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Study of common hypertriglyceridaemia genetic variants and subclinical atherosclerosis in a group of women with SLE and a control group

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ABSTRACT

Objective SLE is associated with increased cardiovascular risk (CVR). High serum concentrations of triglyceride-rich lipoproteins and apolipoprotein B-rich particles constitute the characteristic dyslipidaemia of SLE.

Methods A cross-sectional study was conducted to study the relationship between genetic variants involved in polygenic hypertriglyceridaemia, subclinical atherosclerosis and lipoprotein abnormalities. 73 women with SLE and 73 control women age-matched with the case group were recruited (age range 30–75 years). Serum analysis, subclinical atherosclerosis screening studies for the detection of plaque, and genetic analysis of the *APOE*, *ZPR1*, *APOA5* and *GCKR* genes were performed.

Results Triglyceride concentrations and the prevalence of hypertension, dyslipidaemia and carotid atherosclerosis were higher in women with SLE than in the control group. Multivariate logistic regression showed that CC homozygosity for the *GCKR* rs1260326 gene (OR=0.111, 95% CI 0.015 to 0.804, p=0.030) and an increase of 1 mmol/L in triglyceride concentrations were associated with a greater risk of carotid plaque in women with SLE (OR=7.576, 95% CI 2.415 to 23.767, p=0.001).

Conclusions *GCKR* CC homozygosity (rs1260326) and serum triglyceride concentrations are independently associated with subclinical carotid atherosclerosis in women with SLE. Subclinical carotid atherosclerosis is also more prevalent in these women compared with the control group. The study of *GCKR* rs1260326 gene variants may contribute to more precise assessment of CVR and modulation of the intensity of lipid-lowering treatment in patients with SLE.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ SLE is associated with polygenic hypertriglyceridaemia, but the effect that hypertriglyceridaemia polymorphisms may have on cardiovascular risk (CVR) in these patients is unknown.

WHAT THIS STUDY ADDS

⇒ CC homozygosity for GCKR rs1260326 was shown to have a protective effect against carotid atherosclerosis compared with TT homozygosity in patients with SLE.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The study of GCKR polymorphisms can improve CVR stratification in patients with SLE who could benefit from more intensive lipid-lowering pharmacological strategies.

INTRODUCTION

SLE is a systemic autoimmune disease that predominantly affects adult women and is associated with increased cardiovascular risk (CVR).[1 2] The interaction of classical cardiovascular risk factors (CVRFs) with the chronic inflammatory state of SLE has been linked to acceleration of the atherosclerotic process and premature cardiovascular events (CVEs) in these patients. The most characteristic dyslipidaemia of SLE is atherogenic dyslipidaemia, which is characterised by an increase in plasma triglyceride concentrations, a decrease in the concentration of high-density lipoprotein cholesterol (HDL-C), and a generalised disorder of structure and function of all lipoproteins that worsens within disease flares.[3] Triglycerides are transported in the plasma by very low-density lipoproteins (LDLs), chylomicrons and their remnants. Similar to LDLs, all these lipoprotein particles have one apolipoprotein B molecule per particle and may enter the arterial wall and cause atherosclerotic cardiovascular disease (ASCVD). Under these circumstances, low-density lipoprotein cholesterol (LDL-C) concentrations may be virtually normal but with an increase in small dense LDL particle concentrations.[4]

According to the Spanish Society of Arteriosclerosis (SEA) and the European Atherosclerosis Society, hypertriglyceridaemia is defined as a plasma triglyceride concentration

greater than 1.7 mmol/L or 150 mg/dL.[4 5] Most cases of hypertriglyceridaemia have a polygenic nature. The so-called polygenic hypertriglyceridaemia can be caused by the sum of rare genetic variants inherited heterozygously that encode essential proteins in the metabolism of triglycerides, including the *LPL*, *APOAV*, *APOCII*, *LMF1* and *GPIHBP1* genes.[6] However, the Global Lipids Genetics Consortium genome-wide association studies have demonstrated that polygenic hypertriglyceridaemia is more frequently the result of an excessive aggregation of single nucleotide polymorphisms.[7] More specifically, a study carried out by our group concluded that the pathogenic variants of the *ZPR1*, *APOA5* and *GCKR* genes most frequently conditioned the development of hypertriglyceridaemia in patients in our area. To date, the effect that these polymorphisms may have on the lipoprotein metabolism of patients with SLE is unknown. Likewise, it is not known whether their interaction with the remaining CVRFs influences the development of cardiovascular disease (CVD) in patients with SLE.

The objective of this study was to analyse the relationship between the genetic variants of the *ZPR1*, *APOA5* and *GCKR* genes, subclinical atherosclerosis and lipoprotein abnormalities in a population of patients with and without SLE.

