Determinants of early-onset cardiovascular disease: a case-control study of young myocardial infarction patients

Deodato Assanelli, Andrea Bonanome*, Mario Grassi**, Silvana Archetti***, Riccardo Negrini***, Alessandro Pezzini§, Salvatore Curello§§, Francesco Visioli§§§

*Chair of Internal Medicine, University of Brescia, Brescia, *Chair of Internal Medicine, University of Padua, Padua, **Section of Medical Statistics and Epidemiology, Department of Health Sciences, University of Pavia, Pavia, ***Biotechnologies III Laboratory, Spedali Civili, Brescia, §Department of Neurology, University of Brescia, Brescia, §§Department of Cardiology, University of Brescia, Brescia, §§§Department of Pharmacological Sciences, University of Milan, Milan, Italy*

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Background. **The present case-control investigation was undertaken with the aim of thoroughly assessing the risk profile of young coronary patients and to correlate it with their endothelium-dependent vasodilation and with the presence of atherosclerotic lesions.**

Methods. **Forty-eight subjects (age < 41 years) diagnosed with myocardial infarction were screened. They were matched 1:1, for age and sex, with controls. We evaluated the serum total, HDL-, and LDL-cholesterol, triacylglycerols, fibrinogen, homocysteine, folic acid, vitamin B12, vitamin E, antioxidant capacity, and uric acid levels, and we also analyzed the patients for the presence of *Helicobacter pylori* and of methylenetetrahydrofolate reductase and cystathionine β-synthase genetic mutations. Post-ischemic vasodilation of the brachial artery was evaluated and the intima-media thickness of the carotid arteries was measured at echo-Doppler.**

Results. **A statistical modeling selection between block variables revealed that smoking, the apoε genotype, dyslipidemia, fibrinogen, vitamin E concentrations, and intima-media thickness were important predictors of cardiovascular disease, with an accuracy of 91.7%.**

Conclusions. **On the basis of these results, we believe that acute coronary events in young subjects should be followed by a screening of their siblings, as they might be at higher risk for cardiovascular disease.**

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Address:
Dr. Francesco Visioli
Dipartimento di Scienze Farmacologiche
Università degli Studi
Via Balzaretti, 9
20133 Milano
E-mail:
francesco.visioli@unimi.it

Introduction

The incidence of cardiovascular disorders increases with life, usually due to the progression of atherosclerotic lesions and the contribution of other risk factors (often none of which lethal *per se*). Infrequently, a single genetic condition, e.g. familial hypercholesterolemia or high plasma fibrinogen, may by itself induce a precocious and severe development of cardiovascular disease[1]. In fact, although the highest incidence of coronary heart disease (CHD) is observed later in life, between 2 and 6% of myocardial infarctions occur in subjects < 40 years[2]. Atherosclerosis contributes to approximately 80% of juvenile myocardial infarctions but hardly ever are lesions individually so severe as to lead to myocardial infarction.

A large body of data shows that the traditional risk factors for atherosclerosis such as cigarette smoking, hypertension, hypercholesterolemia, hypertriglyceridemia, low plasma HDL concentrations, and overweight are involved in the development of CHD in youth[3-5]. Further, the impaired production of prothrombotic factors and elevated plasma concentrations of homocysteine has been held responsible for acute coronary events in young subjects[1]. Yet, although CHD may often be attributed to some of the aforementioned risk factors, a significant number of coronary events remain of unknown origin, even when the risk profile of these patients is thoroughly assessed. There is evidence, however, that the younger the age at which CHD manifests, the greater the impact that genetic factors play on its pathogenesis[6] as suggested, for example, by the family history of these patients. Some genetic polymorphisms, such as those of apolipoprotein E, have been correlated with higher cardiovascular risk. In particular, the presence of the apoε4 genotype may increase the myocar-

dial infarction risk by increasing the levels of LDL-cholesterol and LDL oxidation[7].

Endothelial dysfunction is a co-factor of cardiovascular mortality[8,9] and may be induced by most risk factors for atherosclerosis even in the absence of severe atherosclerotic lesions and before symptoms of coronary atherosclerosis become clinically apparent. Thus, the early detection of endothelial dysfunction may provide a clue for the early diagnosis of cardiovascular disease, in turn leading to better treatment of young patients and to more efficient prevention of CHD in their families[8].

