

# Asymptomatic Carotid Atherosclerosis Cardiovascular Risk Factors and Common Hypertriglyceridemia Genetic Variants in Patients with Systemic Erythematosus Lupus

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## Research article

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# Abstract

**Background and aims:** SLE is a systemic autoimmune disease associated with an increased cardiovascular risk which is related with the characteristic dyslipidemia of SLE. This consists of an alteration of triglyceride-rich lipoprotein metabolism and an increased concentration of apoB containing particles. The objective of this study is to know the prevalence of carotid atherosclerosis and to analyze its relationship with dyslipidemia and its related genetic factors in a population of SLE patients.

**Methods:** Seventy-one SLE female were recruited. A carotid ultrasound was performed to evaluate the presence of atheromatous plaques and cIMT. Lipid profile and analysis of ZPR1, APOA5 and GCKR genes were carried out. SLE patients were analyzed according to the presence or absence of carotid plaques. Statistical analyses were performed to evaluate the relationship between lipid parameters and these allelic variants involved in triglyceride metabolism.

**Results:** SLE patients with carotid plaque had higher concentrations of plasma triglyceride than SLE patients without carotid plaque (1.5 vs 0.9 mmol/L, respectively,  $p=0.001$ ), Non-HDLc (3.5 vs 3.1 mmol/L,  $p=0.025$ ) and apoB (1.0 vs 0.9 g/L,  $p=0.010$ ). GCKR (c.1337C>T) C-allele was observed in 83.3% and 16.7% ( $p=0.047$ ) of patients with and without carotid plaque, respectively. The GCKR (c.1337C>T) CC genotype (OR= 0.03; [95% CI] [0.002 to 0.53],  $p=0.016$ ), and triglyceride concentrations (OR= 7.57; [95% CI] [1.43 to 40.19],  $p=0.017$ ) were independently associated with the diagnosis of carotid plaque.

**Conclusions:** plasma triglyceride concentration and GCKR CC homozygosity for GCKR gene are independent predictive factors of carotid atherosclerosis in women with systemic lupus erythematosus. **Keywords:** triglycerides, GCKR gene, Non-HDLc, atherosclerosis, systemic lupus erythematosus.

## 1 Introduction

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease characterized by inflammation and tissue damage through an exaggerated response of the immune system due to the binding of auto antibodies to the body's cells and the deposit of antigen-antibody complexes. SLE most commonly presents in women in the reproductive age, has a highly variable clinical course and is associated with a 3-fold increased risk of premature death, mostly due to infection, renal impairment or cardiovascular disease (1). Even during inactive disease SLE patients may have a characteristic dyslipidemia that is known as "Lupus pattern" consisting of hypertriglyceridemia and decreased levels of high-density lipoprotein-cholesterol (HDLc) (2). In active SLE, this "Lupus pattern" may be aggravated by the presence of Anti-lipoprotein lipase (LPL) antibodies and the consequent decrease in lipolysis that results in accumulation of triglyceride-rich lipoproteins (3). Furthermore, HDL efflux capacity is significantly impaired and there is an increased expression of apolipoprotein E (apoE) (4).

The increase in cardiovascular risk of patients with SLE is not justified by traditional risk factors alone. It has been suggested that chronic inflammation related to SLE plays a role in the development of

atherosclerosis (5). Also other, more subtle lipid metabolism disorders may play a role. Among them, it is observed an alteration in the structure and composition of lipoproteins, which occurs as triglyceride concentrations increase, associating decreasing HDLC concentration and raising small and dense low-density lipoprotein-cholesterol (LDLC) particles proportion, which is known as the atherogenic lipoprotein phenotype (6).

Different methods for quantification and characterization of lipoprotein subfractions have been used. Ultracentrifugation is not usually used due to its complexity and new methods such as the nuclear magnetic resonance (NRM) or several electrophoresis techniques still are not available for clinical routine

As of today, there are only a few reports on atherogenic lipoprotein phenotype and its significance in SLE. Also, not only SLE disease but environmental and lipid factors are influenced by polygenic disorders. This genetic role has not been fully explained, and its relation with dyslipidemia and cardiovascular disease in SLE subjects is unknown.

