Assessment of Asymptomatic Coronary Artery Disease in Apparently Uncomplicated Type 2 Diabetic Patients

A role for lipoprotein(a) and apolipoprotein(a) polymorphism

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OBJECTIVE — In patients with uncomplicated diabetes, there is low probability of finding significant coronary artery disease (CAD) by noninvasive tests. Therefore, screening for its presence is not justified, and it is important to find reliable predictors of silent CAD to identify patients with uncomplicated diabetes for further screening. The relationship between lipoprotein(a) [Lp(a)], apolipoprotein(a) [apo(a)] polymorphism, and silent CAD has never been studied. We investigated the association of Lp(a) and apo(a) polymorphism with angiographically documented asymptomatic CAD in type 2 diabetic patients without evident complications.

RESEARCH DESIGN AND METHODS — A total of 1,323 diabetic patients without any clinical and electrocardiographic evidence of CAD were evaluated. Of 121 patients with highly positive results of exercise electrocardiography (ECG) (n = 30) or positive results on exercise thallium scintigraphy (n = 91), 103 subjects showed angiographically documented CAD (CAD group). Of 1,106 patients with negative results on exercise ECG, 103 subjects without CAD (NO CAD group) were selected and matched by age, gender, and duration of diabetes to patients in the CAD group. In patients in the NO CAD group, results of exercise ECG, 48-h ambulatory ECG, and stress echocardiography were negative for CAD.

RESULTS — The CAD group had higher Lp(a) levels (21.7 ± 17.7 vs. 15.2 ± 19.0 mg/dl; P = 0.0093) than the NO CAD group, and a percentage of subjects had at least one small apo(a) isoform (68.9 vs. 29.1%; P = 0.0000) higher than the NO CAD group. Logistic regression analysis showed that apo(a) phenotypes (odds ratio [OR] 8.13, 95% CI 3.65–21.23), microalbuminuria (5.38, 2.44–11.88), smoking (2.72, 1.31–5.64), and Lp(a) levels (2.41, 1.15–5.03) were predictors of asymptomatic CAD.

CONCLUSIONS — Our investigation reports the first evidence of an independent association of Lp(a) and apo(a) polymorphism with asymptomatic CAD. This suggests that Lp(a) levels and apo(a) phenotypes could be used together with other risk factors as markers of asymptomatic CAD in patients with diabetes.

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Abbreviations: AER, albumin excretion rate; apo(a), apolipoprotein(a); CAD, coronary artery disease; EBCT, electron beam computed tomography; ECG, electrocardiography; Lp(a), lipoprotein(a); MW, molecular weight; OR, odds ratio.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Diabetes is associated with a more than threefold–increased risk of coronary artery disease (CAD) (1). In patients with diabetes, CAD is often asymptomatic (2–4). Asymptomatic CAD is a strong predictor of future coronary events and early death, especially in patients with diabetes (5,6). Early identification of diabetic patients with silent CAD can allow reduction of mortality and morbidity for cardiovascular events (7,8). In patients with uncomplicated diabetes, there is low probability of finding significant CAD by noninvasive tests. Therefore, screening for its presence is not justified (2), and it is important to find reliable predictors of silent CAD to identify patients with uncomplicated diabetes for further screening.

Lipoprotein(a) [Lp(a)] is a powerful risk factor for CAD (9). Recent studies report that the polymorphism of the specific apolipoprotein of Lp(a), called apolipoprotein(a) [apo(a)], could play a role independent of Lp(a) levels in the development of CAD (10–14). In patients with type 2 diabetes, the relationship between Lp(a) and known CAD has been evaluated (15,16), but no studies have investigated the association of Lp(a) levels and apo(a) polymorphism with asymptomatic CAD.

The aim of the present study was to investigate whether Lp(a) levels and apo(a) phenotypes are associated with the presence of angiographically documented asymptomatic CAD in patients with type 2 diabetes.