This paper reports the results of a thorough assessment (biochemical and genetic) of the risk profile of young myocardial infarction patients, including the evaluation of their endothelial function and of a large number of variables.

Methods

Subjects. This investigation conforms to the principles outlined in the Declaration of Helsinki and was approved by the University of Brescia Institutional Review Board. Patients gave informed consent to the study. Forty-eight subjects (43 males and 5 females), with a mean age of 35.4 years (range 19-41 years) were consecutively recruited among those admitted – and diagnosed – with *de novo* acute myocardial infarction to the Cardiology Division of the University of Brescia Medical School during 1999. The chosen upper age limit was 41 years and all patients had an elevated ST segment. Coronary angiography was performed in all patients within 4 days of admission, whereas outpatient evaluation was performed 3 months later. Therapy with ACE-inhibitors or calcium antagonists was not interrupted, whereas beta-blockers were gradually discontinued starting from 1 week before the initiation of the study; statin therapy was discontinued 2 months before the beginning of the study. The extent of the coronary stenoses was quantified by using the Gensini score[10], within 1 month of myocardial infarction.

Patients were matched 1:1 (for age and sex) with a random sample of controls recruited (during the same year) using the registry office list of Brescia, i.e. the same city where patients were hospitalized. Control subjects also gave informed consent to the study.

Anthropometric and demographic variables. Participants were interviewed to assess the presence of diseases such as diabetes, hypertension, dyslipidemia, a family history of cardiovascular events, and habits such as smoking and alcohol consumption, as well as education.

Hypertension was coded as pharmacologically treated or not; diabetes was coded as present or not; a positive family history for an early event (age < 50 years) without the proband was coded as the number of affected siblings; a positive family history for an early event (age < 50 years) was coded as present or not; a positive family history for events including the proband was coded as the number of affected siblings (1, 2, 3, etc.); smoking was coded as non-smoking < 10 cigarettes/day, between 10 and 20 cigarettes/day, > 20 cigarettes/day, smoking cessation < 2 years, and smoking cessation > 2 years; dyslipidemia was coded as present or not and included high cholesterolemia, triglyceridemia or both, low HDL-cholesterol levels, or treatment with statins; alcohol consumption was coded as normal or absent (abstemious); education was coded as primary school, high school and university education; civil status was coded as single, married, divorced or widow.

Laboratory analyses. Blood was drawn in Vacutainer™ tubes after a 12-hour fast and serum was separated by centrifugation at 800 rpm for 15 min. Total, LDL- and HDL-cholesterol, and triacylglycerol concentrations were determined using standard laboratory methods. Clottable fibrinogen was measured using a modified Clauss method[11]. Serum homocysteine concentrations were determined by means of reverse-phase high-performance liquid chromatography (HPLC) coupled with fluorescence detection[12]. Serum folic acid was quantified by using ion capture technology (AxSIM System, Abbott Laboratories, Abbott Park, IL, USA). The antioxidant capacity of serum was evaluated according to the instructions of a commercially available kit (OxyResearch, Oxys, OR), which is based on the reduction of Cu(II) to Cu(I) by antioxidants, using uric acid as the reference compound; results are expressed as mEq uric acid[13]. After protein precipitation with ethanol, vitamin E was extracted from the serum using hexane and quantified by means of reverse-phase HPLC coupled with fluorescence detection[14]. The levels of uric acid were determined using an enzymatic method (Uricase methods). *Helicobacter pylori* was determined by immunoassay[15].

Genetic analysis. The major determinants of homocysteinemia, i.e. methylenetetrahydrofolate reductase (MTHFR) and cystathionine β-synthase (CBS), were evaluated from frozen samples of EDTA-anticoagulated whole blood, by using a standard DNA extraction procedure. For analysis of the CBS gene, a 184 bp DNA fragment containing exon 8 was selectively amplified by polymerase chain reaction (PCR) and was visualized on ethidium bromide-containing agarose gel. After amplification, the heterozygous state for a 68 bp insertion was revealed by a pattern of bands represented, in addition to the normal 184 bp products, by a larger molecular weight (252 bp), slower band. The homozygous state for the insertion was revealed by the presence of only the 252 bp slowly moving bands[16].

