eNOS and *ACE* genes influence peripheral arterial disease predisposition in smokers

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Objective: **Several biologic mediators and genetic predisposing factors may contribute to the development of peripheral arterial disease (PAD). The *eNOS* gene, encoding for endothelial nitric oxide synthase, has been proposed as a candidate gene in the predisposition to the disease. In this study, we evaluated the role of *eNOS-786T>C*, *-894G>T* and *4a/4b* polymorphisms as markers of PAD per se and in the presence of the *ACE D* allele in patients previously investigated.**

Methods: **We analyzed 281 consecutive patients (220 men, 61 women; median age, 72 years) with PAD and 562 healthy controls, comparable for sex and age.**

Results: **eNOS-*786C*, but not *-894T* and *4a*, allele frequency was significantly higher in PAD patients than in controls ($P = .03$). An association with the predisposition to PAD was found for the *eNOS-786C* allele (odds ratio [OR], 1.52; 95% confidence interval [CI], 1.11-2.09; $P = .009$) and the *eNOS-786C/4a* haplotype (OR, 1.41; 95% CI, 1.02-1.94, $P = .04$) at univariate analysis but not after adjustment for traditional risk factors. When smoking habit was considered, we observed that *eNOS-786C/4a* haplotype, but not the *eNOS-786C* allele, influenced PAD predisposition after adjustment for traditional risk factors in smokers (OR, 2.71; 95% CI, 1.38-5.30; $P = .004$). The *eNOS-786C* and *eNOS-786C/4a* haplotype did not modify the susceptibility to PAD in patients carrying the *ACE D* allele. Nevertheless, the presence of the *eNOS-786C/4a* haplotype increased PAD predisposition in smokers also carrying *ACE D* allele (OR, 2.71 to 3.79; $P > .05$ for interaction).**

Conclusions: **This study demonstrated an association between *eNOS* and *ACE* genes in increasing PAD susceptibility in smokers, thus providing evidence for a gene-environment interaction in modulating predisposition to the disease. (J Vasc Surg 2010;52:97-102.)**

Peripheral arterial disease (PAD), a common manifestation of systemic atherosclerosis, represents one of the different localizations of the atherosclerotic process. PAD is associated with an increased risk of cardiovascular events mainly related to the coexistence of coronary artery disease (CAD) and cerebrovascular disease.[1] Apart from traditional cardiovascular risk factors such as hypertension, smoking habit, dyslipidemia, and diabetes, several novel biologic mediators and genetic predisposing factors appear to be relevant in determining the atherogenic process.[1]

Endothelial dysfunction is a key step in the initiation and progression of atherogenesis, and nitric oxide (NO) is able to modulate most of the steps that are important in this process.[2] NO contributes to vascular tone regulation, inhibition of platelet aggregation, leukocyte adhesion to vascular endothelium, and inhibition of smooth muscle cell migration and proliferation. All of these actions likely prevent the development of the atherosclerotic plaque.[2]

NO is synthesized from L-arginine by at least three isoforms of NO synthase (NOS): inducible NOS, neuronal NOS, and endothelial (eNOS).[3] Experimental data concerning the role of eNOS responsible for NO synthesis in a *eNOS* knockout mice model demonstrated that, in the absence of eNOS, luminal remodeling is impaired and vessel wall thickness is doubled. These data suggested that endothelial-derived NO, in addition to its role as a vasodilator, might be involved in controlling vessel wall geometry.[4]