MATERIALS AND METHODS

Population and study design

This was a cross-sectional, observational study that included 146 women in two groups. A total of 73 female subjects with SLE were consecutively recruited from the systemic autoimmune diseases unit of our hospital. The diagnosis of SLE was based on the revised criteria of the American College of Rheumatology. The control group consisted of 73 women without SLE, matched by age (±2 years) with the patient group (age range 30–75 years) and were selected from the outpatient clinics (mainly dermatology or occupational medicine follow-up) of the hospital, where they were visited for illnesses unrelated to autoimmune diseases, atherosclerosis, or any systemic or serious illness. Women with previous CVE, autoimmune diseases other than SLE, non-cardiovascular lower limb amputation and inability to obtain ultrasound (US) images of the carotid arteries were excluded.

Data collection

Clinical and anthropometric information was collected. CVRF was defined according to the SEA[5]; the dietary pattern was assessed using the Mediterranean Diet questionnaire[8]; and SLE disease activity was measured with index scales (Systemic Lupus Erythematosus Disease Activity Index[9] and Systemic Lupus International Collaborating Clinics/ACR Damage Index (SLICC/SDI).[10]

Laboratory data

Blood and urine samples were collected after 8 hours of fasting. All the biochemical analyses were performed in plasma using a COBAS 711 (Roche Diagnostics, Basel, Switzerland), while homocysteine levels were determined using the Immulite 2000 XPi analyser (Siemens Healthcare GmbH, Erlangen, Germany). Haematology values were measured (Sysmex S.L XN 900), and coagulation parameters were determined (ACTLOP analyser).

Genetic analysis of polymorphisms involved in triglyceride metabolism

Variants of the *APOE* gene were genotyped using validated TaqMan MGB probes. Genetic analysis of the allelic variants of the *ZPR1*, *APOA5* and *GCKR* genes, which mainly determine the presence of polygenic hypertriglyceridaemia, was performed. The three possible genotypes were dichotomised in homozygotes so as not to present a risk allele (0) or risk allele carriers (either heterozygous or homozygous) 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).

Evaluation of insulin resistance

The triglyceride/glucose (T&G) index described by Simental-Mendía *et al* was chosen for the evaluation of insulin resistance. It was calculated as the natural logarithm (Ln) of the product of glucose and plasma triglycerides according to the following formula: Ln [fasting triglycerides (mg/dL)×fasting glucose (mg/dL)]/2. As a cut-off point for the diagnosis of insulin resistance, a T&G index greater than 4.65 proposed by the same authors was used.[11 12]

Carotid US and ankle–brachial index (ABI)

Certified vascular technologists measured the carotid US and the ABI using standardised protocols. Carotid US was performed with the commercially available scanner (ACUSON Antares, Siemens Medical Solutions USA) using a 6 MHz linear array transducer. According to the Mannheim consensus, plaque criteria were defined as a focal protrusion in the lumen with a carotid intima–media thickness (cIMT) of >1.5 mm, a protrusion at least 50% greater than the surrounding cIMT or an arterial lumen of >0.5 mm.[13]

The ABI was performed using an automatic waveform analyser (Vascular Handheld Doppler Bidop V.7; Hadeko, Kawasaki, Japan) and was calculated as the ratio of ankle to brachial pressure. The ABI was evaluated according to standardised criteria.[14]

Statistical analyses

Qualitative variables were analysed by χ^2 or Fisher's exact test. Analysis of variance and Mann-Whitney U test were used for normally and not-normally distributed quantitative variables, respectively. To analyse the relationship between genetic variants and the presence of plaque, multivariate logistic regression analysis was performed. The presence of plaque was considered as a dependent variable and genetic variables as independent variables adjusted for different covariates that were related to subclinical atherosclerosis in a population with SLE in a previous study by our group[15]: the group (control and

SLE), arterial hypertension, triglycerides (both presented statistical significance in the bivariate analysis) and age. Carotid US could not be performed in 15 women of the control group due to organisational difficulties caused by the COVID-19 pandemic. To determine whether the sample of controls with carotid US was representative of all the women in the control group, an analysis of the differences between the two subgroups, with and without US, was performed. The results of this analysis are shown in online supplemental table 1. For all analyses, a p value less than 0.05 was considered significant.

RESULTS

Comparison between women with SLE and the control group

The most relevant data of this analysis are shown in table 1. When comparing both groups, it was observed that women with SLE had a higher prevalence of hypertension and dyslipidaemia (45.2% vs 5.5%, p<0.001, and 52.1% vs 34.2%, p=0.030, respectively), performed less physical activity (1 point vs 2 points, p=0.001), had a worse dietary pattern (11 points vs 12 points, p=0.003) and had a higher prevalence of premature menopause (12.3% vs 1.4%, p=0.009) than the control group. None of the subjects in either group exceeded the limit of low-risk alcohol consumption for women (10 g of alcohol per day).