RESEARCH DESIGN AND METHODS

Patients

From November 1998 to March 2001, a total of 1,323 patients with type 2 diabetes but no evident complications were evaluated to identify patients with asymptomatic CAD. Exclusion criteria were age <45 or >70 years; history of coronary events; symptoms of coronary events as

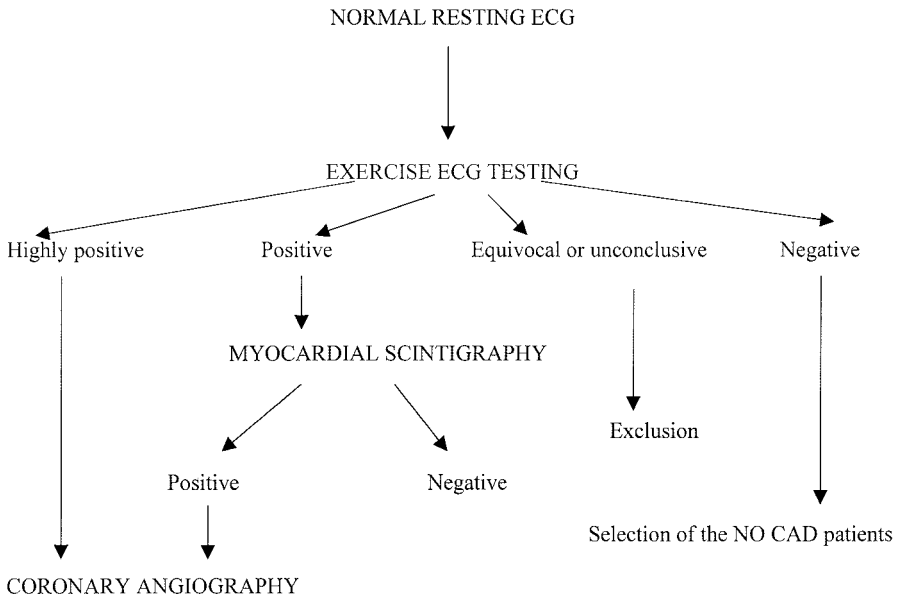

Figure 1—*The study protocol.*

defined by the Rose questionnaire; history of artery revascularization or heart failure; abnormal result of resting electrocardiography (ECG); uncontrolled hypertension (>180/100 mmHg); bundle branch block, atrial fibrillation, or other important arrhythmia; significant valvular disease; duration of diabetes <12 months; cardiomyopathy; chronic or acute disease; pregnancy; liver or kidney disease (creatinine >130 μmol/l); proteinuria (dipstick-positive proteinuria or albumin excretion rate [AER] ≥300 mg/day); diabetic proliferative retinopathy or previous photocoagulation; therapy with digitalis; neoplasia; conditions that prohibit maximal exercise ECG (such as amputation, foot wound, severe obesity, etc.); previous stroke; and intermittent claudication. Diabetes was diagnosed according to American Diabetes Association (ADA) criteria (17). Hypertension was diagnosed according to World Health Organization (WHO) criteria (18) or by presence of specific treatment. Patients with AER <30 mg/day were considered normoalbuminuric; patients with AER 30–299 mg/day were considered microalbuminuric.

Study protocol

The study protocol is shown in Fig. 1. All 1,323 patients underwent exercise stress testing (19). Subjects were requested to discontinue any antihypertensive drug with anti-ischemic properties, including β-blockers and calcium channel blockers. Results of exercise ECG were considered positive if there was an ST segment depression ≥1 mm that was planar or downsloping and persisted for at least 80 ms after the J point. Results of exercise ECG were considered negative if the patient reached 90% of the maximal predicted exercise heart rate for age without symptoms and significant change in ST segment. When results of exercise ECG were highly positive (ST depression in five or more leads, >2 mm maximum ST depression, a positive test with a heart rate <120, hypotension during exercise, or exercise capacity <5 min), the suspicion of CAD was considered strong. In other patients with a positive result of exercise ECG, exercise stress thallium scintigraphy was performed. Initial imaging was made within 5 min after intravenous injection of thallium-201. Four hours later, cardiac imaging was repeated. Five regions of the left ventricle were defined: anterior, apical, septal, inferior, and posterolateral. Results of scintigraphy were considered positive for CAD when the thallium scan exhibited fixed or transient uptake defects. In patients with highly positive results of ECG and those with positive results of scintigraphy, diagnostic coronary angiography was recommended. Coronary angiography was performed as previously reported (19). An atherosclerotic lesion was considered significant when stenosis ≥50% of the lumen in at least one major vessel was documented.