NO availability may be genetically determined, and there is documented evidence that a reduction in NO synthesis, associated with *eNOS* gene polymorphisms, is linked to endothelial dysfunction.[5] The *eNOS* gene (*7q35-q36*) exhibits several polymorphisms, some of which appear to be related to the variability in NO plasma levels. A substitution of guanine to thymine at nucleotide 894 in exon 7 of the *eNOS* gene (*894G>T* polymorphism) is associated with reduced basal NO production;[6] the rare *C* allele of the *-786T>C* polymorphism in the 5′-flanking region of the gene results in a significant reduction in *eNOS* promoter activity;[7] and, finally, a 27-base pair (bp) variable tandem repeat polymorphism in intron 4 (also called *eNOS 4a4b*) has been associated with variations in NO, nitrite, and nitrate plasma levels.[8] Recently, it has demonstrated a functional role for the *eNOS 4a4b* polymorphism: individuals carrying the *4a4a* genotype have lower NO-producing

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activity than individuals carrying the wild-type allele, even if higher *eNOS* messenger RNA levels were observed.[9]

We previously demonstrated a role for the *eNOS* gene in influencing atherosclerosis by modulating the predisposition to carotid atherosclerosis,[10] abdominal aortic aneurysm (AAA),[11] and CAD.[12] To the best of our knowledge, few data are available concerning the influence of the *eNOS* gene in modulating the susceptibility to PAD.[13]

We recently showed that the I/D polymorphism in the gene encoding for angiotensin-converting enzyme (ACE) is involved in modulating the predisposition to PAD.[1] To date, polymorphisms in genes encoding for renin angiotensin system components, such as the *ACE* gene, might contribute to increased angiotensin II levels, which may be crucial in the development of the atherosclerotic process. In particular, we recently documented[14] that the *ACE D/-240T* haplotype modulates PAD susceptibility, apart from other atherosclerotic localizations and traditional cardiovascular risk factors. ACE may affect the atherosclerotic process through bradykinin degradation and NO release reduction, thus possibly inducing PAD phenotype. A reduced NO availability, which may be also genetically determined, promotes endothelial dysfunction, one of the earliest processes in the development of atherosclerosis.[15] Owing to the polygenic nature of PAD, in the present study, we investigated the contribution of the *ACE* and *eNOS* genes as markers of the atherosclerotic process leading to PAD. Therefore, we analyzed *eNOS-786T>C, -894G>T,* and *4a/4b* polymorphisms, which are able to modulate NO availability, as predisposing factors to PAD per se and in the presence of the *ACE* D allele, which previously was demonstrated to influence PAD susceptibility.[14]

MATERIALS AND METHODS

All participants in this study gave informed consent. The study was complied with the Declaration of Helsinki and was approved by the local ethics committee.

Study population. The study population consisted of 281 patients previously investigated[14] with symptoms or signs suggestive for the presence of PAD, who were referred to the Unit of Vascular Surgery of the University of Florence, AOU-Careggi, to be evaluated for possible surgical intervention. This was a retrospective case-control association study. PAD was diagnosed when patients had typical symptoms of intermittent claudication, such as cramping pain of the calves or buttocks during exercise and an ankle-brachial index (ABI) of <0.90 at rest, calculated according to the recommendations of the American Heart Association.[16]

All patients were also evaluated for atherosclerotic disease at other locations. In particular, a cardiologic evaluation including electrocardiogram and echocardiogram was performed in all patients. In patients with symptoms potentially related to ischemic heart disease, additional studies were performed (echocardiogram with drug-induced stress testing, myocardial scintigraphy, and/or coronary angiography) according to American College of Cardiology/American Heart Association guidelines.[17] Carotid artery duplex scanning with color-coded echo-flow imaging was conducted according to North American Symptomatic Carotid Endarterectomy Trial criteria.[18] Rutherford categories were assigned as follows: class 2, moderate claudication; class 3, severe claudication; class 4, rest pain; and class 5-6, ulcers or gangrene.[19]

The patients were compared with a control group of 562 age- and sex-matched individuals without symptomatic PAD recruited from the staff of the University of Florence and the hospital or those who were partners or friends of patients. Expert physicians performed, in the frame of a physical examination, a detailed interview addressed to personal and familial history to identify disease-free controls and to exclude individuals who were thought to have any form of vascular disease (Appendix, online only).