Statin treatment was also more frequent among the group of women with SLE than those in the control group (45.2% vs 11%, p<0.001) and, consequently, the women in the SLE group presented lower total cholesterol concentrations (4.9 mmol/L vs 5.3 mmol/L, p=0.007), LDL-C (2.7 mmol/L vs 3.0 mmol/L, p=0.002), apolipoprotein B (0.9 mmol/L vs 1.0 mmol/L, p=0.014) and non-HDL-C (3.2 mmol/L vs 3.5 mmol/L, p=0.026) than the women in the control group. However, women with SLE had higher triglyceride concentrations than the control group (1.0 mmol/L vs 0.8 mmol/L, p=0.008). Despite the absence of differences in creatinine values, parathyroid hormone (PTH) concentrations (5.6 pmol/L vs 4.4 pmol/L, p=0.024) and proteinuria detected by the albumin:creatinine ratio (0.8 g/mol vs 0.0 g/ mol, p<0.001), all these values were higher in the patients with SLE. C reactive protein (CRP) (2.7 mg/L vs 1.0 mg/L, p<0.001) and homocysteine (11 µmol/L vs 9 µmol/L, p<0.001) concentrations were higher, while albumin (44.0 g/L vs 46 g/L, p<0.001) and vitamin B_{12} (324 pmol/L vs 384 pmol/L, p=0.006) concentrations were lower in the women in the SLE group compared with the women in the control group. The remaining variables studied are shown in online supplemental tables S1 and S2.

As shown in table 2, there were no significant differences between patients with SLE and control women in either the analysis of the *APOE* gene polymorphisms or in that of the genetic variants of the *ZPR1*, *APOA5* and *GCKR* genes. The Hardy-Weinberg equilibrium remained constant in both groups.

The study of subclinical atherosclerosis revealed that there were more women with carotid plaque in the SLE group than in the control group (20.5% vs 6.9%, p=0.028, respectively) with a statistically significant difference (table 3). There were no significant differences in ABI values. The maximum and minimum ABI data are shown in online supplemental table S2. The statistical analysis of the genetic variants of the *ZPR1*, *APOA5* and *GCKR* genes with carotid plaque is shown in online supplemental table S3.

A multivariate logistic regression model was made to evaluate the contribution of the genetic variants of the *GCKR* gene, SLE, plasma triglyceride concentrations, hypertension and age to the risk of having carotid plaque (table 4). The whole model explained the 40.3% (Nagelkerke R^2) risk of having carotid plaque. CC homozygosis for the *GCKR* rs1260326 gene (OR=0.111, 95% CI 0.015 to 0.804, p=0.030) showed that it has a protective effect against carotid atherosclerosis compared with TT homozygosis. In addition, a 1 mmol/L increase in plasma triglyceride concentrations was associated with a 7.6-fold increase in the risk of presenting carotid plaque (OR=7.576, 95% CI 2.415 to 23.767, p=0.001). Hypertension was associated with a trend towards increased risk of carotid plaque (OR=3.577, 95% CI 0.872 to 14.676, p=0.077). In addition, SLE was associated with a trend towards a higher risk of having carotid plaque (OR=1.588, 95% CI 0.352 to 7.168, p=0.548). The same model was analysed, adding the interaction between the group (SLE and control) and the *GCKR* gene, and there was no significant interaction.

Logistic regression analysis was performed to evaluate the effect of the *ZPR1* and *APOA5* genes, but non-significant results were obtained due to the small number (there was only one patient) of the reference group (homozygous GG) for both genes.

Different models adjusted for several variables were also calculated: for disease severity (SLICC/SDI greater or less than 0), accumulated dose of corticosteroids, insulin resistance, basal glycaemia, plasma homocysteine concentrations and the dietary questionnaire score. The results of all these analyses were similar and are shown in online supplemental tables S4–11. Statistical analysis of the allelic variants of the *GCKR* gene and the lipid profile adjusted for lipid-lowering drugs and the results are shown in online supplemental table S12.

DISCUSSION

To date, this is the first study that strengthens knowledge of the effect of the presence of polygenic variants of hypertriglyceridaemia on the development of subclinical atherosclerosis not only in a population with SLE but also in a control group. Women with SLE had higher concentrations of triglycerides as well as a higher prevalence of carotid plaque than women without SLE. CC homozygosity for *GCKR* rs1260326 and an increase of 1 mmol/L in

