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Silent coronary artery disease in type 2 diabetes mellitus: the role of Lipoprotein(a), homocysteine and apo(a) polymorphism

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Abstract

Background: There is little data on the relationship between novel cardiovascular risk factors and silent coronary artery disease (CAD) in diabetic patients. We investigated whether Lipoprotein(a), homocysteine and apolipoprotein(a) polymorphism are associated with angiographically assessed asymptomatic coronary artery disease (CAD) in diabetic patients.

Methods: 1,971 type 2 diabetic patients without clinical signs of cardiovascular diseases and with a negative history of CAD were consecutively evaluated. Among them, 179 patients showed electrocardiographic abnormalities suggestive of ischemia or previous asymptomatic myocardial infarction. These 179 patients were subjected to a non-invasive test for CAD (ECG stress testing and/or scintigraphy). Among patients with a highly positive stress testing (n = 19) or a positive scintigraphy (n = 74), 75 showed an angiographically documented CAD (CAD group). Seventy-five patients without CAD (NO CAD group) were matched by age, sex and duration of diabetes to CAD patients. In NO CAD patients an exercise ECG test, a 48-hour ambulatory ECG and a stress echocardiogram were negative for CAD.

Results: Lipoprotein(a) levels (22.0 ± 18.9 versus 16.0 ± 19.4 mg/dl; $p < 0.05$), homocysteine levels (13.6 ± 6.6 versus 11.4 ± 4.9 mmol/l; $p < 0.05$) and the percentage of subjects with at least one small apolipoprotein(a) isoform (70.7% versus 29.3%; $p < 0.0001$) were higher in CAD than NO CAD group. Logistic regression analysis showed that apolipoprotein(a) polymorphism (OR:8.65; 95%CI:3.05–24.55), microalbuminuria (OR:6.16; 95%CI:2.21–17.18), smoking (OR:2.53; 95%CI:1.05–6.08), HDL (OR:3.16; 95%CI:1.28–7.81), homocysteine (OR:2.25; 95%CI:1.14–4.43) and Lipoprotein(a) (OR:2.62; 95%CI:1.01–6.79) were independent predictors of asymptomatic CAD.

Conclusions: The present investigation shows an independent association of Lipoprotein(a), homocysteine and apo(a) polymorphism with silent CAD. Other studies are needed to establish whether these parameters are suitable for CAD screening in diabetic patients.

Background

Lipoprotein(a) -Lp(a)- and plasma total homocysteine (tHcy) are risk factors for coronary artery disease (CAD) [1,2]. The specific apolipoprotein of Lp(a), called apo(a), could play a role independent of Lp(a) levels in the development of CAD [3–8]. Among diabetic patients silent CAD is quite frequent [9–11]. Silent CAD is a strong predictor of future coronary events and early death, particularly in diabetic patients [12,13]. This suggests that the early identification of diabetic patients with silent CAD could permit the reduction of mortality and morbidity for coronary events by the implementation of specific preventive programs [14]. Nevertheless, the diagnosis of silent CAD is quite difficult, since few risk factors are known. In diabetic patients the relationship of Lp(a) and tHcy with overt CAD has been analysed [15–22]. An association of high Lp(a) levels and apo(a) phenotypes with silent CAD has been found in diabetic patients with normal resting ECG [23]. No studies investigated the relationship between Hcy and silent CAD.

In the present study we investigated whether in a group of type 2 diabetic patients without a personal history of cardiovascular events and without current clinical signs of CAD Lp(a), Hcy and apo(a) polymorphism are associated with angiographically assessed silent CAD.