Participants were considered to have hypertension if they had been diagnosed as hypertensives (systolic blood pressure ≥140 mm Hg, diastolic blood pressure ≥90 mm Hg) according to the guidelines of European Society of Hypertension/European Society of Cardiology[20] or were taking antihypertensive drugs. Dyslipidemia was defined as total cholesterol levels >190 mg/dL, low-density lipoprotein >115 mg/dL, high-density lipoprotein <46 mg/dL in women and <40 mg/dL in men, and triglyceride levels >150 mg/dL, according to the third report of the National Cholesterol Education Program (NCEP).[21] Diabetes was defined in agreement with the American Diabetes Association.[22] Smokers were defined as current or recent (ex-smokers who stopped ≤5 years previously). A positive family history was defined as the presence of at least one first-degree relative who had developed cardiovascular disease before the age of 55 years for men and 65 years for women.

Detection of *ACE* and *eNOS* polymorphisms. Genomic DNA was isolated from whole blood by using the FlexiGeneDNA kit (QIAGEN, Germany), which permits DNA purification through precipitation after isopropanol addition and recovery by centrifugation. Recovered DNA is washed in 70% ethanol, dried, and resuspended in a hydration buffer.

The *ACE* I/D polymorphism has been evaluated by polymerase chain reaction (PCR) and electrophoresis on agarose gel to permit genotyping discrimination (DD, ID, and II genotypes), as previously described.[23]

The *eNOS-786T>C (rs2070744)* and *-894G>T (rs1799983)* polymorphisms were evaluated through electronic microchip technology (NanoChip Molecular Biology Workstation; 10 × 10 array NanoChip cartridge; Nanogen, San Diego, Calif), a DNA microarray technology based on the application of an electric field that allows the rapid deposition of biotinylated PCR products, derived from the amplification of the genomic sequence containing each polymorphism through PCR reaction, on a streptavidin-coated array. The PCR products hybridize to fluorescent-specific probes to permit genotype discrimination.[24]

Table I. Demographic and clinical characteristics of the study populations

Variable	*Patients (n = 281)*	*Controls (n = 562)*	P *value*
Age, y[a]	72 (30-93)	71 (24-95)	.4
Males	220 (78)	422 (75.0)	.3
Hypertension	167 (59.4)	112 (19.9)	<.0001
Diabetes	47 (16.7)	28 (5.0)	<.0001
Dyslipidemia	135 (48)	107 (19.0)	<.0001
Smokers (current or recent)[b]	176 (62.6)	118 (21.0)	<.0001
Family history	56 (19.9)	34 (6.0)	<.0001
Coronary artery disease	70 (24.9)	. . .	. . .
Abdominal aortic aneurysm	34 (12.1)	. . .	. . .
Carotid atherosclerosis	28 (10.0)	. . .	. . .
Rutherford category			
2	28 (10.0)	. . .	. . .
3	188 (66.9)	. . .	. . .
4	48 (17.1)	. . .	. . .
5-6	17 (6.0)	. . .	. . .

[a]Age is presented as median (range); all other data are number (%).
[b]Ex-smokers who stopped <5 years previously.

Table II. Genotype distribution and allele frequencies of *eNOS* gene polymorphisms of the study populations[a]

Genotype	*Allele*	*Patients (n = 281) No. (%)*	*Controls (n = 562) No. (%)*	P *value*
eNOS				
−786TT		74 (26.3)	198 (35.2)	
−786TC		153 (54.4)	269 (47.9)	
−786CC		54 (19.2)	95 (16.9)	.03
	−786C	0.46	0.41	.03
894GG		120 (42.7)	228 (40.6)	
894GT		134 (47.7)	259 (46.1)	
894TT		27 (9.6)	75 (13.3)	.3
	894T	0.33	0.36	.2
4b/4ba		188 (66.9)	388 (69.0)	
4a/4b		81 (28.8)	152 (27.0)	
4a/4a		10 (3.6)	22 (3.9)	.8
	4a	0.18	0.17	.7