Table 1 Baseline characteristics of the SLE and control groups

Variables	Control group (n=73)	SLE group (n=73)	P value
Age (years)	52.0 (9.5)	52.3 (9.8)	0.851
Hypertension	4 (5.5)	33 (45.2)	**<0.001**
Diabetes mellitus	3 (4.1)	5 (6.8)	0.719
Dyslipidaemia	25 (34.2)	38 (52.1)	**0.030**
Smoking (packs/year)	0.0 (0.0–9.8)	2.5 (0.0–16.3)	0.130
Dietary questionnaire (score)	12 (10–13)	11 (9–12)	**0.003**
Physical activity (score)	2 (1–3)	1 (0–2)	**0.001**
Menopause	43 (58.9)	37 (50.7)	**0.031**
Premature menopause, <40 years	1 (1.4)	9 (12.3)	
Family history of CVD	35 (47.9)	33 (45.2)	0.740
Statins	8 (11.0)	33 (45.2)	**<0.001**
Antiplatelets/anticoagulants	0 (0.0)	20 (27.4)	**<0.001**
Waist circumference (cm)	89.3 (13.2)	88.8 (12.9)	0.812
Body mass index (kg/m^2)	24.8 (22.6–28.9)	25.5 (23.1–29.7)	0.386
Glycated haemoglobin (%)	5.40 (5.2–5.7)	5.45 (5.3–5.7)	0.196
Glucose (mmol/L)	5.0 (4.7–5.3)	4.9 (4.6–5.2)	0.087
T&G index (*score*)	4.4 (0.3)	4.5 (0.2)	0.069
Insulin resistance	10 (13.9)	18 (24.7)	0.100
Total cholesterol (mmol/L)	5.3 (0.9)	4.9 (0.7)	**0.007**
HDL-C (mmol/L)	1.8 (0.5)	1.7 (0.4)	0.262
LDL-C (mmol/L)	3.0 (0.8)	2.7 (0.6)	**0.002**
Apolipoprotein A (g/L)	1.7 (0.3)	1.6 (0.3)	0.361
Apolipoprotein B (g/L)	1.0 (0.2)	0.9 (0.2)	**0.014**
Triglycerides (mmol/L)	0.8 (0.6–1.1)	1.0 (0.8–1.4)	**0.008**
Non-HDL-C (mmol/L)	3.5 (0.9)	3.2 (0.7)	**0.026**
Lipoprotein (a) (g/L)	0.20 (0.08–0.53)	0.18 (0.07–0.49)	0.582
Creatinine (µmol/L)	63.0 (57.3–68.8)	63.0 (55.0–74.0)	0.542
Albumin (*mmol/L*)	46.0 (44.0–47.8)	44.0 (42.0–46.0)	**<0.001**
C- reactive protein (*mg/L*)	1.0 (0.5–2.1)	2.7 (1.1–5.6)	**<0.001**
Homocysteine (*µmol/L*)	9 (8–11)	11.0 (8.9–14.7)	**<0.001**
Albumin/creatinine (g/mol)	0.0 (0.0–0.5), n=66	0.8 (0.1–4.1), n=52	**<0.001**
Vitamin B_{12} (pmol/L)	384 (293–450)	324 (259–376)	**0.006**
Folic acid (nmol/L)	20.5 (19.9–29.7)	18.9 (14.3–24.1)	0.066
Calcidiol (nmol/L)	68.0 (45.4–81.1)	58.6 (39.8–76.6)	0.131
Parathyroid hormone (pmol/L)	4.4 (3.1–5.7), n=66	5.6 (3.9–7.3), n=33	**0.024**

Data are expressed as n (%) for qualitative variables and analysed by the χ^2 or Fisher test; mean (SD) for normally distributed quantitative variables and analysed by analysis of variance; median (IQR) for non-normally distributed variables and analysed by non-parametric tests (Mann-Whitney U). Data highlighted in bold indicate p<0.05.
CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T&G, triglyceride and glucose.

triglyceride concentrations were associated with a greater risk of carotid plaque in women with SLE.

Subclinical atherosclerosis at an early age is common in patients with SLE. The detection of atherosclerotic carotid plaque by US has been associated with a fourfold increased risk of CVD in patients with SLE.[16] This degree of risk is comparable to the risk of presenting CVD in patients with diabetes mellitus.[17] Furthermore, the prevalence of carotid plaque in these patients with SLE is higher than in the general population.[16] In our cohort,

Table 2 Statistical analysis of the genetic variants most commonly related to hypertriglyceridaemia in both the SLE and control groups

Gene	Control group (n=73)	SLE group (n=73)	P value
ZPR1 (c.*724C>G)			
CC	50 (68.5)	51 (69.9)	
CG	23 (31.5)	21 (28.8)	NA
GG	0 (0)	1 (1.4)	
Grouped:			
CG or GG	23 (31.5)	22 (30.1)	0.858
APOA5 (c.56C>G)			
CC	66 (90.4)	60 (82.2)	
CG	7 (9.6)	12 (16.4)	NA
GG	0 (0.0)	1 (1.4)	
Grouped			
CG or GG	7 (9.6)	13 (17.8)	0.149
GCKR (c.1337C>T)			
CC	20 (27.4)	28 (38.4)	
CT	37 (50.7)	33 (45.2)	0.344
TT	16 (21.9)	12 (16.4)	
APOE E2/E3-4	8 (11.1)	4 (5.5)	0.218
APOE E4/E2-3	13 (18.1)	13 (17.8 %)	0.969

Data are expressed as n (%).
NA, not statistically applicable.

patients with SLE had a higher prevalence of carotid plaque than control women, although the percentages in both groups were lower than those in previously published series (6.9% vs 17.0%–26.6% and 20.5% vs 16.0%–37.0%, respectively).[18–21] The study of peripheral vascular disease using the ABI showed no differences between the two groups. Only three controls and five patients with SLE presented a pathological ABI, although it is considered that this test is only useful for detecting advanced atherosclerotic disease.

The pathogenesis of ASCVD in SLE is complex and involves numerous factors. Although the presence of classical CVRF in patients with SLE is associated with a worse cardiovascular prognosis, these CVRFs do not fully explain the increase in CVR compared with patients without SLE. Historically, patients with SLE have a higher prevalence of conventional CVRF than the general population.[22] In the present study, hypertension, sedentary lifestyle, early menopause and a non-healthy dietary pattern were more common in women with SLE than in those in the control group.