MTHFR genotypes were determined in accordance with the method of Frosst et al.[17], using PCR amplifi-

cation and restriction digestion with Hinf I to distinguish mutant from wild type alleles.

Genetic variations of apoε were determined by restriction isotyping, using PCR amplification and subsequent digestion with Hha I. As nucleotide substitutions that result in arg-cys interchanges at positions 112 and 158 also alter the Hha I cleavage sites, each genotype may be distinguished by unique combinations of Hha I fragment sizes in all homozygotic and heterozygotic combinations. Mutation analyses of the homozygous form were confirmed by DNA sequencing on an ABI 377 DNA Sequencer (Applied Biosystems Inc., Foster City, CA, USA). Sequence reactions were performed by using the PRISM™ Ready Reaction DyeDeoxy™ Terminator Cycle Sequencing Kit (Applied Biosystems Inc., Foster City, CA, USA). The presence of the factor V Leiden genotype mutation was determined on whole blood by means of PCR[16].

Endothelium-dependent vasodilation. Post-ischemic vasodilation of the brachial artery was studied as the index of endothelial function[18], by a well-established procedure[19]. The same physician performed all tests. Patients were studied in the morning, after a 10 min rest on the stretcher, and were advised not to consume alcohol or caffeine and not to smoke before the test. A high-resolution echograph and Doppler (Siemens Sonoline Elegra, Malvern, PA, USA) equipped with a 7.5 MHz linear probe was employed. Linear scans of the brachial artery were taken, while the depth and gain settings of the instrument were optimized before the test. The focus zone was positioned in coincidence of the proximal wall of the brachial artery and machine-operating parameters were conserved throughout the study. When an optimal segment of the brachial artery was identified, the skin was marked in order to determine the baseline post-ischemic variations at the same site. Measurements of the arterial diameters were taken, from the proximal to the distal wall, at the end of diastole, as identified in coincidence of the R wave of the ECG, which was continuously recorded throughout the study[20,21].

Having taken the baseline measurements of the brachial artery diameter, the blood flow was stopped by inflating a cuff placed at the arm at a pressure of 300 mmHg. The cuff was then deflated after 4.5 min and the brachial artery diameter was continuously recorded from 30 s before deflation to 90 s after deflation. The diameter of the brachial artery at 60 s was considered as being representative of the maximum post-ischemic vasodilation. The change in the diameter of the brachial artery was expressed as the percentage change in comparison with the baseline values[20,21].

Carotid ultrasound analysis. High-resolution B-mode ultrasound images acquired with a 7.0 MHz linear array transducer (Acuson 128 XP, Mountain View, CA, USA) were used to evaluate the intima-media thickness (IMT). All scans were performed on supine patients by the same sonograph technician who was unaware of the clinical status of the patient. Images of the right and left common carotid arteries, the carotid bulb and the internal carotid arterial wall segments were acquired from a fixed lateral transducer angle[22].

All patients underwent an echo color Doppler study of the common and internal carotid arteries, starting from the clavicle and proceeding cranially[23]. The carotid bifurcation and the internal and external carotid arteries were identified in all cases. The intima-media complex in the distal wall was identified as two echogenic lines separated by an anechoic space, as defined by Poli et al.[24]. Three measurements of the sites of greatest thickness were taken on digitized images at the end of systole. The highest mean value of three measurements (performed by independent physicians/technicians) of the two carotid arteries was considered as being representative of the atherosclerotic status of the patient. Stenosis severity of the internal carotid artery was estimated by blood flow velocity, according to the following criteria: < 50% stenosis when mild spectral broadening and a peak systolic flow velocity < 125 cm/s were recorded; 50-79% stenosis in the presence of spectral broadening throughout systole and a peak systolic flow velocity > 125 cm/s; 80-99% stenosis in case of an end-diastolic flow velocity > 35 cm/s. Occlusion was determined by the absence of a Doppler signal.

Statistical analysis. Results for continuous and categorical variables are expressed as means ± SD and absolute frequencies respectively. Categorical variables with three or more categories are also expressed as binary (1 = present, 0 = not present) variables.

Univariate comparisons between cases and control subjects were evaluated using the two-sample Student's t-test and the Fisher exact test for continuous and binary variables respectively. A p value < 0.05 was considered as statistically significant. Pearson's correlation coefficients were used to evaluate the bivariate association between variables within case/control groups.