Diabetic patients without CAD (NO CAD group) were selected from the diabetic patients with negative results of maximal exercise test; NO CAD patients were matched by age, sex, and duration of diabetes to patients with angiographically documented asymptomatic CAD (CAD group). NO CAD patients underwent exercise ECG again; they also underwent 48-h ambulatory ECG (Holter) monitoring and stress exercise echocardiography. The 48-h Holter ECG monitoring consisted of a two-channel recording (leads V_1 and V_5). Results of 48-h Holter ECG monitoring were considered negative for CAD if no significant ST segment depression was found. Real-time two-dimensional echocardiography was performed before and immediately after exercise ECG. Postexercise echocardiography was started immediately after termination of exercise (<30 s) and was completed within 3 min. Simultaneous comparison of the echocardiograms at rest and postexercise was performed using a quad-screen format to identify regional wall motion abnormalities. Results of stress echocardiography were considered negative if no regional wall motion abnormalities were observed. In CAD and NO CAD patients, five standard repeatable tests were performed to study autonomic function (20). The study was approved by the ethic committee.

Laboratory procedures

Venous blood samples were taken from subjects after fasting for 12 h. In subjects treated with lipid-lowering drugs or estrogen and progestogen, blood samples were collected after a washout period of at least 3 months from the above drugs. For the quantification of Lp(a) and the characterization of apo(a) isoforms, we used plasma obtained by addition of EDTA and low-speed centrifugation at 4°C for 12 min. Immediately after centrifugation, plasma samples were frozen and stored at −80°C. Cholesterol, HDL, and triglycerides were measured by an automatic analyzer Hitachi 737 (Hitachi, Tokyo, Japan). LDL was calculated by Friedewald's formula, considering the contribution of Lp(a) on LDL levels (21). HbA_{1c} was measured by high-performance liquid chromatography (Biorad, Richmond, CA). AER was measured by nephelometry (Beckmann, Milan, Italy). Lp(a) plasma

concentrations were determined by a sandwich enzyme-linked immunosorbent assay method (Macra-Lp(a); SDI, Newark, DE). Apo(a) isoforms were detected by a high-resolution immunoblotting technique (13,22).

Statistical analysis

By using ANCOVA, all data regarding lipid parameters were adjusted for sex, BMI, smoking, drug intake, and presence of hypertension, microalbuminuria, and menopause. To assess differences in cholesterol, LDL, HDL, and BMI, Student's *t* test was used. Because of the highly "skewed" distribution of Lp(a) and triglyceride levels, to compare Lp(a) and triglycerides values, Mann-Whitney *U* test was used. The Pearson χ^2 test was used for comparison of frequency. The relationship between Lp(a) levels and AER was evaluated by Pearson's correlation coefficient after log-transformation. A multiple logistic regression analysis with the presence of asymptomatic CAD as the dependent variable was performed. Odds ratios (ORs) were estimated, and the results were given as OR and 95% CI. Data were presented as means ± SD, unless otherwise stated. $P < 0.05$ was considered significant.