Table 3 Statistical analysis of subclinical atherosclerosis in both the SLE and control groups

Variable	Control group (n=73)	SLE group (n=73)	P value
Pathological ABI	3 (5.8)	5 (6.8)	>0.999
Carotid plaque	4 (6.9), n=58	15 (20.5), n=73	**0.028**

Data are expressed as n (%). Data highlighted in bold indicate p<0.05.
ABI, ankle–brachial index.

Table 4 Multivariate analysis of risk factors for carotid plaque in both the SLE and control group

Variable	OR (95% CI)	P value
SLE	1.588 (0.352 to 7.168)	0.548
GCKR (c.1337C>T) TT	Ref.	0.065
CC	0.111 (0.015 to 0.804)	**0.030**
CT	0.665 (0.147 to 3.011)	0.597
Triglycerides (mmol/L)	7.576 (2.415 to 23.767)	**0.001**
Hypertension	3.577 (0.872 to 14.676)	0.077
Age	1.050 (0.983 to 1.122)	0.145

Nagelkerke R^2: 40.3%. Data highlighted in bold indicate p<0.05.
Ref., reference.

Dyslipidaemia was also more frequent among patients with SLE. In fact, the prevalence of dyslipidaemia in SLE ranges from 36% at diagnosis to 60% or even higher after 3 years.[23] The recommendations of scientific societies regarding cardiovascular prevention in patients with SLE have led to a greater use of statins in this group of patients (45.2% vs 11 %). These drugs decrease LDL-C concentrations by 25%–50% and triglyceride concentrations by 14%–29%, and increase HDL-C concentrations by 4%–10%.[7] The higher use of statins in patients with SLE explains why they had lower concentrations of total cholesterol, LDL-C, apolipoprotein B and non-HDL-C than women in the control group. However, despite the higher statin use, the patients in the SLE group had higher triglyceride concentrations and also lower HDL-C concentrations than the control group, indicating a lipid metabolism disorder known as atherogenic dyslipidaemia.

The prevalence of atherogenic dyslipidaemia is high among patients with SLE.[24] The causes of atherogenic dyslipidaemia in SLE are diverse, including insulin resistance and the inability of insulin to stimulate glucose metabolism.[25] Insulin resistance hinders the lipolysis and storage of fatty acids in adipocytes and alters the metabolic pathway dependent on PI 3 kinases which, among other functions, contribute to the degradation of apolipoprotein B. As a result of the massive supply of fatty acids and the defect in the degradation of apolipoprotein B, hypertriglyceridaemia and the atherogenic lipoprotein phenotype appear. This pattern is also associated with a reduction in HDL-C concentrations and the loss of antioxidant capacity.[26] Inadequate secretion of a set of hormones and proteins called adipokines that modify insulin sensitivity may also play a role.[27] In addition, the

appearance of antilipoprotein–lipase antibodies induces a decrease in the lipolytic activity of this enzyme.[28] In the current study, a trend to a higher prevalence of insulin resistance assessed by the T&G index was more frequent among patients with SLE. This index is a good diagnostic tool for insulin resistance,[29] even in patients with SLE and rheumatoid arthritis,[30] and has been associated with a higher prevalence of CVE and subclinical atherosclerosis in the general population.[31 32]

Hypertriglyceridaemia and atherogenic dyslipidaemia are the result of an interaction between genetic factors, constituted by the aggregation of multiple common and rare genetic variants, and environmental factors.[5] Apolipoprotein A5 activity deficiency has been linked to diabetic dyslipidaemia[33] and the rs964184 (c.*724C>G) variant of the *ZPR1* gene (also called *ZNF259*), hypertriglyceridaemia, coronary artery disease and metabolic syndrome.[34] However, in the present study, there were no significant differences between the group with SLE and the control group. There were also no differences related to the allelic variants of the *APOE* gene.

The last gene studied was the *GCKR* gene that encodes the glucokinase regulatory protein (GKRP). This protein is released into the cytoplasm in the postprandial phase and stimulates de novo glycogen deposition and lipogenesis. The presence of the TT rs1260326 (p.P446L) allele of *GCKR* destabilises the glucokinase binding interface. In the fasting state, increased hepatic glucokinase activity results in higher triglyceride concentrations and lower glucose concentrations.[35] In addition, the *GCKR* rs1260326 variant has been shown to be associated with an increased risk of coronary artery disease (OR per risk allele 1.02, 95% CI 1.00 to 1.04).[36] In a previous study with a large number of women with SLE, it was observed that the *GCKR* rs1260326 variant was related to the presence of carotid atherosclerosis in these patients. In that study, CC homozygosity of the *GCKR* gene was independently associated with carotid plaque in patients with SLE (OR=0.026, 95% CI 0.001 to 0.473, p=0.014).[15] In the current work, CC homozygosity of the *GCKR* rs1260326 gene (OR=0.111, 95% CI 0.015 to 0.804, p=0.030) and plasma triglyceride concentrations (OR=7.576, 95% CI 2.415 to 23.767, p=0.001) in patients with SLE were found to be independently associated with the presence of subclinical carotid plaque compared with the control group. Although hypertension did not reach statistical significance, the association of this CVRF with subclinical atherosclerosis in patients with lupus has been demonstrated in other studies.[37] As observed in the different analysis models, the T&G index and insulin resistance lost value as covariates due to their relationship with triglyceride concentrations. Nonetheless, the statistical trend corroborates the usefulness of both as markers of atherogenic dyslipidaemia in patients with SLE. Likewise, the chronicity of SLE, the cumulative dose of corticosteroids and age are some of the clinically relevant CVRFs in SLE and are usually included in CVR calculators for these patients.[38] However, in our study, no significant differences were obtained when they were included in the multivariate models.