The objective of this study is to know the prevalence of carotid atherosclerosis and to analyze its relationship with dyslipidemia and its related genetic factors in a population of patients with SLE

## 2 Materials And Methods

### 2.1 Study population

Seventy-one female patients with SLE diagnosis, based on revised American College of Rheumatology (ACR) classification criteria for SLE (24), were recruited from the systemic autoimmune diseases unit of the Hospital Universitari de Bellvitge (L'Hospitalet de Llobregat, Barcelona). The research protocol was approved by the ethics committee of the hospital. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and all patients gave their informed consent to participate in the study. The exclusion criteria were as follows: history of a previous atherosclerotic cardiovascular event and autoimmune disease other than SLE, non-cardiovascular lower limb amputation and when it was not possible to obtain an ultrasound image of the carotid bifurcation. The clinical information that was retrieved for each study participant included:

1. Demographic data: age and ethnic
2. Cardiovascular risk factors: smoking status, arterial hypertension, dyslipidemia, diabetes mellitus, body mass index (BMI) and family history of early cardiovascular disease (7), premature menopause before the age of 40 years (8)
3. Drugs: antithrombotic medications, statins, immunosuppressive
4. Carotid ultrasound
5. Laboratory data: serum glucose, lipid profile, liver and kidney parameters, hematology and coagulation tests.
6. Ankle-brachial index

7. Lifestyle: A Mediterranean Diet questionnaire of SEA (*Sociedad Española de Aterosclerosis*) (9) and physical activity
8. SLE: onset and time evolution of the disease, analytical immunological activity, SLE disease activity index scales SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) (10) and Systemic Lupus International Collaborating Clinics/ACR Damage Index (SDI) (11), secondary antiphospholipid syndrome, history of immunosuppressive drugs and analytical parameters of inflammation.

Samples were collected in tubes containing the anticoagulant EDTA-K4 and were centrifuged for 15 minutes at 1500 g and at 4°C then plasma was separated and stored at -80° until analyzed.

## 2.2 Measurement of small and dense LDL cholesterol concentration

Small and dense LDL (sd-LDL) particles were isolated using the previously and validated reported precipitation method adapted for use in our laboratory to process samples containing elevated concentrations of triglycerides (12). The surfactant magnesium heparin was prepared as a solution of 150U/mL of Heparin-Na<sup>+</sup> (Sigma Aldrich H3393) and 90 mmol/L of MgCl<sub>2</sub> 300μL of Magnesium Heparin was added to each 300μL of plasma and incubated for 10 minutes at 37°C. The mixture was kept at 0°C for 15 minutes and centrifuged at 21913g for 15 minutes at 4°C (6K15 SIGMA centrifuge). At this moment, lipoproteins with a density <1.044g/mL were precipitated. The supernatant contained HDL and sd-LDL particles that had densities of between 1.044 and 1.063 g/mL. Supernatant HDL-C and total cholesterol were measured using a Cobas 8000 modular analyzer (Roche® Diagnostics GmbH, Mannheim, Germany by homogeneous assay) (13).

## 2.3 Genetic analysis of polymorphisms involved in triglyceride metabolism

Functional variants in *APOE* gene were genotyped using validated TaqMan™ MGB Probes. We performed the genetic analysis of the most prevalent allelic variants that most determine the presence of polygenic hypertriglyceridemia (14) adapted to the population of our area and therefore making the three most relevant genetic variants corresponding to the *ZPR1*, *APOA5* and *GCKR* genes. The three possible genotypes will be dichotomized in homozygotes so as not to present a risk allele (0) or risk allele carriers (either hetero or homozygous) (1) as shown below: c\*724C>G (*ZPR1*); (0=CC vs 1=G), c.56C>G (*APOA5*); (0=CC vs 1=G), c.1337T>C (*GCKR*); (0=CC vs 1=T).