RESULTS — Of the 1,323 diabetic patients, results of exercise stress ECG were positive in 121 patients (9.1%). In 1,106 patients (83.6%), the results were negative. A total of 96 subjects (7.3%) exhibiting equivocal or inconclusive results were excluded from the study. In 30 patients, results of exercise ECG were highly positive. In 91 patients with positive results of exercise ECG, exercise stress thallium scintigraphy was performed. Of these 91 patients, results of scintigraphy were positive in 85. A total of 110 patients (29 subjects with highly positive results of exercise ECG and 81 with positive results of scintigraphy) gave informed consent for coronary angiography. Coronary angiography showed a significant lesion in 103 subjects (27 with highly positive results of exercise ECG and 76 with positive results of scintigraphy). Angiography showed no significant lesions in seven patients. Of 103 patients with angiographically documented asymptomatic CAD (CAD group), monovessel disease was shown in 50 subjects (48.5%), bivessel disease was shown in 35 (34.0%), and multivessel disease was shown in 18 (17.5%). A total of 103 diabetic patients without CAD (NO CAD group) were selected from the 1,106 diabetic patients with a negative results of maximal exercise ECG. NO CAD patients were matched by age, sex, and duration of diabetes to 103 CAD patients. In the NO CAD patients, results of exercise ECG, 48-h ECG Holter monitoring, and stress echocardiography were negative for CAD. Of the CAD patients, 51 (49.5%) were treated with oral agents, 26 (25.2%) were treated with insulin, 9 (8.8%) were treated with insulin and oral agents, and 17 (16.5%) were treated with diet alone. Among the NO CAD patients, 61 (59.2%) were treated with oral agents, 21 (20.4%) were treated with insulin, 8 (7.8%) were treated with insulin and oral agents, and 13 (12.6%) were treated with diet alone. No differences in diabetes treatment were found between the groups ($\chi^2 = 2.017$; df = 3; NS).

The characteristics of diabetic patients with and without CAD are shown in Table 1. The percentage of microalbuminuric subjects and smokers was significantly higher and HDL levels were significantly lower in the CAD group than in the NO CAD group.

Glycemic control

Table 1 shows that HbA_{1c} did not differ between the two study groups at recruitment. HbA_{1c} was measured quarterly. We did not find any differences in the average values of HbA_{1c} recorded in the year before the recruitment (7.4 ± 1.3 vs. 7.7 ± 1.3%; NS).

Lp(a) levels and apo(a) phenotypes

Table 1 shows that Lp(a) levels were significantly higher in the CAD group than in the NO CAD group; the percentage of subjects with Lp(a) levels >30 mg/dl was higher in the CAD group than in the NO CAD group.

Of the 206 patients selected, we detected 26 apo(a) isoforms with an apparent molecular weight (MW) varying from 400 to 835 kDa; 107 subjects (52.0%) showed two electrophoretic bands, and 98 (47.6%) showed only one electrophoretic band. One patient (0.4%) in the NO CAD group had the so-called "null phenotype" (no band detectable by electrophoresis). This subject had a Lp(a) plasma concentration of 0 mg/dl. The cutoff between 640 and 655 kDa was used to divide apo(a) isoforms of low and high MW (13,16).

Table 1 shows that the percentage of subjects with at least one apo(a) isoform of low MW was significantly higher in the CAD group than in the NO CAD group.

No significant correlation between Lp(a) levels and AER was found in the CAD group ($r = 0.027$; $P = 0.393$) or in the NO CAD group ($r = 0.017$; $P = 0.433$).

Multivariate analysis

A multiple logistic regression analysis was performed with presence/absence of asymptomatic CAD as the dependent variable and the following as predictive variables: hypertension, family history of CAD, smoking, microalbuminuria, HbA_{1c}, BMI, Lp(a), cholesterol, triglycerides, LDL, and HDL. Analysis showed that microalbuminuria (OR 5.37, 95% CI 2.58–11.20; $P = 0.000$), smoking (2.67, 1.37–5.21; $P = 0.004$), and high Lp(a) levels (>30 mg/dl) (2.41, 1.15–5.03; $P = 0.019$) were significant predictors of asymptomatic CAD in diabetic patients. When apo(a) phenotypes (presence of at least one isoform of low MW or presence of only isoforms of high MW) were added to the list of potential predictors, the analysis showed that apo(a) polymorphism (8.13, 3.65–21.23; $P = 0.000$), microalbuminuria (5.38, 2.44–11.88; $P = 0.000$), and smoking (2.72, 1.31–5.64; $P = 0.007$) were independent predictors of asymptomatic CAD in type 2 diabetes. Lp(a) levels did not enter the model.