The association between osteoporosis and ASCVD is well documented both in the general population and in patients with autoimmune diseases. Lack of sun exposure, a sedentary lifestyle and especially chronic kidney disease favour a decrease in plasma concentrations of calcium and vitamin D and an increase in phosphate and fibroblast growth factor 23. These modifications induce a greater secretion of PTH, which, together with the reduction of the renal synthesis of klotho, promotes the deposit of calcium at the vascular level.[39] Patients in the SLE group had higher PTH concentrations than those in the control group (5.6 pmol/L vs 4.4 pmol/L, p=0.024), although plasma PTH could only be analysed in a subsample of patients and control subjects. No statistically significant differences were observed in plasma concentrations of creatinine or vitamin D, despite the latter being lower in the SLE group than in the control group (58.6 nmol/L vs 68.0 nmol/L, p=0.131). These results are consistent with the potential role of plasma PTH as a biomarker of atherosclerosis in these patients.[40]

The combination of conventional CVRF with other pathogenic factors that emerge in the chronic inflammatory context of SLE may explain the development of accelerated atherosclerosis. In our cohort, women with SLE had higher CRP concentrations and lower albumin concentrations than those in the control group. Similarly, patients with SLE had higher concentrations of plasma homocysteine. There were no differences in folic acid concentrations, but vitamin B_{12} concentrations were lower in women with SLE than women in the control group. Homocysteine is a sulfur amino acid resulting from the metabolism of methionine that requires vitamin B_{12} and folic acid as cofactors to be eliminated. In a recent meta-analysis including 50 studies and 4396 patients with SLE, plasma homocysteine concentrations in patients with SLE were higher compared with the population without SLE.[41] Homocysteine exerts its atherogenic role through different mechanisms. It is speculated that the inhibition of endothelial nitric oxide synthase produced by homocysteine reduces the bioavailability of nitric oxide and leads to endothelial dysfunction.[42] In addition, homocysteine increases the activity of HMG CoA reductase and cholesterol synthesis.[43] The association of hyperhomocysteinaemia with ASCVD is well documented both in the general population and in patients with SLE,[44] but in our study, this variable did not provide additional information to the model.

The main limitation of this study is the small sample size, especially with regard to the association analysis of genetic variants. On the other hand, the sample was only made up of women, although it should be taken into account that SLE predominantly affects the female sex. Furthermore, the control group was not selected from the general population census, and this may limit its representativeness. Finally, a high percentage of patients in the group of patients with SLE and 11% of the control group

were treated with statins, drugs that have a moderate hypotriglyceridaemic effect. This effect could have attenuated the magnitude of the influence of allelic variants of the *GCKR* gene on triglyceride concentrations and atherosclerosis, but despite this, a significant relationship was observed. As observed in online supplemental table S13, there were no patients with plaque and without lipid-lowering treatment in the CC allele group. These results should be evaluated in a larger population.

Despite the aforementioned limitations, this research reaffirms the independent association of CC homozygosis of the *GCKR* gene with carotid atherosclerosis in patients with SLE. Therefore, our results demonstrate the relationship between the genetic variants of one of the genes related to polygenic hypertriglyceridaemia, the *GCKR* gene, and subclinical atherosclerosis in patients with SLE. It should be noted that the increased risk (OR=7.576) of having carotid plaque attributed to an increase of 1 mmol/L in the concentration of triglycerides occurs even with concentrations lower than 150 mg/dL or 1.7 mmol/L. There is a direct relationship between triglyceride concentrations and CVE even with triglyceride levels below 150 mg/dL,[45] and in the past years, the optimal triglyceride concentration has been defined as below 100 mg/dL.[46] The recommendations of the treatment of dyslipidaemia in patients with autoimmune diseases are not uniform; however, cardiovascular prevention clinical guidelines consider the presence of these diseases as a CVR-enhancing factor. More precise stratification of CVR in these patients may be possible by studying variants of the *GCKR* rs1260326 gene in addition to evaluating conventional CVRFs and carotid US. Moreover, the study of *GCKR* rs1260326 gene variants may contribute to modulating the intensity of lipid-lowering treatment in patients with SLE.

CONCLUSIONS

The results of this study demonstrate that CC homozygosity of the *GCKR* gene and plasma triglyceride concentrations are independently associated with subclinical carotid atherosclerosis in women with SLE. Women with SLE have a higher prevalence of subclinical carotid atherosclerosis. More studies are needed to define the role of triglycerides in the residual risk of ASCVD in these patients.