eNOS, Endothelial nitric oxide synthase.
-786TT: homozygotes for T allele; *-786TC:* heterozygotes for *T* and *C* alleles; *-786CC:* homozygotes for the rare allele C.
894GG: homozygotes for G allele; *894GT:* heterozygotes for *G* and *T* alleles; *894TT:* homozygotes for the rare allele *T.*
4b/4b: homozygotes for *4*b allele; *4a/4b:* heterozygotes for *4a* and *4b* alleles; *4a/4a*: homozygotes for the rare allele *4a.*
[a]Two patients heterozygous for the rare *d* allele were not included in the analysis.

The *eNOS 4a/4b* polymorphism was analyzed through PCR reaction as previously described.[12]

Statistical analysis. Statistical analysis was performed by using SPSS 11.5 software (SPSS Inc, Chicago, Ill). Continuous variables were expressed as median (range), and the nonparametric Mann-Whitney test for unpaired data was used for comparison between single groups, as appropriate. The χ^2 test was used to test for deviation of genotype distribution from Hardy-Weinberg equilibrium. The χ^2 test was also used to evaluate the differences in genotype distribution and allele frequency and in the prevalence of traditional risk factors between patients and controls.

During genotyping of the *eNOS 4a/4b* polymorphism in PAD patients, we found the presence of a rarer *d* allele previously described in acute coronary syndrome patients.[25] Patients carrying this allele were excluded to avoid confounding effects on statistical analysis. The association between *eNOS* polymorphisms and PAD was assessed by using logistic regression analysis under a dominant genetic model of inheritance that compares individuals with one or more polymorphic alleles with a baseline group with no polymorphic alleles (eg, *eNOS-786CC* + *-786TC* vs *-786TT*). Variables that showed a statistically significant association with PAD susceptibility at univariate analysis were introduced in a multivariate model. The odds ratio (OR) with 95% confidence interval (CI) was determined. A value of $P < .05$ was considered to indicate statistical significance.

Only one study has investigated the prevalence of *eNOS* polymorphisms in white patients[13]; therefore, in a priori sample size calculation, we referred to our previous observations in white patients with atherosclerosis.[10-12] Accordingly, a sample size of at least 250 individuals in each group was deemed sufficient to prove or exclude an association between *eNOS* polymorphisms and PAD with a statistical power (β) of 80% and significance value of 0.05 (α).

RESULTS

Demographic and clinical characteristics of the study population are described in Table I. The prevalence of traditional cardiovascular risk factors was significantly higher in patients than in controls (Table I), and 91 of 281 PAD patients had at least one other atherosclerotic localization (CAD, carotid atherosclerosis) and AAA.

No deviation from the expected genotype proportion predicted by the Hardy-Weinberg equilibrium was observed for the polymorphisms analyzed (Table II). Concerning the *eNOS 4a4b* polymorphism, we excluded from the analysis two patients carrying the previously identified rare *d* allele[25] to avoid confounding effects.

A significant difference in genotype distribution and allele frequency between PAD patients and controls was observed for *eNOS-786T>C* but not for *-894G>T* and the *4a/4b* polymorphism (Table II).

We analyzed genotype distribution and allele frequency for all *eNOS* polymorphisms investigated, according to the presence or absence of at least any other atherosclerotic localization. We observed no difference between the two groups ($P > .05$).