Assessment of the CHD risk profile was performed using logistic regression modeling[25]. We conducted the model selection using a "blockwise" procedure: the response variable was case-control status and predictor variables were divided into six clusters: personal, inherited, metabolic, prothrombotic, antioxidant, or Doppler; within each block, an initial logistic regression model was fitted and the "best" model was determined with one variable per block subsequently added or removed until a $p < 0.05$ and narrow confidence intervals (CI) were reached. This model selection procedure allows a within-group multivariate analysis of causal variables with the supposedly same pathophysiological mechanism, providing advantages over the usual forward/backward stepwise procedure in which variables are considered as single blocks.

We investigated the statistical performance between block variables by two model goodness-of-fit indices: Akaike's information criterion (AIC)[26] and accuracy. AIC [-2 log-likelihood (model) + 2 number of parameter (model)] combines a measure of the discrepancy between the fitted values and the observed data (determined by -2 logL) and of the simplicity of the model as reflected by the number of parameters. The "best" model, i.e. the most parsimonious, is identified by the smallest AIC. The accuracy is defined as the correct classification of the case-control status by using the model fitted values, which form non-linear combinations of the predictor variables. We considered ≥ 90% as "good" accuracy.

Goodness-of-fit indices, p values, and maximum likelihood estimates of the odds ratio (with 95% CI) were computed using SPSS statistical software (version 10.0.1).

Results

Subject characteristics. The anamnestic and biochemical data from cases and controls are reported in table I. HDL-cholesterol and vitamin E concentrations and antioxidant capacity were lower in CHD patients, whereas body mass index, systolic and diastolic blood pressure, and fibrinogen concentrations were significantly higher in CHD patients. Serum homocysteine concentrations were higher in cases than in controls but this difference did not reach statistical significance.

Cases under drug treatment had a higher blood pressure, were heavier smokers, had a higher incidence of dyslipidemia and a lower level of education. They also had a more frequent familial history of events (both as total and at a young age), and were more frequently found to have a homozygous muta-

Table I. Comparison of cases and controls on continuous/binary variables.

Variable	Cases (n=48)	Controls (n=48)	p
Personal			
Education (1=secondary and higher)	17 (35.4%)	36 (75.0%)	< 0.001
Civil status (1=married)	33 (68.8%)	35 (72.9%)	0.411
Smoking (1=smoker)	43 (89.6%)	27 (56.2%)	< 0.001
Alcohol (1=abstainer)	12 (25.0%)	10 (20.8%)	0.404
Body mass index (kg/m^2)	27.5 ± 3.3	25.3 ± 3.2	0.002
Inherited			
Family history < 50 (1=1+)	15 (31.3%)	5 (10.4%)	0.011
Family history (1=1+)	22 (45.8%)	13 (27.1%)	0.045
MTHFR (1=C/T and T/T)	38 (79.2%)	28 (58.3%)	0.023
CBS (1=+/- and +/+)	3 (6.3%)	7 (14.6%)	0.158
Factor V Leiden (1= G/A and A/A)	4 (8.3%)	1 (2.1%)	0.181
Apoε genotype (1=3/4 and 4/4)	19 (39.6%)	5 (10.4%)	0.001
Metabolic			
Arterial hypertension (1=yes)	13 (27.1%)	5 (10.4%)	0.033
Treated hypertension (1=yes)	10 (20.8%)	0	0.001
Systolic blood pressure (mmHg)	133.0 ± 15.1	124.0 ± 13.2	0.002
Diastolic blood pressure (mmHg)	87.6 ± 9.6	81.7 ± 7.6	0.003
Total cholesterol (mmol/l)	205.0 ± 36.7	204.0 ± 38.1	0.917
HDL-cholesterol (mmol/l)	39.7 ± 7.9	47.3 ± 10.9	< 0.001
LDL-cholesterol (mmol/l)	134.1 ± 32.0	127.5 ± 38.1	0.357
Triglycerides (mmol/l)	155.0 ± 72.7	146.1 ± 87.6	0.593
Diabetes (1=yes)	4 (8.3%)	0	0.059
Dyslipidemia (1=yes)	30 (62.5%)	10 (20.8%)	< 0.001
Prothrombotic			
Helicobacter pylori (1=+)	29 (60.4%)	27 (56.3%)	0.418
Fibrinogen (mg/l)	337.8 ± 72.6	284.0 ± 54.5	< 0.001
Folic acid (ng/ml)	6.8 ± 3.7	6.6 ± 2.3	0.733
Homocysteine (mmol/l)	16.1 ± 11.6	13.2 ± 8.2	0.157
Antioxidant			
Uric acid (mg/dl)	5.2 ± 1.1	5.2 ± 1.5	0.967
Vitamin E (mg/ml)	15.9 ± 4.2	18.6 ± 3.5	0.001
Antioxidant power (mEq uric acid)	94.4 ± 22.5	110.4 ± 12.6	< 0.001
Doppler			
Fibrous thickening (1=yes)	8 (16.7%)	1 (2.1%)	0.015
Intima-media thickness (mm)	0.74 ± 0.19	0.55 ± 0.12	< 0.001
Vasodilation (mm)	0.34 ± 0.22	0.60 ± 0.21	< 0.001
Vasodilation change (%)	7.3 ± 5.7	16.0 ± 7.4	< 0.001