## 2.4 Carotid ultrasound and ankle-brachial index

Certified vascular technologists measured the carotid ultrasound (US) and the ankle-brachial index (ABI) using standardized protocols. Carotid US was performed to assess carotid intima-media wall thickness (cIMT) in the far wall of the common carotid artery and to detect focal plaques in the extracranial carotid tree with the commercially available scanner (ACUSON Antares™ Siemens Medical Solutions USA, Inc.) ultrasound system using a 6 MHz linear array transducer. Based on the Mannheim consensus, plaque criteria were defined as a focal protrusion in the lumen measuring at least cIMT > 1.5 mm, a protrusion at least 50% greater than the surrounding cIMT or an arterial lumen encroaching > 0.5 mm (15). The cIMT

was defined as the distance between the leading edge of the lumen–intima echo and the leading edge of the media–adventitia echo (16). The normal values of cIMT in Spanish women corresponding to the 75th percentile are 0.60mm for < 46 years, 0.84mm for < 65 years and 1.02 for > 65years. At inclusion, cIMT measurements above those values were considered pathologic. When a plaque was observed in the region of the CCA measurements, the cIMT was not measured (17).

The ABI was performed after 5 minutes of resting in the supine position. Systolic blood pressure was measured in the brachial arteries and at the ankle level and on both sides of the body using an automatic waveform analyzer (Vascular Handheld Doppler Bidop 7 Hadeko® Kawasaki, Japan). The ABI was calculated as ratio of ankle pressure to brachial pressure. Ratios of > 0.90 to < 1.4 are considered normal, ratios  $\leq$  0.9 indicate the presence of peripheral artery disease (18) and ratios  $\geq$  1.4 usually indicate the presence of arterial calcification, a situation that is also associated with an increased risk of cardiovascular complications and increased risk of all-cause mortality (7, 19).

## 2.5 Statistical analyses

Qualitative variables were described as absolute frequency (percentage) and were analyzed by the Chi-square test or Fisher's exact test. Normally distributed quantitative variables were described as mean (standard deviation), and analyzed by analysis of variance (ANOVA). Variables that were not distributed normally were described as median (interquartile range) and analyzed with nonparametric tests (Mann-Whitney U). To analyze the relationship between genetic variables and the presence of plaque, a binary logistic regression model was carried out, with the presence of plaque as a dependent variable, genetic variables as independent variables and adjusting for covariates: age, hypertension, SLICC, triglycerides (mmol/L) and presence of nephritis.  $p < 0.05$  was considered significant.

# 3 Results

## 3.1 Subjects characteristics

Seventy-one SLE female patients met inclusion criteria and were enrolled. As shown in Table 1, the mean age of subjects at enrollment was 52.2 years (30-75) with a median BMI of 25.5 kg/m<sup>2</sup> and mean waist circumference of 88.4 cm (58-129). Thirty subjects (42.3%) had a family history of cardiovascular disease, 46.5% were hypertensive, 53.5% had dyslipidemia and 7% were diabetic. The mean time of SLE disease duration was 20.7 years. Antiphospholipid syndrome was present in 14.1% of subjects. Thirty four (47.9%) subjects had a history of lupus nephritis. Regarding treatments, 46.4% of patients were on statins and 28.1% on antithrombotic drugs. Sixty five (91.5%) subjects were on hydroxychloroquine. Sixty three (88.7%) subjects had been treated with immunosuppressive drugs at some point since SLE diagnosis.

## 3.2 Comparisons between subgroups of SLE-patients

Two groups of SLE patients according to the presence or absence of carotid plaques were considered. Carotid plaques were present in 15 patients (21.1%). No significant differences were observed between both groups with and without carotid atherosclerosis with reference to age, race, educational level, dietary pattern, smoking and alcohol consumption. There were also no significant differences with reference to early menopause and other gynecological history including contraceptive use, menarche, early menopause, pregnancies, miscarriages and breastfeeding, nor with respect to factors directly related with SLE, such as antiphospholipid syndrome, nephritis and SLE disease activity scores assessed using the SLEDAI and SDI scores.