At univariate logistic regression analysis, the *eNOS-786C* allele was significantly associated with PAD; nevertheless, the *-786C* allele did not influence the susceptibility to PAD after an adjustment for age, sex, and traditional risk factors (Table III). No significant association was observed in the other two polymorphisms analyzed (Table III). In

Table III. Univariate and multivariate analyses for *eNOS* and *ACE* gene polymorphisms and susceptibility to PAD

	Univariate analysis		*Multivariate analysis*[a]	
Variable	*OR (95% CI)*	P *value*	*OR (95% CI)*	P *value*
Age	1.01 (0.99-1.02)	.06	. . .	. . .
Sex	0.80 (0.56-1.13)	.2	. . .	. . .
Hypertension	6.27 (4.56-8.63)	<.0001	. . .	. . .
Smoking habit	6.72 (4.89-9.23)	<.0001	. . .	. . .
Diabetes	3.95 (2.41-6.47)	<.0001	. . .	. . .
Dyslipidemia	3.33 (2.43-4.56)	<.0001	. . .	. . .
eNOS -786C	1.52 (1.11-2.09)	.009	0.99 (0.65-1.52)	.9
eNOS 894T	0.92 (0.68-1.22)	.5	. . .	. . .
eNOS 4a	1.10 (0.81-1.50)	.5	. . .	. . .
eNOS -786C /4a	1.41 (1.02-1.94)	.04	1.20 (0.81-1.82)	.4
eNOS -786C and *4a* variants in patients carrying *ACE D* allele				
ACE D	1.90 (1.29-2.81)	.001	1.90 (1.16-3.10)	.01
ACE D/eNOS-786C	1.50 (1.11-2.02)	.009	1.30 (0.89-1.91)	.2
ACE D/eNOS-786C/ 4a	1.40 (0.99-1.98)	.05	. . .	. . .

ACE, Angiotensin-converting enzyme; *eNOS,* endothelial nitric oxide synthase.
[a]Adjusted for age, sex, and traditional cardiovascular risk factors.

Table IV. Univariate and multivariate analyses for *eNOS* and *ACE* gene polymorphisms and susceptibility to peripheral arterial disease according to smoking habit

	Smokers (n = 176)		*Non smokers (n = 105)*	
Analysis	*OR (95% CI)*	P *value*	*OR (95% CI)*	P *value*
Univariate analysis				
eNOS –786C	1.84 (1.10-3.08)	.02	1.23 (0.77-1.97)	.4
eNOS 894T	0.78 (0.48-1.27)	.3	0.79 (0.51-1.22)	.3
eNOS 4a	1.40 (0.84-2.33)	.2	0.95 (0.59-1.52)	.8
eNOS -786C/4a	2.14 (1.22-3.76)	.008	1.13 (0.69-1.84)	.6
Multivariate analysis[a]				
eNOS -786C	1.54 (0.80-2.98)	.2	. . .	. . .
eNOS -786C/4a	2.71 (1.38-5.30)	.004	. . .	. . .
				. . .
eNOS -786C and *4a* variants in individuals carrying *ACE D* allele				
Univariate analysis				
ACE D	2.84 (1.49-5.43)	.002	1.22 (0.71-2.11)	0.5
ACE D/eNOS -786C	2.06 (1.2-3.43)	.006	1.01 (0.65-1.58)	0.9
ACE D/eNOS -786C/ 4a	2.45 (1.27-4.71)	.007	1.02 (0.60-1.74)	0.9
Multivariate analysis[a]				
ACE D	2.82 (1.33-5.99)	.007	. . .	. . .
ACE D/eNOS -786C	2.26 (1.25-4.09)	.007	. . .	. . .
ACE D/eNOS -786C/4a	3.79 (1.78-8.06)	.001	. . .	. . .

ACE, Angiotensin-converting enzyme; *eNOS,* endothelial nitric oxide synthase.
[a]Adjusted for age, sex, and traditional cardiovascular risk factors.

individuals carrying both the *eNOS*-786C and *4a* alleles, we observed a significant association with PAD susceptibility at univariate but not at multivariate analysis (Table III).

No significant difference was observed in genotype distribution for the polymorphisms analyzed, according to Rutherford categories (data not shown).