Sensitivity, specificity, and positive or negative predictive values have been calculated for Lp(a) (sensitivity 33.9%, specificity 81.6%, positive predictive value 64.8%, negative predictive value 55.3%), apo(a) polymorphism (sensitivity 68.9%, specificity 70.8%, positive predictive value 70.3%, negative predictive value 69.5%), microalbuminuria (sensitivity 44.7%, specificity 86.4%, positive predictive value 76.7%, negative predictive value 60.9%), and smoking (sensitivity 46.6%, specificity 76.6%, positive predictive value 66.6%, negative predictive value 58.9%).

Risk factors in nonsmokers and normoalbuminuric patients

Table 2 shows risk factors in subgroups of nonsmokers and normoalbuminuric subjects with and without CAD. In both subgroups, Lp(a) levels in nonsmokers (OR

Table 1—*Characteristics of diabetic patients with asymptomatic CAD and without CAD*

	CAD	NO CAD	*P*
N	103	103	
Sex (male/female)	84/19	84/19	1.000
Age (years)	59.4 ± 6.3	59.2 ± 6.9	0.9785
Duration of diabetes (years)	6.6 ± 5.2	6.7 ± 5.5	0.9581
BMI (kg/m^2)	26.6 ± 3.3	25.9 ± 3.8	0.1761
HbA_{1c} (%)	7.5 ± 1.2	7.3 ± 1.4	0.4853
Cholesterol (mmol/l)	5.6 ± 0.9	5.4 ± 0.9	0.0668
LDL (mmol/l)	2.8 ± 0.8	2.7 ± 0.9	0.2369
HDL (mmol/l)	1.1 ± 0.2	1.2 ± 0.2	0.0026
Triglycerides (mmol/l)	1.8 ± 0.6	1.7 ± 0.6	0.2167
Microalbuminuria (%)	44.7	13.6	0.0000
AER (mg/day)			
Mean	52.6 ± 62.5	32.3 ± 51.7	
Median	25.0	15.0	0.0010
Range	4.0–291.0	3.0–269.0	
Smokers (%)	46.6	23.3	0.0005
History of CAD (%)	39.8	28.2	0.0775
Hypertension (%)	45.6	56.3	0.1256
Autonomic neuropathy (%)	8.7	5.3	0.4212
Lp(a) levels (mg/dl)			
Mean	21.7 ± 17.7	15.2 ± 19.0	
Median	18.0	7.0	0.0093
Range	0.5–62	0–96.0	
Subjects with Lp(a) levels >30 mg/dl (%)	34.0%	18.4%	0.0113
Subjects with at least one isoform of low MW (%)	68.9	29.1	
Subjects with two isoforms of low MW (%)	10.7	1.0	
Subjects with one isoform of low MW (%)	58.2	28.1	0.0000
Subjects with only isoforms of high MW (%)	31.1	70.9	

Data are means ± SD unless otherwise indicated.

5.47, 95% CI 1.84–16.21; $P = 0.002$) and normoalbuminuric patients (3.60, 1.47–8.77; $P = 0.005$) and apo(a) phenotypes in nonsmokers (17.13, 5.19–56.48; $P = 0.000$) and normoalbuminuric patients (8.12, 2.98–22.12; $P = 0.000$) remained strongly and independently associated with silent CAD. Other independent predictors were microalbuminuria (7.76, 2.25–25.55; $P = 0.001$) in nonsmokers and smoking (2.20, 1.00–4.81; $P = 0.047$) in normoalbuminuric patients.

CONCLUSIONS — In diabetic patients with complications, a noninvasive screening for CAD is currently recommended (8). On the contrary, in patients without complications, it is very difficult to identify subjects for screening. Screening for CAD is also recommended when two or more cardiovascular risk factors are present; nevertheless, this recommendation does not address the potential impact of some powerful risk factors, including Lp(a), when data are not available (8).