Methods

Patients

We evaluated 1,971 type 2 diabetic patients to find subjects with silent CAD. Exclusion criteria were: age <45 or >70 years, symptoms of coronary events as defined by Rose questionnaire, history of coronary events, artery revascularization, stroke, claudicatio intermittens, heart failure, uncontrolled hypertension (>180/100 mmHg), significant valvular diseases, cardiomyopathy, chronic or acute diseases, 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 digital, neoplasia, duration of diabetes < 12 months, conditions which did not permit maximal exercise ECG (amputation, foot wound, severe obesity, etc). Diabetes was diagnosed according to ADA criteria [24]. Hypertension was diagnosed according to WHO criteria [25] or in presence of a specific treatment. Patients with AER<30 mg/day were considered normoalbuminuric; patients with AER between 30 and 299 mg/day were considered microalbuminuric.

Study protocol

The study protocol is depicted in Figure 1. All the patients underwent a standard 12-lead resting ECG interpreted according to Minnesota Code [26]. According to resting ECG, patients were subdivided in four subgroups: 1) normal ECG; 2) ST-T abnormalities; 3) abnormalities suggestive of infarction; 4) other abnormalities. Patients with ST-T abnormalities underwent an exercise stress testing [27]. Subjects were requested to discontinue any antihypertensive drug with antiischemic properties, including β-blockers and calcium channel blockers. An exercise ECG test was considered positive if there was an ST segment depression equal to or greater than 1 mm which was planar or downsloping and persisted for at least 80 ms after the J point. A test was considered negative when the patient reached 90% of the maximal predicted exercise heart rate for age without symptoms and significant ST segment change. When exercise ECG test was highly positive (ST depression in 5 or more leads; >2 mm maximum ST depression; a positive test with a heart rate <120; hypotension during exercise; exercise capacity <5 min) the suspicion of CAD was considered strong. In other patients with a positive or equivocal exercise ECG test an exercise stress thallium scintigraphy was performed. Initial imaging was made within 5 minutes 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 postero-lateral. The scintigraphy was considered positive for CAD when the thallium scan exhibited fixed or transient uptake defects. In patients with highly positive ECG and those with a positive scintigraphy a diagnostic coronary angiography was recommended. Angiography was performed as previously reported [27]. An atherosclerotic lesion was considered significant when a stenosis ≥50% of the lumen in at least one major vessel was documented. In patients with ECG abnormalities suggestive of myocardial infarction an exercise stress thallium scintigraphy was performed. In patients with a positive scintigraphy a diagnostic coronary angiography was recommended. Patients with other ECG abnormalities were excluded from the study.

Diabetic patients without CAD (NO CAD group) were selected from the patients with a normal resting ECG; NO CAD patients were matched by age, gender and duration of diabetes to patients with angiographically documented asymptomatic CAD (CAD group). NO CAD patients underwent a stress ECG testing, a 48-hour ambulatory ECG (Holter) monitoring and a stress exercise echocardiography. The 48-h Holter ECG monitoring consisted of a 2 channel recording (leads V1 and V5). Real time two dimensional echocardiografic examinations were performed before and immediately after an exercise ECG testing. The post-exercise echocardiografic study was started immediately after the termination of the exercise (<30 s) and completed within 3 minutes. In CAD and NO CAD patients the following five standard repeatable tests were performed to study autonomic function [28]: heart-rate response to Valsalva manoeuvre, heart-rate variation during deep breathing, blood-pressure response to sustained