CBS = cystathionine β-synthase; MTHFR = methylenetetrahydrofolate reductase.

tion of MTHFR and the apoε4 genotype. Fibrous thickening on the common carotid artery was more frequent in cases. The IMT, fibrous thickening of the carotid artery and the percentage of carotid stenosis were higher in cases than in controls. Post-ischemic vasodilation was lower in cases than in controls, both when expressed as absolute values and as percent of basal values. The extent of atherosclerotic lesions was not particularly severe in the patients recruited in this study: that is, the absolute value of IMT was not abnormal. Non-significant inverse and direct correlations were found between post-ischemic vasodilation and IMT both in patients and control subjects (r = -0.071, p = 0.630 and r = 0.054, p = 0.716, respectively). Moreover, the Gensini score of patients was not correlated with IMT (r = 0.154, p = 0.307) and with vasodilation (r = 0.083, p = 0.585).

Logistic regression modeling. Table II summarizes the goodness-of-fit indices and conditional p values (i.e. the Student's t-test of no effect of each variable adjusted for the other variables in the block) of the block logistic regression modeling.

Doppler variables were the most parsimonious model (AIC 86.7), while metabolic and prothrombotic variables were the most accurate (accuracy 80.2%). The variables (one variable per block) included in the "best" model were: smoking (personal), apolipoprotein E (inherited), dyslipidemia (metabolic), fibrinogen (prothrombotic), vitamin E (antioxidant). and IMT (Doppler). The "best" model had the smallest AIC index (44.62), i.e., a rebound of 45% as compared with the Doppler model, and a higher accuracy (91.7%), i.e., 44 cases and 44 controls were correctly classified as case and control, whereas only 4 cases and 4 controls were misclassified. The odds ratios (95% CI) for CHD, computed using the "best" model, are reported in table III. For continuous variables the odds ratios are of 1 SD for each increase. The pooled SDs (n = 96) were used and were as follows: fibrinogen 60 g/l, and vitamin E 4 mg/dl. The odds ratios for binary variables were: 1 = present vs 0 = not present. For computing convenience, IMT was re-expressed as the binary variable by the median cut-off point (0.50 mm). Smoking subjects exhibited a 9-fold higher CHD risk (95% CI 0.02 to 0.73), whereas the apoε 3/4 or 4/4 genotypes exhibited a 7-fold increase in CHD risk (95% CI 1.1 to 51.1). Moreover, dyslipidemia was associated with a 17-fold higher CHD risk (95% CI 2.6 to 116.1). An IMT > 0.050 nm was associated with a 5-fold increase in CHD (95% CI 1.1 to 24.6) and an increase of 1 SD in the plasma fibrinogen concentration was associated with an 11-fold increase in CHD risk (95% CI 2.9 to 39.6). Conversely, an increase of 1 SD in the plasma vitamin E concentration was associated with a 10-fold decrease in CHD risk (95% CI 0.03 to 0.35).

Table II. Goodness-of-fit indices and conditional p values of block logistic regression models.