With this in mind, we have evaluated a large group of type 2 diabetic patients without any clinical and electrocardiographic evidence of CAD to find subjects with asymptomatic CAD. Patients with clinical conditions often associated with asymptomatic CAD were excluded. Therefore, we have selected a diabetic population at relatively low risk of silent CAD. In this group, some cardiovascular risk factors, including Lp(a) and its polymorphism, were tested as potential predictors of silent CAD.

Methodological considerations

Nesto reported that a positive result of a noninvasive test carried a relatively low positive predictive value for significant CAD on angiography (7). For this reason, we have performed statistical analysis only on subjects with angiographically documented CAD.

Noninvasive tests currently used for screening diabetic patients for CAD are able to identify only subjects with myocardial ischemia due to significant coronary stenosis, but they cannot exclude nonocclusive CAD. We aimed to find predictors of silent CAD that can be detected by the noninvasive tests currently recommended. In the NO CAD group, the presence of myocardial ischemia was excluded by three noninvasive tests. Therefore, the parameters independently associated with silent CAD in our survey should be helpful to discriminate patients with apparently uncomplicated diabetes for further screening with tests currently used. It is interesting to note that significant differences in Lp(a) levels and apo(a) phenotypes between diabetic patients with significant angiographic CAD and a subgroup of diabetic patients with absence of angiographic coronary lesions have been shown (16). Therefore, one might hypothesize that the possible presence of some diabetic patients with nonocclusive CAD in our control group may

Table 2—*Clinical features of nonsmokers and normoalbuminuric patients with and without CAD*

	Nonsmokers			Normoalbuminuric patients		
	CAD	NO CAD	*P*	CAD	NO CAD	*P*
N	55	79		57	89	
Sex (male/female)	39/16	62/17	0.4255	43/14	73/16	0.4529
Age (years)	60.0 ± 5.7	59.3 ± 7.0	0.3323	60.1 ± 6.4	59.6 ± 6.8	0.4446
Duration of diabetes (years)	6.6 ± 4.8	6.5 ± 5.3	0.8751	5.9 ± 4.8	6.8 ± 5.4	0.8157
BMI (kg/m^2)	26.3 ± 3.5	25.6 ± 3.9	0.1738	26.3 ± 3.3	26.0 ± 3.7	0.8697
HbA1c (%)	7.4 ± 1.6	7.3 ± 1.4	0.4932	7.2 ± 1.2	7.2 ± 1.5	0.8328
Cholesterol (mmol/l)	5.6 ± 0.9	5.4 ± 0.9	0.0948	5.6 ± 0.9	5.5 ± 1.0	0.7584
LDL (mmol/l)	2.9 ± 0.9	2.7 ± 1.0	0.0726	2.7 ± 0.8	2.8 ± 0.9	0.6286
HDL (mmol/l)	1.2 ± 0.3	1.2 ± 0.2	0.1407	1.2 ± 0.3	1.2 ± 0.2	0.4785
Triglycerides (mmol/l)	1.7 ± 0.7	1.7 ± 0.7	0.4165	1.8 ± 0.7	1.7 ± 0.6	0.4281
Microabuminuria (%)	41.8	12.6	0.0003	0	0	
AER (mg/day)						
Mean	55.6 ± 66.3	32.5 ± 54.8		14.6 ± 7.0	14.9 ± 7.3	
Median	26.0	14.0	0.0119	12.0	14.0	0.7561
Range	4.0–291.0	3.0–269.0		4.0–28.0	3.0–28.0	
Smokers (%)	0	0		43.8	22.4	0.0109
History of CAD (%)	41.8	24.0	0.0464	38.6	24.7	0.1101
Hypertension (%)	45.4	55.7	0.3216	54.3	52.8	0.9870
Autonomic neuropathy (%)	12.7	6.3	0.2020	7.0	6.7	0.9487
Lp(a) levels (mg/dl)						
Mean	24.0 ± 18.8	14.2 ± 19.0		24.0 ± 18.9	14.5 ± 18.7	
Median	20.5	7.0	0.0096	20.0	7	0.0399
Range	0.5–62	0–96		0.5–62	0–96	
Subjects with Lp(a) levels >30 mg/dl (%)	40.0	13.9	0.0012	40.3	16.3	0.0017
Subjects with at least one apo(a) isoform of low MW (%)	76.3	22.7	0.0000	73.6	28.0	0.0000