Compared to patients without carotid plaque, SLE patients with carotid plaque had higher concentration of plasma triglyceride (TG) (1.5 vs 0.9 mmol/L,  $p=0.001$ ), non-HDL-cholesterol (Non-HDL-C) (3.5 vs 3.1 mmol/L,  $p=0.025$ ), apolipoprotein B (apoB) (1.0 vs 0.9 g/L,  $p=0.010$ ) and homocysteine (13 vs 10.5,  $p=0.037$ ). Patients with carotid plaque also had a higher prevalence of hypertension than patients without carotid plaque (80% vs 37.5%,  $p=0.003$ ), as well as a higher prevalence of dyslipidemia (86.7% vs 44.6%,  $p=0.004$ ). The clinical course of SLE was longer in patients with carotid plaque than in those without it (24.1 vs 19.8 years,  $p=0.063$ ) although it was not statistically significant. As it is shown in Table 1, cholesterol-lowering drugs were more frequently used by SLE patients with carotid plaque than those without carotid plaque, being on statins 86.7% vs 35.7% ( $p<0.001$ ), respectively, and on ezetimibe 20% vs 0% ( $p=0.008$ ), respectively.

#### **Table 1. Baseline characteristics of patients**

Variables	Total (n=71)	No Carotid Plaque (n=56)	Carotid Plaque (n=15)	<i>p</i>
Age ( <i>years</i> )	52 (9.9)	51 (10.3)	56 (7.3)	0.067
BMI ( <i>kg/m<sup>2</sup></i> )	25.5 (23.0 to 29.6)	25.4 (22.9 to 29.0)	25.9 (23.2 to 30.7)	0.460
Waist circumference ( <i>cm</i> )	88.4 (12.8)	88.0 (12.2)	90.0 (15.2)	0.611
Dietary questionnaire SEA ( <i>score</i> )	11 (9 to 12)	11 (9 to 12)	11 (10 to 12)	0.585
Smoking ( <i>pack/years</i> )	0,9 (0 to 15.7)	3.0 (0 to 15.9)	0 (0 to 14.5)	0.268
Hypertension	33 (46.5%)	21 (37.5%)	12 (80%)	<b>0.003</b>
Diabetes mellitus	5 (7%)	5 (8.9%)	0	0.577
Dyslipidemia	38 (53.5%)	25 (44.6%)	13 (86.7%)	<b>0.004</b>
Cardiovascular familial history	30 (42.3%)	21 (37.5%)	9 (60%)	0.117
Antiphospholipid syndrome	10 (14.1%)	8 (10.7%)	4 (26.7%)	0.202
Nephritis	34 (47.9%)	24 (42.9%)	10 (66.7%)	0.101
SLE disease length ( <i>years</i> )	20.7 (8.1)	19.8 (7.2)	24.1 (10.1)	0.063
Pathological ABI	5 (7%)	5 (8.9%)	0	0.577
Glomerular filtration rate CKD-EPI ( <i>ml/min/1,73 m<sup>2</sup></i> )	90 (78 to 90)	90 (84.5 to 90)	89 (64 to 90)	0.068
Total cholesterol ( <i>mmol/L</i> )	4.9 (0.7)	4.8 (0.7)	5.2 (0.8)	0.091
LDLC ( <i>mmol/L</i> )	2.6 (0.6)	2.6 (0.6)	2.8 (0.6)	0.431
HDLC ( <i>mmol/L</i> )	1.7 (0.4)	1.7 (0.5)	1.6 (0.4)	0.489
TG ( <i>mmol/L</i> )	1.0 (0.8 to 1.4)	0.9 (0.7 to 1.3)	1.5 (1.1 to 2.1)	<b>0.001</b>
Non-HDLC ( <i>mmol/L</i> )	3.2 (0.7)	3.1 (0.7)	3.5 (0.7)	<b>0.025</b>
ApoB ( <i>g/L</i> )	0.9 (0.8 to 1.0)	0.9 (0.8 to 1.0)	1.0 (0.9 to 1.1)	<b>0.010</b>
Sd-LDL ( <i>mmol/L</i> )	1.2 (0.3)	1.2 (0.3)	1.2 (0.4)	0.572
Lipoprotein (a) ( <i>g/L</i> )	0.17 (0.07 to 0.47)	0.17 (0.07 to 0.37)	0.25 (0.05 to 1.30)	0.430
Homocysteine ( <i>μmol/L</i> )	11 (9 to 14.9)	10.5 (8 to 14.5)	13 (11 to 18)	<b>0.037</b>