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Patient consent for publication Consent obtained directly from patient(s)

Ethics approval This study involves human participants and was approved by the ethics committee of Hospital Universitari de Bellvitge PR179/19. The participants gave informed consent to participate in the study before taking part.

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Supplementary Material

Table S1: Statistical analysis of the clinical and analytical variables of the women of the control group without and with ultrasound (US).

Variable	**Control without US (n= 15)**	**Control with US (n= 58)**	***p***
Age *(years)*	54.5 (12.6)	51.4 (8.6)	0.266
Statins	3 (20.0 %)	5 (8.6 %)	0.348
Hypertension	2 (13.3 %)	2 (3.4 %)	0.185
Diabetes mellitus	0 (0.0 %)	3 (5.2 %)	> 0.999
Dyslipidemia	5 (33.3 %)	20 (34.5 %)	0.933
Body mass index *(kg/m²)*	23.5 (4.5)	26.4 (4.0)	**0.018**
Weight *(kg)*	61.0 (10.8)	68.8 (12.0)	**0.026**
GFR CKD-EPI *(mL/min/1.73 m²)*	89.5 (87 to 90)	90 (90 to 90)	0.099
Glucose *(mmol/L)*	5.0 (0.6)	5.3 (1.6)	0.586
T&G index	4.40 (4.24 to4.54)	4.37 (4.25 to 4.56)	0.943
Insulin resistance	2 (14.3 %)	8 (13.8 %)	< 0.999
Total cholesterol *(mmol/L)*	4.7 (0.8)	5.4 (1.0)	0.077
HDL-C *(mmol/L)*	1.6 (0.7)	1.8 (0.5)	0.290
LDL-C *(mmol/L)*	2.8 (0.8)	3.1 (0.8)	0.194
Apolipoprotein A *(g/L)*	1.6 (1.4 to 1.7)	1.7 (1.5 to 1.9)	0.099
Apolipoprotein B *(g/L)*	0.9 (0.8 to 1.1)	1.1 (0.9 to 1.2)	0.183
Triglycerides *(mmol/L)*	0.9 (0.6 to 1.1)	0.8 (0.6 to 1.1)	0.989
Non-HDL-C *(mmol/L)*	3.2 (0.8)	3.5 (0.9)	0.213
Lipoprotein (a) *(g/L)*	0.5 (0.1 to 1.7)	0.2 (0.6 to 0.5)	0.099
Albumin/creatinine *(g/mol)*	0.6 (0.1 to 0.8)	0.0 (0.0 to 0.4)	**0.007**
APOE E2/E3-4	0 (0.0 %)	8 (14.0 %)	0.191
APOE E4/E2-3	4 (26.7 %)	9 (15.8 %)	0.450
ZPR1 (c.*724C>G) CC CG	 9 (60.0 %) 6 (40.0 %)	 41 (70.7 %) 17 (29.3 %)	0.427
APOA5 (c.56C>G) CC CG	 12 (80.0 %) 3 (20.0 %)	 54 (93.1 %) 4 (6.9 %)	0.124
GCKR (c.1337C>T) CC CT TT	 3 (20.0 %) 9 (60.0 %) 3 (20.0 %)	 17 (29.3 %) 28 (48.3 %) 13 (22.4 %)	0.694
Family history of premature CAD Family history of non-premature CAD	0 (0.0 %) 3 (20.0 %)	6 (10.3 %) 10 (17.2 %)	0.335 0.723
Family history of premature stroke Family history of non-premature stroke	0 (0.0 %) 5 (33.3 %)	5 (8.6 %) 11 (19.0 %)	0.576 0.295
Active smoking	5 (33.3 %)	13 (22.4 %)	0.502