Because experimental data demonstrated that the *eNOS-786C/4a* haplotype is differently able to modulate *eNOS* gene expression in the presence or absence of smoking habit,[26] we analyzed the role of the *eNOS* gene in modulating PAD susceptibility separately in smokers and in nonsmokers. In smokers, but not in nonsmokers, we observed that the *eNOS-786C* allele, but not *-894T* and *4a*, was associated with PAD at univariate analysis (Table IV); however, this association was not confirmed after adjustment for age, sex, and the other risk factors (Table IV). In smokers, the contemporary presence of the *eNOS-786C* and *4a* alleles significantly modulated the predisposition to PAD at both univariate and multivariate analysis (Table IV).

Because we had previously demonstrated in the same patients[14] that the *ACE D* allele influenced the predisposition to PAD, in the present study, we searched for a role of *eNOS* alleles in modulating PAD susceptibility in patients also carrying the *ACE D* allele. In these patients, neither the *eNOS-786C* allele nor the *eNOS-786C/4a* haplotype modified the susceptibility to PAD (Table III). Nevertheless, the presence of the *eNOS-786C/4a* haplotype increased

PAD predisposition in smokers also carrying the *ACE D* allele (OR, 2.71-3.79; $P > .05$ for interaction; Table IV).

DISCUSSION

Owing to the multifactorial nature of PAD, in which genetic components and environmental factors interact in determining its pathogenesis, in the present study, we evaluated the influence of genetic variants in another candidate gene, such as *eNOS*, as predisposing factors to PAD, and we provided the novel finding of the effect of *eNOS* in increasing the predisposition to the disease in smokers. This effect was evident also in those smokers carrying the *ACE D* allele. We provided further evidence of a combined effect of two candidate genes, *ACE* and *eNOS,* with the known effect on endothelial dysfunction,[27] in conferring a significant risk of PAD developing in smokers. Therefore, our findings contribute to highlight a gene–environment interaction that is able to modulate PAD predisposition.

We recently demonstrated a role for the *ACE* gene in predisposing to PAD.[14] The activity of the NO pathway is reduced through ACE-induced bradykinin degradation,[27] thus contributing to endothelial dysfunction. Data from previous reports suggest that eNOS-derived NO acts as an antiatherogenic molecule,[2] and a reduced endogenous NO synthesis facilitates the progression of atherosclerosis.[2] A genetically determined reduced NO availability contributes to promote endothelial dysfunction, which reflects a specific atherogenic vascular milieu.

Established cardiovascular risk factors, such as smoking, can cause endothelial damage severe enough to be followed by atherosclerotic or thrombotic changes.[28] Therefore, environmental factors such as smoking and the genetic predisposition to endothelial dysfunction may interact in affecting the atherosclerotic process.

Functional polymorphisms in the *eNOS* gene modulate NO availability. Data from an experimental study demonstrated a *cis*-acting role of the *eNOS 4a* allele on the promoter functional activity, therefore, the *4a* and *-786C* variants cooperate in regulating transcription efficiency in a haplotype-specific fashion.[26] Moreover, the effect of the *-786C/4a* haplotype in regulating *eNOS* gene expression is further modified by cigarette smoking.[26] As expected, we found that a high percentage of PAD patients were smokers (63%). On the basis of the experimental data from Wang et al,[6] we investigated the relationship between the *eNOS-786/4a* haplotype and smoking and demonstrated an interaction affecting PAD susceptibility.

The role of the *eNOS* gene in modulating PAD susceptibility has been poorly investigated. The Edinburgh artery study,[13] which analyzed the *eNOS 4a/4b* polymorphism, did not demonstrate its role as a susceptibility factor to PAD. Our results on the influence of this polymorphism in a larger white population are in keeping with those from the Edinburgh artery study.[13]

We previously demonstrated an association between the *eNOS* gene and other atherosclerotic localizations, such as CAD, carotid atherosclerosis, and AAAs.[10-12] The weak association between the *eNOS* gene and PAD, not confirmed after adjustment for traditional risk factors, may be related to the different percentage of cardiovascular risk factors observed in the present study compared with those observed in the different populations that have been investigated.[10-12]

This study has some limitations. The main limitation was that we were not able to perform a Doppler examination with ABI measurement or diagnostic procedures to evaluate asymptomatic atherosclerotic lesions in controls. A percentage of individuals with PAD are clinically asymptomatic, thus the possibility that our control group was disease-free cannot be excluded.

