF expression, supporting a protective effect for VEGF in atherosclerosis. These findings require replication in an independent study group, combined with study of additional candidate polymorphisms in the *VEGF* gene.

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