SDI ( <i>score</i> )	0 (0 to 1)	0 (0 to 1)	0 (0 to 1)	0.578
SLEDAI ( <i>score</i> )	4 (2 to 8)	5 (2 to 8)	4 (2 to 6)	0.083
C3 complement ( <i>mg/L</i> )	1049 (233)	1050 (241)	1042 (212)	0.904
C4 complement ( <i>mg/L</i> )	172 (84)	175 (88)	162 (71)	0.601
Prothrombin time ( <i>ratio</i> )	1.0 (0.9 to 1.0)	1.0 (0.9 to 1.0)	1.0 (0.9 to 2.1)	0.703
Fibrinogen ( <i>g/L</i> )	3.2 (2.7 to 3.7)	3.2 (2.6 to 3.7)	3.4 ( 2.8 to 3.8)	0.489

Table 1. Data are expressed as n (%); mean (*SD*= standard deviation) for normally distributed quantitative variables and median (interquartile interval) for non-normally distributed variables. Value data highlighted in bold indicate P values  $p < 0.05$ . BMI= body mass index, LDLC= low-density lipoprotein cholesterol, HDLC= high-density lipoprotein cholesterol, TG= triglycerides, Non-HDLC = non-HDL-cholesterol, Apo B= apolipoprotein B, sd-LDL= small and dense LDL, SDI: Systemic Lupus International Collaborating Clinics/ACR Damage Index, SLEDAI= Systemic Lupus Erythematosus Disease Activity Index, ABI= ankle brachial index.

Results of the genetic analysis of the three common allelic variants that most influence the plasma concentration of triglycerides in patients from our area are shown in Table 2. Comparable proportions of subjects had carotid plaque across the *ZPR1* and *APOA5* genotypes. Patients homozygote and heterozygote for a protective *GKCR* C-allele had a lower prevalence of carotid plaque than homozygote patients with the *GCKR* (c.1337C>T) TT variant (16.7% vs 45.5%,  $p = 0.047$ ).

**Table 2. Comparison of carotid plaque across genotypes.**

Gene	Allelic variant	No Carotid Plaque		Carotid Plaque		Total	<i>p</i>
<i>ZPR1</i> (c.*724C>G)	Homozygous CC	38	(76%)	12	(24%)	50	0,617
	Heterozygous CG	17	(85%)	3	(15%)	20	
	Homozygous GG	1	(100%)	0	(0%)	1	
<i>APOA5</i> (c.56C>G)	Homozygous CC	46	(78%)	13	(22%)	59	0.838
	Heterozygous CG	9	(81.8%)	2	(18.2%)	11	
	Homozygous GG	1	(100%)	0	(0%)	1	
<i>GCKR</i> (c.1337C>T)	Homozygous CC	23	(85.2%)	4	(14.8%)	27	NA
	Heterozygous CT	27	(81.8%)	6	(18.2%)	33	
	Homozygous TT	6	(54.5%)	5	(45.5%)	11	
Grouped <i>GCKR</i> (c.1337C>T)	Homozygous CC	50	(83.3%)	10	(16.7%)	60	<b>0.047</b>
	or Heterozygous CT						
	Homozygous TT	6	(54.5%)	5	(45.5%)	11	