Predictor	Personal	p	Inherited	p	Metabolic	p	Prothrombotic	p	Antioxidant	p	Doppler	p	Best	p
1	Education	0.001	Family < 50	0.007	Arterial hypertension	0.698	Smoking	0.001	Uric acid	0.838	Fibrous thick	0.790	Smoking	0.022
2	Civil status	0.405	MTHFR	0.079	Treated hypertension	0.401	MTHFR	0.165	Vitamin E	0.005	IMT	0.001	ApoE	0.043
3	Smoking	0.005	CBS	0.644	Systolic BP	0.041	CBS	0.298	Antioxidant capacity	0.001	Vasodilation	0.001	Dyslipidemia	0.003
4	Alcohol	0.513	Factor V Leiden	0.320	Diastolic BP	0.680	Factor V Leiden	0.054					Fibrinogen	0.001
5	BMI	0.001	Apoε	0.008	HDL	0.002	*H. pylori*	0.110					Vitamin E	0.001
6					LDL	0.158	Fibrinogen	0.001					IMT	0.042
7					Triglycerides	0.005	Folic acid	0.299						
8					Dyslipidemia	0.001	Homocysteine	0.437						
9					BMI	0.566								
10														
(-2 logL)	92.1		109.18		73.41		74.85		106.21		80.7		44.62	
m	5		5		10		9		3		3		6	
AIC	102.1		119.18		93.41		92.85		112.21		86.7		56.62	
Accuracy	75		69.8		80.2		80.2		77.1		77.1		91.7	

Accuracy = percentage of correct classification; AIC = Akaike's information criterion (-2 logL+2m); BMI = body mass index; BP = blood pressure; CBS = cystathionine β-synthase; IMT = intima-media thickness; L = likelihood of the model; m = number of parameters of the model; MTHFR = methylenetetrahydrofolate reductase.

Table III. Odds ratios (OR) and 95% confidence intervals (CI) from the "best" logistic regression model.

	OR	95% CI	p
Smoking (1=smoker)	9.09	1.37-50	0.022
ApoE (1=3/4 and 4/4)	7.38	1.07-51.08	0.043
Dyslipidemia (1=yes)	17.42	2.61-116.12	0.003
Fibrinogen (1 SD=60 g/l)	10.68	2.88-39.61	< 0.001
Vitamin E (1 SD=4 mg/dl)	0.10	0.03-0.35	< 0.001
IMT (1= < 0.50 mm)	5.10	1.06-24.58	0.042

IMT = intima-media thickness.

Discussion

The causes of premature cardiovascular event in juvenile CHD are often elusive. Yet, a thorough investigation of subjects presenting with early-onset cardiovascular disease is very important to establish the best treatment and a preventive strategy aimed at their siblings.

In the present study, we took a novel approach by matching a group of patients who had had a myocardial infarction before 41 years of age with a group of controls. With the exception of smoking and overweight, traditional risk factors had a very similar prevalence in the two groups. For example, patients and controls had almost identical plasma total cholesterol concentrations, significantly lower, but still within normal range, HDL-cholesterol concentrations, and slightly higher triglyceride concentrations. The association of moderate hypertriglyceridemia with lower HDL-cholesterol levels has been suggested to increase CHD risk[27]; however, the lipid profile of the patients included in this study was not severely skewed in this direction.

Both the systolic and diastolic blood pressure values were higher in patients than in controls, but the mean values were not abnormally elevated and only 16% of patients were on antihypertensive medications. Plasma fibrinogen was also higher in cases than in controls, yet not abnormally high in absolute terms; the ARIC study[28] showed that elevated plasma levels of fibrinogen (a prothrombotic factor and a marker of inflammation) was associated with cardiovascular disease in both men and women.

Taken together, these biochemical data indicate that young CHD patients do not exhibit a peculiar risk profile. Yet, their family history was characterized by a significant record of premature cardiovascular events, consistent with other published data[29]. Thus, a subtle genetic predisposition may have contributed to the development of cardiovascular disease in these patients.

The homocysteine concentrations did not significantly differ between cases and controls, although homocysteinemia was more elevated in young coronary patients. Accordingly, the prevalence of homozygous and heterozygous MTHFR mutations was much greater in patients than in controls. However, the CBS gene was homogeneous in both the case-control groups.