Our study also lacked of measurements of nitrite/nitrate (NOx) levels, which might have allowed us to evaluate NO production and might have contributed to the overall interpretation of data.

CONCLUSIONS

This study showed an association between the *eNOS* and *ACE* genes and PAD in smokers and provides evidence of a gene-environment interaction that modulates PAD predisposition. Nevertheless, the lack of ABI measurements and our ability to exclude asymptomatic PAD in controls might dim our results. Actually, the moderate occurrence of asymptomatic PAD in individuals aged >70 years might mask the true effect of a genetic predisposition. On the other hand, we observed the influence of the *eNOS* gene on PAD predisposition only in smokers, whose percentage was threefold higher in the patient group than in controls. To date, the Framingham study documented that the risk of PAD is twice as high in smokers as in nonsmokers.[29] These data might permit a hypothesis that the weight of smoking in determining the atherosclerotic process was relevant independently of the presence of potential asymptomatic forms. Moreover, the combined effect of smoking and genetically determined reduced NO availability might further contribute in promoting the endothelial dysfunction relevant in atherosclerosis, thus dimming potential confounding effects related to the presence of asymptomatic forms.

Finally, the combined information from more genetic variants may permit the identification of individuals at high risk of developing a complex disorder; in particular, as concerns PAD, this information may be useful to identify those individuals among smokers at even higher risk to develop the disease. Nevertheless, further studies performed on larger samples are needed to support our intriguing finding. Pharmacologic strategies, such as supplementation of the substrate for NO synthesis, L-arginine, and NO donors, may offer a better control of endothelial dysfunction, in particular, in smokers.

AUTHOR CONTRIBUTIONS

Conception and design: ES, RA, CF
Analysis and interpretation: ES, FS, GP, CF
Data collection: IR, GP, RP, CP
Writing the article: ES, FS, CF
Critical revision of the article: ES, CP, RA, CF

Final approval of the article: ES, FS, IR, GP, RP, CP, RA, CF
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APPENDIX (online only)

DETAILED INTERVIEW FORM

ID number . . . . . .
Age . . . . . .
Gender . . . . . .

Family history (cardiovascular disease in first-degree relatives: males: ≤55 yrs, females: ≤65 yrs)
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. . . . . .
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Risk factors for cardiovascular disease:
Weight (Kg): . . . . . .
Height (m): . . . . . .
BMI: (kg/m^2): . . . . . .

Physical activity: Yes . . . . . No . . . . . .
Grade of physical activity:
Light . . . . . .
Moderate. . . . . .
Intense . . . . . .

Smoking Habit:
No: . . . . . .
Yes: . . . . . Current: . . . . . Ex-smoker: . . . .

Blood pressure: SBP (mm Hg): . . . . DBP (mm Hg): . . . .
Hypertension: Yes . . . . . No . . . . . .
Therapy: . . . . . .
Total cholesterol: . . . . . HDL-C: . . . . . .
LDL-C: . . . . . Triglycerides: . . . . . .
Dyslipidemia: Yes . . . . . No . . . . . .
Therapy . . . . . .
Diabetes: Yes. . .. . .No. . .. .
Therapy. . .. .
Previous CAD: Yes . . . . . No . . . . . when . . . . . .
Previous stroke: Yes . . . . . No . . . . . when . . . . . .
Previous AAA: Yes. . . . . No . . . . . when . . . . . .
Carotid atherosclerosis: Yes . . . . . No . . . . . .
Symptomatic PAD: Yes . . . . . No . . . . . .