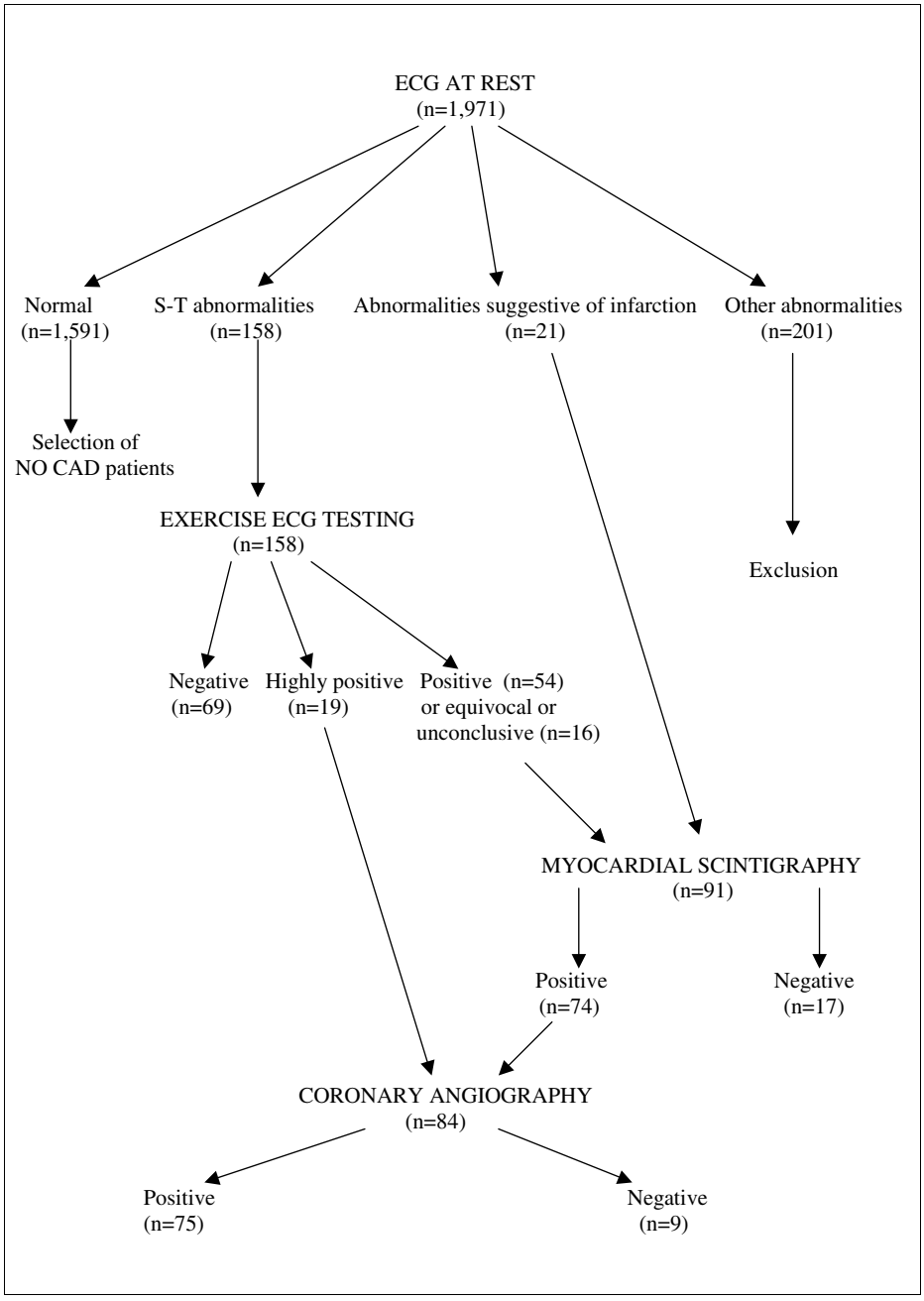

Figure 1
The study protocol. The number (n=) of patients at each point of the flow diagram is reported. Coronary angiography was recommended in 93 subjects, but 84 angiographies were performed, since 12 patients did not give their consent.

Table 1: Schematic outcomes of a test

TEST	CAD	n	NO CAD	n
Positive	True positive	a	False positive	c
Negative	False negative	b	True negative	d

Sensitivity = a / (a+b); specificity = d / (c+d); positive predictive value = a / (a+c) Negative predictive value = d / (b+d)

handgrip, immediate heart-rate response to standing, blood pressure to standing. The first three tests mainly investigate parasympathetic damage, while the latter two tests evaluate damage of sympathetic system. We considered the diagnosis of impaired autonomic function in the presence of abnormal findings in at least one of the tests [28]. The study was approved by the ethic committee. Invasive testing was performed only when there was a clear clinical indication according to the daily clinical practice in our Institute. All patients gave their informed consent both to perform each test and to participate in the study.