Table 2. Data are expressed as n (%) and value data highlighted in bold indicate *P* values  $p < 0.05$ . NA= non-applicable

In the multivariate logistic regression (Table 3) we observed the protecting *GCKR* rs1260326 CC genotype, reduced the risk of having carotid plaque (Odds Ratio-OR= 0.03; [95% confidence interval-CI] [0.002 to 0.53],  $p=0.016$ ). Furthermore, a one millimole per liter-unit increase of triglycerides was associated with increasing risk for carotid plaque (OR= 7.57; [95% CI] [1.43 to 40.19],  $p=0.017$ ). The whole model explained the 54% (Nagelkerke  $R^2$ ) of the risk for developing carotid plaque in our SLE patients.

**Table 3. Multivariate analysis of risk factors for carotid plaque in SLE patients**

Variables	OR (IC 95%)	<i>p</i>
<i>GCKR</i> (c.1337C>T)		
- CC	0.03 (0.002 to 0.53)	<b>0.016</b>
- CT	0.15 (0.02 to 1.46)	0.103
Triglycerides	7.57 (1.43 to 40.19)	<b>0.017</b>
Hypertension	7.57 (0.98 to 58.17)	0.052
Nephritis	1.88 (0.38 to 9.37)	0.440
SLE disease length ( <i>years</i> )	1.05 (0.96 to 1.16)	0.289
SDI	1.00 (0.60 to 1.67)	0.997
Age ( <i>10 years</i> )	2.10 (0.89 to 4.94))	0.090

Table 3. OR=odds ratio; CI=confidence interval. Age (10 years) indicates ten years age difference, SDI: Systemic Lupus International Collaborating Clinics/ACR Damage Index. Value data highlighted in bold indicate *P* values  $p < 0.05$ .

## 4 Discussion

To our knowledge, this is the first study to link common genetic variants associated with the concentration of plasma triglyceride to subclinical carotid atherosclerosis in SLE.

Detection of carotid plaques is one of the most common findings in the study of subclinical atherosclerosis and a strong predictor of future cardiovascular events. More specifically, the presence of carotid plaque at baseline has been associated with a greater than four-fold increased risk for any hard cardiovascular event in SLE patients (20). Although intimal thickening has also been linked to increased cardiovascular risk, it is well established that presence of an atherosclerotic plaque is a better surrogate marker of cardiovascular risk than carotid intima-media thickness also in SLE patients (21-23). In this sense, we only analyzed the data on the presence of carotid plaque. Our SLE population had a similar prevalence of carotid plaque (21.1% vs 16% to 37%) than what was reported in previous series of SLE patients (24, 25). Patients with carotid plaque were more hypertensive and dyslipidemic than those without plaque. These, are two modifiable conventional cardiovascular risk factors that increase the risk of cardiovascular events in SLE (26-29). In concordance to previous results, patients with plaque have higher plasmatic concentrations of TG which was consistent with the more common dyslipidemia of SLE (30). Although Non-HDL-C and apoB were higher in patients with plaque, there were no statistically differences in low-density lipoprotein cholesterol (LDL-C). Non-HDL-C includes the atherogenic potential of remnant lipoproteins and offers a more accurate risk estimation than does LDL-C in hypertriglyceridemic subjects by adding remnant-cholesterol (remnant-cholesterol= total cholesterol - HDL-C - LDL-C) to LDL-C (31). These results are in agreement with current knowledge of the role of TG-rich lipoproteins and their

remnants as a strong proatherogenic factor for the initiation and the progression of atherosclerosis determined by the number of circulating concentration of apoB-containing particles (32). On the other hand, we did not find statistically significant differences in HDL-C concentrations. This result is in agreement with the data from Mendelian randomization studies that do not provide compelling evidence that HDL-C is causally associated with the risk of atherosclerotic cardiovascular disease (33).