Data are means ± SD unless otherwise indicated.

have even diluted the potential impact of Lp(a) and apo(a) polymorphism in predicting silent CAD. However, further studies should confirm our data.

Nonocclusive CAD represents an important clinical problem because it may be very dangerous (16). Nevertheless, it is very difficult to identify nonocclusive CAD in clinical practice. An interesting new approach to find subclinical nonocclusive CAD seems to be represented by electron beam computed tomography (EBCT), which may allow the identification of patients with coronary calcification (23). The weight of evidence for a powerful association of EBCT with increased risk of CAD events has been rapidly accumulating, although clinical relevance of calcified plaque as seen by EBCT is not universally accepted (23). Indeed, EBCT is not currently recommended for screening diabetic patients for CAD (8). However, EBCT may contribute to better clarify the association of Lp(a) with nonocclusive CAD. An association between Lp(a) levels and coronary calcification has been found in nondiabetic subjects (23). It is of interest to also investigate the role of apo(a) polymorphism. Moreover, further studies should clarify the possible association of both Lp(a) and apo(a) polymorphism with coronary calcification in diabetic patients.

Koistinen (24) found that asymptomatic CAD is associated with the duration of diabetes. So, when data are analyzed, duration of diabetes might represent a confounding factor. Therefore, to avoid this confounding factor, the NO CAD group was matched by duration of diabetes to the CAD group.

Another methodological problem is represented by the lack of standardization of the methods for Lp(a) measurement. Lp(a) assays may be differently affected by apo(a) size heterogeneity, because available commercial assays may underestimate Lp(a) levels for smaller apo(a) isoforms and overestimate Lp(a) levels for larger apo(a) isoforms (25). Therefore, there may be some difficulties both in establishing the exact predictive power of Lp(a) levels and in comparing different studies.

Lp(a) levels, apo(a) polymorphism, and silent CAD

Our study reports the first evidence of an independent association of Lp(a) levels and apo(a) polymorphism with the presence of angiographically documented asymptomatic CAD in diabetic patients. When apo(a) polymorphism was not included in the list of potential predictors, Lp(a) was an independent predictor of asymptomatic CAD. When apo(a) polymorphism was added to the list, Lp(a) levels did not enter into the model, because Lp(a) level is strongly dependent on apo(a) polymorphism (13). This suggests that apo(a) polymorphism may have a predictive value that is higher than that of Lp(a) levels, as also indicated by estimation of ORs. This finding confirms previous studies and indicates that apo(a)

polymorphism could add incremental information on the cardiovascular risk linked to the apo(a) gene (10–14).

Although the role of Lp(a) and apo(a) polymorphism in the development of CAD in patients with type 2 diabetes has not been fully elucidated (15,16), Lp(a) levels and apo(a) phenotypes are powerful risk factors for coronary atherosclerosis in the general population (9–14). Therefore, diabetic patients with silent CAD also may have a prevalence of high Lp(a) levels and apo(a) phenotypes of low MW that is higher than that in diabetic patients without CAD. Therefore, if our data are confirmed by larger studies, apo(a) phenotypes could be used together with Lp(a) levels as powerful markers of asymptomatic CAD in patients with diabetes. In particular, our data suggest that the evaluation of both Lp(a) levels and apo(a) polymorphism provides a more complete characterization of the risk for silent CAD linked to the apo(a) gene than the evaluation of Lp(a) levels alone. Lp(a) levels are considerably easier to test on a large scale than apo(a) size and, particularly among Caucasians, small apo(a) size correlates well with high Lp(a) levels; therefore, Lp(a) levels should be measured in all patients with diabetes. However, as previously suggested (13), apo(a) polymorphism should be analyzed at least in patients with Lp(a) levels near the cutoff (10–30 mg/dl) and when conditions modifying Lp(a) levels are present.