Laboratory procedures

Venous blood samples were taken from subjects after 12-hour fasting. In subjects treated with lipid-lowering drugs or oestrogen and progestagen, blood samples were taken after a wash-out period of at least 3 months from above drugs. No patient was receiving Vitamin B or folic acid supplementation. For the quantification of Lp(a) and tHcy and the characterization of apo(a) isoforms we used plasma obtained by addition of EDTA and centrifugation within 1 hour after collection. Immediately after centrifugation, plasma samples were frozen and stored at -80°C. Cholesterol, HDL and triglycerides were measured by an automatic analyzer HITACHI 737 (Tokio-Japan). LDL was calculated by Friedewald's formula, considering the contribution of Lp(a) on LDL levels [29]. Glycated haemoglobin (HbA1c) 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-ELISA method (Macra-Lp(a) SDI, Newark, Delaware). Plasma tHcy level was measured by high-performance liquid chromatography. Apo(a) isoforms were detected by a high-resolution immunoblotting technique [7,30]. The investigators who performed laboratory procedures were blind to the status of the patients (presence or absence of CAD). Physicians who analysed and/or performed resting ECG, exercise ECG testing, myocardial scintigraphy and coronary arteriography were blind to the following biological parameters of the patients: levels of Lp(a) and tHcy, apo(a) phenotypes.

Statistical analysis

By using an analysis of covariance, all data regarding lipid parameters and tHcy were adjusted for sex, BMI, smoking, drug intake, and presence of hypertension, microalbuminuria and menopause. To assess differences in cholesterol, LDL, HDL, BMI, the Student t-test was utilized. Because of the highly "skewed" distribution of Lp(a), tHcy, and triglycerides levels, to compare Lp(a), tHcy, and triglycerides values the Mann-Whitney U-test was used. The Pearson chi-squared test was exploited for frequency comparison. The relationship of AER with Lp(a) levels and tHcy levels 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 ORs and 95%CI. Sensitivity (probability that a test will be positive when the disease is present), specificity (probability that a test will be negative when the disease is not present), positive predictive value (probability that the disease is present when the test is positive) and negative predictive value (probability that the disease is not present when the test is negative) have been calculated for some parameters. Table 1 reports the schematic outcomes of a test. Data were presented as means ± SD, unless otherwise stated. $P < 0.05$ was considered significant.

Results

Out of the 1,971 type 2 diabetic patients, 1591 (80.7%) showed a normal resting ECG, 158 (8.1%) ST-T abnormalities, 21 (1.0%) signs suggestive of infarction and 201 (10.2%) other ECG abnormalities (bundle branch block, atrial fibrillation or other important arrythmias, atrioventricular block, fascicular anterior block, left ventricular hypertrophy).

Patients with ST-T abnormalities

Out of 158 type 2 diabetic patients with ST-T abnormalities, exercise stress ECG test was positive in 73 patients, negative in 69, equivocal or unconclusive in 16. In 19 patients the exercise ECG test was highly positive and therefore the angiography was recommended. Other 54 patients with a positive exercise ECG and 16 patients with equivocal or unconclusive test underwent an exercise

Table 2: Characteristics of diabetic patients with asymptomatic CAD and without CAD.