The study of small dense LDL in SLE patients with atherosclerosis has been addressed in different investigations. By using the Lipoprint LDL system, it was demonstrated that patients with SLE have a population of LDL particles smaller and denser than those from healthy controls (34) and another study reported that circulating lipoprotein remnant particles measured by nuclear magnetic resonance were better predictor of carotid atherosclerosis in SLE subjects than other lipoprotein variables (30). In spite of the higher TG and Non-HDL-C plasma concentrations that SLE patients with carotid atherosclerosis from this study displayed, we did not find any differences in small dense LDL concentrations by using the modified heparin-Mg<sup>2+</sup> precipitation method (13) between SLE patients with and without carotid atherosclerosis. It has also been suggested the association of  $\epsilon_2$  APOE allele with increased cIMT in SLE patients (30), but this was not observed in SLE patients from this study.

Triglycerides are carried in plasma by very low-density lipoproteins (VLDL), chylomicrons and their remnants which are highly atherogenic. There is a direct relationship between the concentration of plasma triglyceride and concentration of cholesterol in remnant particles. Strong evidence suggests that elevated TG-rich lipoproteins are causal risk factors for low-grade inflammation and atherosclerosis (35, 36). Mild-to-moderate hypertriglyceridemia is a polygenic disorder, being the consequence of a cumulative burden of common and rare genetic variants that are usually exacerbated by non-genetic factors (37). In this study, the analysis of the *ZPR1*, *APOA5* and *GCKR* genes displayed that *GCKR* rs1260326 have a strong relationship with the presence of carotid plaque in SLE patients. Glucokinase regulator *GCKR* gene encodes glucokinase regulatory protein (GKRP), which is released in postprandial phase to cytoplasm and stimulates glycogen deposition and de novo lipogenesis. The homozygous TT allele of the *GCKR* rs1260326 led to a destabilization of the glucokinase binding interface. Increased hepatic glucokinase activity results in higher fasting serum TG concentration and lower glucose concentration (38, 39). In a meta-analysis of 46 genome-wide association studies a significant association between the homozygous TT allele of *GCKR* rs1260326 and higher circulating TG levels was observed (40) although the atherogenic effect of this variant is controversial (41-43). The evaluation of non-traditional cardiovascular risk factors revealed statistically significant differences in plasma homocysteine concentrations in patients with carotid plaque ( $p=0.037$ ) which is consistent with the reported role of homocysteine as a potential contributor to the increased burden of atherosclerotic disease in SLE (44, 45). Antiphospholipid antibodies and SLE nephritis are tightly related to the risk of cardiovascular events in SLE patients (24, 26, 46), although neither antiphospholipid syndrome nor lupus nephritis or the complement were statistically relevant in our population.

Regarding severity and chronicity of SLE, average SLEDAI score index disease activity was moderate (4 of 2 to 6). Nevertheless, subjects included in this study had less accumulated structural damage

evaluated using SLICC index than cited in former cardiovascular risk reports. Length of SLE disease was longer (20.7 years) than described in other investigations. Evolution time of SLE disease would be a parameter which could be correlated with longer time of chronic inflammation but published evidences do not implicate it as a strong risk factor for cardiovascular events appearance (47, 48).

In this study, the multivariate logistic regression analysis that was performed to identify the independent predictors of carotid atherosclerosis, showed that the *CGKR* CC homozygosity for *CGKR* gene and plasma triglycerides (*OR*=0.03, and 7.57, respectively) remained independently associated with plaques. Remarkably, risk of having carotid plaques was directly related to the highest concentration of plasma TG, despite mean TG concentrations were within the reference intervals (< 150mg/dL or < 1.7 mmol/l). As reported, there was direct relationship between triglyceride levels and mortality in patients with established coronary heart disease even in patients with TG plasma < 1.7 mmol/l (49).