Other cardiovascular risk factors and silent CAD

Our data confirm previous investigations that reported microalbuminuria as an independent predictor of silent CAD in both type 1 and type 2 diabetes (26,27). In our survey, smoking also seems to be an independent discriminator of asymptomatic CAD. Some studies found that microalbuminuria could increase Lp(a) levels (15), although in our survey, no significant correlation between Lp(a) levels and AER was found. It is unclear whether smoking can affect Lp(a) levels (28). The effects of environmental factors on Lp(a) levels could cause two consequences: *1*) the risk linked to circulating Lp(a) might be modified; and 2) Lp(a) levels could not reliably express the genetic risk linked to apo(a) gene. Nevertheless, even when conditions modifying Lp(a) levels are present, apo(a) polymorphism remains a reliable marker of the genetic risk linked to apo(a) gene, because apo(a) isoforms usually are not modified by environmental factors. Nevertheless, Rainwater et al. (29) demonstrated that patients with type 2 diabetes have larger apo(a) size than nondiabetic subjects and their matched nondiabetic relatives. Therefore, in diabetic patients, environmental factors could influence apo(a) isoform size by the general process of nonenzymatic glycation of plasma proteins (29). The modification of the apo(a) isoform size due to hyperglycemia may also explain why apo(a) polymorphism seems to have a lower predictive value for CAD in diabetic subjects than in nondiabetic subjects (16). However, in the present study, there is no bias due to a different glycation of apo(a) isoforms between CAD and NO CAD patients; indeed, the two study groups are matched by diabetes duration and do not show any differences in glycemic control at recruitment and in the year before the recruitment. Therefore, in diabetic patients, the association between apo(a) polymorphism and silent CAD is likely. However, other studies should clarify whether in diabetics apo(a) genotypes show a greater predictive value than apo(a) phenotypes. Another interesting finding of our study is that Lp(a) levels and apo(a) polymorphism are able to discriminate diabetes with silent CAD also in nonsmokers and normoalbuminuric patients. No independent associations between lipids and asymptomatic CAD were found; this finding confirms some previous studies (3,24,30). In the Milan Study on Atherosclerosis and Diabetes (MiSAD) study, an independent association between cholesterol and silent myocardial ischemia was found in men (4). However, in our survey, the CAD group showed HDL levels significantly lower than the NO CAD group in univariate analysis; cholesterol was higher in the CAD group, but the difference was not statistically significant. On the contrary, the role of autonomic neuropathy as predictor of silent CAD remains unclear (8,31). Indeed, although some studies documented that autonomic neuropathy involving the cardiac system may be the cause of silent CAD in diabetic patients, others did not (31). In our survey, a significant association between impairment of autonomic function and angiographically documented asymptomatic CAD was not found.

It is well known that no cardiovascular risk factor alone is able to identify patients to screen for CAD among those with uncomplicated diabetes (8). This seems to also be true for Lp(a) and apo(a) polymorphism, as suggested by their sensitivity and specificity. Therefore, to better estimate the global risk of silent CAD, Lp(a) and apo(a) polymorphism should be used together with other risk factors.

Clinical implications

The early identification of diabetic patients with silent CAD is very important. Indeed, in these individuals, a significant reduction of mortality and morbidity for cardiovascular diseases can be obtained by the implementation of preventive programs, initiation of treatment with antiischemic medications, and early identification of the patient for whom revascularization is appropriate (8). The present investigation suggests that Lp(a) and apo(a) polymorphism could contribute effectively to the identification of diabetic patients with silent CAD. Therefore, if our findings are confirmed by other studies, Lp(a) levels and apo(a) polymorphism should be added to the list of cardiovascular risk factors that warrant a noninvasive screening for CAD in diabetic patients.

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