	CAD	NO CAD	p-value
Number	75	75	
Sex (M/F)	62/13	62/13	NS
Age (years)	56.6 ± 6.2	56.7 ± 6.2	NS
Duration of diabetes (years)	7.9 ± 5.7	8.0 ± 4.9	NS
BMI	26.4 ± 3.4	26.1 ± 3.7	NS
HbA1c (%)	7.8 ± 1.4	7.4 ± 1.4	NS
Cholesterol (mmol/l)			
Mean	5.6 ± 0.9	5.4 ± 1.0	NS
Median	5.6	5.2	
Range	3.8–9.0	3.4–8.6	
LDL (mmol/l)			
Mean	2.7 ± 1.0	2.5 ± 1.0	NS
Median	2.8	2.6	
Range	1.0–5.9	0.5–6.0	
HDL (mmol/l)			
Mean	1.1 ± 0.2	1.2 ± 0.2	<0.05
Median	1.1	1.3	
Range	0.6–2.0	0.9–2.1	
Triglycerides (mmol/l)			
Mean	1.7 ± 0.6	1.6 ± 0.6	NS
Median	1.7	1.5	
Range	0.8–3.2	0.6–3.7	
Microalbuminuria (%)	42.7	10.7	<0.0001
AER (mg/day)			
Mean	53.0 ± 67.8	22.5 ± 37.5	<0.01
Median	25.0	10.0	
Range	4.5–293.1	4.9–258.3	
Smokers (%)	57.3	29.3	<0.001
Family history of CAD (%)	36.0	30.7	NS
Hypertension (%)	53.3	57.3	NS
Autonomic neuropathy (%)	29.3	22.7	NS
Lp(a) levels (mg/dl)			
Mean	22.0 ± 18.9	16.0 ± 19.4	<0.05
Median	18.0	7.0	
Range	0.5–85	0–88.5	
Subjects with Lp(a) levels >30 mg/dl (%)	33.3%	17.3%	<0.05
tHcy levels (mmol/l)			
Mean	13.6 ± 6.0	11.4 ± 4.9	<0.05
Median	12.0	10.0	
Range	7.4–41.1	5.1–35.6	
Subjects with tHcy levels >14.0 mmol/l (%)	42.7	26.7	<0.05
Subjects with at least one isoform of low MW (%)	70.7	29.3	<0.0001

LDL = Low Density Lipoprotein; HDL = High Density Lipoprotein; BMI = Body Mass Index; AER = Albumin Excretion Rate. Lp(a) = Lipoprotein(a); tHcy = total homocysteine; Apo(a) = Apolipoprotein(a); CAD = Coronary Artery Disease; tHcy = total homocysteine; MW = Molecular Weight

stress thallium scintigraphy. Among these patients, scintigraphy was positive in 58 subjects. Seventy-two (17 subjects with highly positive exercise ECG and 55 with a positive scintigraphy) gave their informed consent to perform coronary angiography. Angiography showed a significant atherosclerotic lesion in 65 subjects (16 with a highly positive exercise test and 49 with a positive scintigraphy) and no significant lesions in 7 patients.

Patients with ECG suggestive of infarction

Out of 21 patients with ECG abnormalities suggestive of myocardial infarction, 16 had a positive scintigraphy. Among them, 12 gave their informed consent to perform

Table 3: Results of a multiple logistic regression analysis with the presence of asymptomatic CAD as the dependent variable in diabetic patients

PREDICTORS	Odds Ratio	95% Confidence Intervals	p-value
	MODEL 1		
Microalbuminuria	7.09	2.65–18.96	<0.001
Smoking	2.72	1.17–5.85	<0.02
High Lp(a) levels	2.62	1.01–6.79	<0.05
Low HDL	2.39	1.06–5.38	<0.05
High tHcy levels	2.04	1.03–4.06	<0.05
	MODEL 2		
Apo(a) phenotypes	8.65	3.05–24.55	<0.001
Microalbuminuria	6.16	2.21–17.18	<0.01
Low HDL	3.16	1.28–7.81	<0.05
Smoking	2.53	1.05–6.08	<0.05
High tHcy levels	2.25	1.14–4.43	<0.05

Model 1, potential predictors: hypertension, family history of CAD, smoking, microalbuminuria, HbA1c, BMI, Lp(a), tHcy, cholesterol, triglycerides, LDL, HDL. Model 2, potential predictors: hypertension, family history of CAD, smoking, microalbuminuria, HbA1c, BMI, Lp(a), tHcy, cholesterol, triglycerides, LDL, HDL, Apo(a) phenotypes.

coronary angiography. Angiography showed a significant atherosclerotic lesion in 10 subjects and no significant lesions in two patients.