The association between hypertension and atherosclerosis in general population and in SLE patients is a well-known major contributor to cardiovascular disease (46) as well as age that in this study was not statistically significant, but showed a trend. Although nephritis history, and SLE disease duration and activity (SDI) were included in the model because of its clinical relevance, they lost their significance when *CGKR* genetic study, triglycerides and hypertension were included in the model.

This study has some limitations. Only women were included. In addition, a model adjusted for SLE and dyslipidemia treatments would have given more precise information. Corticosteroids treatments are one of the most important factors related to cardiovascular events in SLE patients in a dose and time depending way (28, 29) and other immunosuppressive agents as azathioprine had been related to the cardiovascular risk in these patients (26). It would have been interesting to perform an analysis on the independent relationship of corticosteroid dose and atherosclerosis beyond disease activity, but it was not possible to calculate cumulative dosages of steroids because the information to perform this calculation was not available.

Higher prevalence of statin treatment in SLE subjects with carotid plaque is the consequence of dyslipidemia. Despite atherogenic dyslipidemia is a well-demonstrated cardiovascular risk factor (50), the mechanisms by which the treatment of hypertriglyceridemia and the lipoprotein disorders associated would reduce cardiovascular disease have not yet been fully established. Currently, there are several ongoing clinical trials to assess the benefit of hypertriglyceridemia treatment in cardiovascular disease. It would be interesting to verify the cardiovascular protective effect of these drugs in lupus patients with asymptomatic atherosclerosis and atherogenic dyslipidemia where TG-rich lipoproteins play a major role. Finally, other limitations of this study that may be considered are the cross-sectional design and its sample size.

In summary, this study shows that plasma triglyceride concentration and *CGKR* CC homozygosity for *CGKR* gene are independent predictive factors of carotid atherosclerosis in women with SLE. These data suggest that strict triglyceride management may be useful to prevent atherosclerosis in SLE. Finally, this study also suggests that the diagnosis of different genetic variants related with hypertriglyceridemia can

be useful to better stratify the cardiovascular risk in patients with SLE. Further study on the role of genetic markers in the study of cardiovascular risk of SLE patients is needed.

## List Of Abbreviations

SLE: systemic lupus erythematosus; HDLC: high-density lipoprotein-cholesterol; LPDL: lipoprotein lipase; apoE: apolipoprotein E; LDLC: low-density lipoprotein-cholesterol; NRM: nuclear magnetic resonance; ACR: American College of Rheumatology; BMI: body mass index; SLEDAI: Systemic Lupus Erythematosus Disease Activity Index; SDI: Systemic Lupus International Collaborating Clinics/ACR Damage Index; sd-LDL: small and dense LDL; US: ultrasound; ABI: [ankle-brachial index](#); cIMT: carotid intima-media wall thickness; ANOVA: analysis of variance; Non-HDLC: non-HDL-cholesterol; apoB: apolipoprotein B; TG: triglyceride, SEA: *Sociedad Española de Aterosclerosis*; SD: standard deviation; NA: non-applicable; OR: Odds Ratio; CI: confidence interval; VLDL: very low-density lipoprotein; GGRP: glucokinase regulatory protein.

## Declarations

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### Authors' contributions:

Study conception and design: Fanlo-Maresma M, Candás-Estébanez B, Pintó-Sala X. Acquisition of data: Fanlo-Maresma M, Esteve-Luque V, Escrihuela-Vidal F, Carratini-Moraes M. Analysis and interpretation of data: Fanlo-Maresma M, Candás-Estébanez B, Padró-Miquel A, Corbella-Ingles E, Pintó-Sala X. Drafting of manuscript: Fanlo-Maresma M. Critical revision: Candás-Estébanez B, Corbella-Virós X, Pintó-Sala X.

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The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate:

The research protocol was approved by the ethics committee of Hospital Universitari de Bellvitge (L'Hospitalet de Llobregat, Barcelona). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and all patients gave their informed consent to participate in the study.

### Consent for publication:

Not applicable.

### Competing interests:

The authors declare that they have no competing interests.

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