The extent of atherosclerotic lesions was not particularly severe in the patients recruited in this study. Fibrous thickening of the common carotid artery was more frequent in cases and IMT, fibrous thickening of the carotid artery and the percentage of carotid stenosis were higher in cases than in controls. Angiograms showed diffuse atherosclerotic lesions in their coronary tree but, usually, only one clinically critical stenosis or occlusion was observed. Data from the literature demonstrate that the Gensini score is not correlated with IMT and vasodilation[30,31]. Furthermore, while IMT measurement of the common carotid arteries scored a significantly greater value in patients than in controls, this parameter was not abnormal in absolute terms. Consequently, had this been the only parameter obtained from these patients, it would have been impossible to detect precocious signs of atherosclerosis in these arteries. This observation suggests that, in young patients, IMT measurement may be a very useful marker of atherosclerosis. Besides macroscopic lesions, even the arterial endothelium was impaired in these young patients, as suggested by the tests of post-ischemic vasodilation. This finding might partially explain the occurrence of acute events in arteries that, *per se*, did not show particularly severe atherosclerosis.

In these patients, one parameter that probably contributed to the impairment of endothelial function was a decrease in antioxidant defense, as indicated by the lower vitamin E concentrations and lower antioxidant capacity: oxidative processes are indeed thought to be implicated in the development of atherosclerosis and endothelial dysfunction.

Many CHD-related variables (Table II) were divided into blocks, according to their physiological mechanism of action. Most significant variables were extrapolated from each block and included: smoking habit (as personal variable), apolipoprotein E genotype (as inherited variable), presence of dyslipidemia (as metabolic variable), the level of fibrinogen (as prothrombotic variable) and of vitamin E (as antioxidant power marker), and IMT (as Doppler marker). These selected variables may allow us to discriminate between cases and controls in a young population and determine the global risk assessment with an accuracy of 91.7%. According to the odds ratio ranks (Table III), the categorical risk factors followed this ranking: first, the presence of lipid disorders; second, smoking habits; and last, the apoε genotype. The results concerning smoking habits agree with those of other investigations[32], while lipid disorders and their genetic determinants (such as the apoε genotype) confirm that their pathological role in young patients is more important than in older patients. In agreement with the results of large prospective studies[28], fibrinogen concentrations were found to be important continuous risk factors for CHD, probably because fibrinogen acts as a prothrombotic

agent and an inflammatory mediator. IMT was also confirmed as an important predictor of CHD[33,34]. High serum vitamin E concentrations are hypothesized to exert protective roles: in our study, an increase of 4 mg/dl in vitamin E concentrations was found to be associated with a 10-fold reduction in the CHD risk.

Our "blockwise" modeling approach is a novel one. Most studies focus primarily on the inflammatory, thrombotic or atherogenic etiology of CHD, while only a few analyzed the different pathogeneses and antioxidant/Doppler measurements together[35]. Obviously, the limitation of the present analysis should be pointed out. The number of cases is relatively small and, due to the low statistical power of this study, we cannot entirely exclude that the results we recorded are due to chance; the panel of evaluated polymorphisms is not complete, mainly on the inflammatory side; some traditional phenotypes are still lacking; the levels of physical activity were not recorded. However, as these factors mostly influence the "classic" risk factors for CHD, their contribution to the overall risk we report should be negligible.

In summary, these data allow for some speculation, both from applicative and theoretical viewpoints. First, the premature occurrence of acute coronary events strongly calls for the screening of premature cardiovascular disease in the siblings of such patients. The optimal screening procedure in asymptomatic siblings is yet to be fully determined: carotid IMT measurement alone appears to be sufficient and – even if expensive and labor intensive – should be added to the assessment of the more traditional risk factors. In addition, although we did not find any statistically significant difference in the homocysteine levels between cases and controls, about one third of the recruited patients had high serum homocysteine concentrations, suggesting that this parameter could be routinely evaluated in juvenile CHD, in order to better integrate the risk assessment. From a therapeutic point of view, the optimal treatment of these patients and their relatives is yet to be fully established. Obviously, a secondary prevention strategy should be directed to patients, but the application of a similar therapy to their healthy relatives, even if exhibiting similar risk profiles, is, to date, unwarranted and debatable. Nevertheless, it appears appropriate to suggest that the correction of overweight and smoking cessation should always be pursued. Finally, a healthful diet may not only ameliorate major alterations consequent to metabolic syndromes, but may also improve the antioxidant protection of the organism and provide adequate folate intake, therefore contributing to restore a proper endothelial function.

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