CAD and NO CAD groups

Among 75 patients with angiographically verified asymptomatic CAD (CAD group), 23 subjects (30.7%) showed a mono-vessel disease, 32 (42.6%) a bi-vessel disease, and 20 (26.7%) a multi-vessel disease. Seventy-five diabetic patients without CAD (NO CAD group) were selected from the 1,591 diabetic patients with a negative resting ECG. NO CAD patients were matched by age, gender and duration of diabetes to 75 CAD patients. In NO CAD patients exercise ECG testing, 48-h ECG Holter and stress echocardiography were negative for CAD. Among CAD patients, 30 (40.0%) were treated with oral agents, 31 (41.3%) with insulin, 2 (2.7%) with insulin and oral agents, and 12 (16.0%) with diet alone. Among NO CAD patients, 36 (48.0%) were treated with oral agents, 24 (32.0%) with insulin, none (0%) with insulin and oral agents, and 15 (20.0%) with diet alone. No differences in diabetes treatment were found between the groups (χ^2 = 3.770; df = 3; NS). Patients receiving metformin were 25 (33.3%) among CAD patients and 31 (41.3%) among NO CAD patients (χ^2 = 1.03; df = 1; NS).

Table 2 shows biological and clinical features of patients with and without CAD. CAD group showed a percentage of microalbuminuric subjects and smokers significantly greater and HDL levels significantly lower than NO CAD group.

Glycemic control

Table 1 shows that the two study groups did not differ in HbA1c at recruitment. In our diabetic patients HbA1c was measured quarterly. We did not find differences in the average values of HbA1c measurements performed in the year before the recruitment (7.8 ± 1.3 versus 7.6 ± 1.2%; NS).

Autonomic function

Among CAD patients, 22 showed abnormal autonomic function. In particular, in 10 patients one of the parasympathetic tests was abnormal, in 8 two of the parasympathetic tests were abnormal, in 2 one of the sympathetic tests was abnormal; in 2 patients both one of the parasympathetic tests and one of the sympathetic tests were abnormal. Among NO CAD patients, 17 showed abnormal autonomic function. In particular, in 10 patients one of the parasympathetic tests was abnormal, in 6 two of the parasympathetic tests were abnormal; in 1 patient both one of the parasympathetic tests and one of the sympathetic tests were abnormal.

Lipoprotein(a) levels, tHcy levels, and apolipoprotein(a) phenotypes

Table 2 shows that CAD group had Lp(a) levels, tHcy levels and a prevalence of subjects with high Lp(a) levels and high tHcy levels significantly higher than NO CAD group. Out of the 150 patients enrolled, we detected 25 apo(a) isoforms with an apparent MW varying from 400 to 835 kDa; 56 subjects (37.3%) showed two electrophoretic bands, 93 (62.0%) showed only one electrophoretic band. One patient (0.7%) of the NO CAD group had the so-called "null phenotype" (no band detectable by electrophoresis): this subject had a Lp(a) plasma concentration = 0 mg/dl. The cut-off between 640 and 655 kDa was used to divide apo(a) isoforms of low and high MW [5,7,17,31]. This cut-off appears to be the most efficient in discriminating subjects at higher cardiovascular risk linked to the apo(a) gene [31].

Table 3 shows that the percentage of subjects with at least one apo(a) isoform of low MW was significantly greater in CAD than in NO CAD group.

AER showed no significant correlation with Lp(a) levels (in CAD group: r = 0.0447; NS - in NO CAD group: r = 0.0186; NS) or with tHcy levels (in CAD group: r = 0.0642; NS - in NO CAD group: r = 0.1606; NS)

Multivariate analysis

A multiple logistic regression analysis was performed with presence/absence of asymptomatic CAD as the dependent variable. Table 3 (model 1) shows the results of the analysis. When apo(a) phenotypes (presence of at least one isoform of low MW = 1 / presence of only isoforms of high MW = 0) were added to the list of potential predictors, the analysis showed that apo(a) phenotypes, microalbuminuria, smoking, high tHcy levels, and low HDL were independent predictors of asymtomatic CAD in diabetic patients (Table 3 model 2).

Sensitivity, specificity and positive or negative predictive values have been calculated for Lp(a) (Sensitivity:0.33; specificity:0.83; positive predictive value:0.66; negative predictive value:0.55), apo(a) phenotype (Sensitivity:0.71; specificity:0.71; positive predictive value:0.71; negative predictive value:0.71), tHcy (Sensitivity:0.43; specificity:0.73; positive predictive value:0.62; negative predictive value:0.56), and microalbuminuria (Sensitivity:0.43; specificity:0.89; positive predictive value:0.80; negative predictive value:0.61). In addition, we tested the prediction model with Lp(a), tHcy and apo(a) in combination: the presence of at least one positive test showed the following results: sensitivity:0.87; specificity:0.45; positive predictive value:0.61; negative predictive value:0.77)

Discussion

Diagnosing silent CAD is difficult. At the present, screening for CAD is recommended in only patients with complications or with ECG abnormalities suggestive of ischemia or infarction [14]. In patients with uncomplicated diabetes screening for CAD is unjustified, since the probability of finding significant CAD by non-invasive testing is low [9]. In uncomplicated patients the screening for CAD is recommended only when two or more cardiovascular risk factors are present; nevertheless, this recommendation does not address the potential impact of some novel cardiovascular risk factors, including Lp(a) and tHcy [14]. The possible association of Lp(a) and apo(a) polymorphism with silent CAD has been recently investigated in type 2 diabetic patients with normal ECG at rest [23] No studies have analysed the possible role of tHcy. In the present study we have analysed in a different population (type 2 diabetic patients with abnormal resting ECG) not only Lp(a) and apo(a) but also tHcy as potential risk factors for silent CAD.

The present study shows that high Lp(a) levels and apo(a) phenotypes of low MW are associated with silent CAD not only in diabetic patients with normal ECG but also in those with ECG abnormalities. In the multivariate analysis, when apo(a) and Lp(a) were entered into the model at the same time, apo(a) was a significant independent predictor of CAD, while Lp(a) did not. Lp(a) was a significant predictor, when apo(a) was not entered. This behaviour of Lp(a) was expected, considering that Lp(a) level is strongly dependent on apo(a) MW [32]. Therefore our finding confirms not only the association of Lp(a) and apo(a) with silent CAD, but also that apo(a) polymorphism may have a predictive power for silent CAD higher than that of Lp(a) levels [23]. Lp(a) levels and the percentage of subjects with low apo(a) phenotype found in the CAD group are similar to those observed both in diabetic patients with overt CAD [17] and in diabetic patients with silent CAD and normal resting ECG [23]. This suggests that high Lp(a) levels and low apo(a) phenotypes are associated with angiographically assessed atherosclerotic stenosis and that these parameters are not linked to any specific clinical manifestation of CAD.

The original finding of the present investigation is the independent association between high tHcy levels and silent CAD. Little is known about the impact of diabetes on plasma tHcy levels [18]. It has been reported that glycemia, HbA1c, diabetes duration and microalbuminuria are not able to affect plasma Hcy [18,19]. On the contrary, tHcy levels might be influenced by the deterioration of renal function or by the therapy with metformin [18]. In our study a bias due to the above conditions does not seem to be likely. Indeed, the two study groups are matched by diabetes duration and do not show any differences in glyc-

emic control. A deterioration of the renal function is not present, since patients with abnormal creatinenemia or with proteinuria were excluded from the study. There are no differences in the percentages of subjects treated with metformin between the two groups. At last no correlations were observed between AER and tHcy levels.

Our data confirm that microalbuminuria and smoking may predict silent CAD in people with diabetes [23]. The present study shows an independent association between low HDL levels and silent CAD; no previous studies found any independent associations between lipid parameters and presence of asymptomatic CAD [10,33,34], except for MiSAD study, that reported an independent association between total cholesterol and silent myocardial ischemia in men [11]. In our survey an association between autonomic neuropathy and silent CAD was not found. This confirms that the role of autonomic neuropathy as a predictor of silent CAD remains to be clarified [14,35].

Our data show that subjects with Lp(a) or tHcy levels higher than 30 mg/dl and 14 mmol/L respectively are 1.5 to 2 fold higher in the CAD group. Conversely, microalbuminuria is quite different (42.7% versus 10.7%) in CAD and NO CAD, suggesting that microalbuminuria could be more pertinent parameter for CAD screening. The reliability of microalbuminuria as a marker for silent CAD seems to be confirmed both by the logistic regression analysis and by the prediction model. This finding is in agreement with previous studies [23,36,37]. However, Lp(a) and tHcy can provide additional information on the risk for silent CAD, since they could favour the identification of subjects with genetic CAD predisposition.

In the present study, diabetic subjects with negative non-invasive testing for CAD were recruited as a control group. The absence of abnormalities on regular testing or with scintigraphy does not exclude non-occlusive CAD. Considering that in our control group angiography was not performed because of evident ethical implications, we are aware that some controls may have non-occlusive CAD. This may influence the degree of association between risk factors and silent CAD.

The association between some novel risk factors and silent CAD has been observed in a selected group of diabetic patients. This does not imply that these parameters can be used to predict the presence of silent CAD in all diabetic subjects; indeed in our study the parameters were not measured in the vast majority of patients. Moreover, due to the relatively low number of patients recruited, ORs estimated by the logistic regression analysis are significant, but the large intervals indicate a relatively weak precision. In addition, sensitivity and specificity for Lp(a), tHcy, and apo(a) polymorphism showed that none of the parameters is able by itself to identify silent CAD. Lp(a), apo(a) and tHcy seems to be quite specific, but the sensitivity of Lp(a) and tHcy is quite low. Among the three parameters, apo(a) polymorphism appears to be the most efficient. The analysis of the three parameters in combination increases the sensibility, but the specificity is quite low. The present prediction model of silent CAD shows scores similar to that previously published [23]. This suggests that, if other larger studies confirm our findings, Lp(a), tHcy and apo(a) may contribute to identify patients to screen for CAD, only if used together with other recognized cardiovascular risk factors.

Another important problem is the lack of standardization of apo(a) phenotyping methods and Lp(a) kits. In addition, there are no recognized standards for apo(a) [23]. For these reasons at the moment it is not possible an utilization of these parameters in clinical practice.

Another problem regards the cut-off of apo(a) polymorphism. The cut-of between 640 and 655 kDa appeared to be the most efficient in discriminating subjects at higher cardiovascular risk linked to the apo(a) gene [32]. However this cut-off has not been validated for clinical use by other studies. So we are using the cut-off between 640 and 655 Kda for research only, but we are aware that this cut-off cannot be used for clinical purposes.

Conclusions

The present investigation shows an association of Lp(a), tHcy and apo(a) polymorphism with silent CAD in diabetic patients with ECG abnormalities. Other larger studies are needed to confirm our findings and to establish whether Lp(a), tHcy, and apo(a) polymorphism can be used as suitable markers for CAD screening.

Authors' contributions

CG and AG were the principal investigators, designed the study, wrote the article, and participated in all analyses of data; CC also performed statistical analysis; AG also performed the detection of apo(a) isoforms; CF and SG participated in the study design, collected and checked data; PF was responsible for overall supervision of the research and interpretation of data. All investigators have contributed to the writing the paper.

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