Variable	Control without US (n= 15)	Control with US (n= 58)	*p*
Ex-smoker >6 months	0 (0.0 %)	12 (20.7 %)	0.061
Waist circumference *(cm)*	90.8 (13.4)	88.9 (13.2)	0.630
Dietary questionnaire *(score)*	10.7 (7 to 14)	11.7 (5 to 14)	0.086
Physical activity *(puntos)*	1.8 (1.1)	1.7 (1.2)	0.862
Menopause Menopause < 40 years	9 (60.0 %) 0 (0.0 %)	35 (60.3 %) 1 (1.7 %)	0.981 > 0.999
Menarche *(years)*	12.1 (1.8)	12.8 (1.7)	0.199
Pregnancies *(number)*	1 (0 to 3)	2 (1 to 3)	0.197
Miscarriages *(number)*	0 (0 to 1)	0 (0 to 1)	0.967
Contraception	1 (6.7 %)	2 (3.4 %)	0.504
Systolic blood pressure *(mm Hg)*	122.6 (17.9)	122.2 (15.9)	0.932
Diastolic blood pressure *(mm Hg)*	75.1 (11.6)	76.7 (9.0)	0.534
Heart rate *(bpm)*	69.6 (6.5)	69.1 (9.8)	0.853
Urea *(mmol/L)*	4.8 (1.1)	5.6 (1.4)	0.076
Creatinine *(µmol/L)*	62.8 (11.1)	63.1 (8.2)	0.893
Sodium *(mmol/L)*	142.4 (1.9)	141.2 (1.9)	**0.037**
Potassium *(mmol/L)*	4.3 (0.3)	4.4 (0.2)	0.493
Angiotensin-converting enzyme *(ukat/L)*	0.7 (0.3 to 1.4)	0.7 (0.1 to 1.5)	0.987
Lactate dehydrogenase *(µkat/L)*	2.8 (0.5)	2.7 (0.5)	0.411
Aspartate amino transferase *(µkat/L)*	0.3 (0.1)	0.3 (0.1)	0.684
Alanine amino transferase *(µkat/L)*	0.2 (0.1)	0.3 (0.1)	0.297
Albumin *(g/L)*	45.1 (3.0)	46.1 (2.5)	0.178
Bilirubin *(µmol/L)*	6.7 (2 to 12)	9.0 (4 to 27)	0.078
Gamma-glutamyltransferase *(µkat/L)*	0.3 (0.2 to 0.5)	0.3 (0.1 to 1.8)	0.838
Alkaline phosphatase *(µkat/L)*	1.2 (0.7 to 1.6)	1.1 (0.6 to 2.0)	0.306
Magnesium *(mmol/L)*	0.9 (0.1)	0.9 (0.1)	0.647
Urates *(µmol/L)*	263.9 (46.1)	246.1 (64.9)	0.337
Ferritin *(µg/L)*	82.4 (20.3 to 231.6)	102.9 (7.3 to 1151.1)	0.636
Transferrin *(µmol/L)*	31.1 (2.7)	33.9 (9.5)	0.271
Transferrin saturation index *(%)*	22.4 (11 to 36)	28.9 (5 to 85)	0.101
Haptoglobin *(g/L)*	1.2 (0.4)	1.2 (0.4)	0.748
Iron *(µmol/L)*	14.0 (4.5)	17.1 (6.9)	0.123
C-reactive protein *(mg/L)*	2.5 (0.3 to 18.1)	2.3 (0.3 to 43.6)	0.878
Rheumatoid factor *(ku.i./L)*	13.5 (10 to 59)	14.6 (10 to 248)	0.896
Protein/creatinine *(g/mol)*	9.2 (8.1 to 11.1)	8.6 (6.3 to 10.5)	0.269
Vitamin D *(nmol/L)*	77.6 (29.7 to 194.0)	67.3 (23.1 to 147.4)	0.253
Parathormone *(pmol/L)*	4.3 (2.9 to 6.9)	4.4 (3.1 to 5.7)	0.844

Variable	Control without US (n= 15)	Control with US (n= 58)	*p*
Thyrotropin *(mu.int./L)*	1.5 (0.9 to 1.9)	2.0 (1.4 to 2.8)	0.085
Thyroxine *(pmol/L)*	14.3 (13.7 to 16.1)	15.0 (13.9 to 16.8)	0.603
Glycosylated hemoglobin *(%)*	5.6 (0.3)	5.5 (0.9)	0.675
Homocysteine *(µmol/L)*	10.2 (5.0 to 24.0)	9.2 (4.0 to 17.0)	0.347
Vitamin B12 *(pmol/L)*	373 (306.0 to 444.5)	384.0 (286.7 to 455.0)	0.727
Folic acid *(nmol/L)*	25.1 (13.4)	23.2 (8.3)	0.519
Immunoglobulin G *(mg/L)*	10446.1 (1974.0)	11003.0 (3330.7)	0.553
Immunoglobulin A *(mg/L)*	2277.0 (742.8)	1910.8 (1019.9)	0.214
Immunoglobulin M *(mg/L)*	1164.0 (683.3)	1193.9 (517.8)	0.858
C3 complement *(mg/L)*	968.7 (345.3)	1093.5 (222.7)	0.980
C4 complement *(mg/L)*	223.1 (61.3)	236.6 (52.9)	0.408
Hemoglobin *(g/L)*	132.2 (8.6)	135.1 (19.0)	0.586
Platelets *(x10E9/L)*	259.6 (54.1)	259.3 (53.8)	0.985
Leukocytes *(x10E9/L)*	6.4 (1.6)	6.0 (1.4)	0.453
Prothrombin time	0.9 (0.9 to 1.0)	0.9 (0.9 to 1.0)	0.834
Thromboplastin time	1.0636 (0.07762)	1.0133 (0.08249)	**0.043**
Fibrinogen *(g/L)*	3.1 (2.7 to 3.7)	3.2 (2.9 to 3.8)	0.403
Pathological D-dimer	0 (0%)	1 (1.8%)	< 0.999

Data are expressed as n (%) for qualitative variables and analyzed by chi^2 test or Fisher test; mean (standard deviation) for normally distributed quantitative variables and analyzed by analysis of variance (ANOVA); median (interquartile range) for non-normally distributed variables, and analyzed by nonparametric tests (Mann–Whitney U). Data highlighted in bold indicate $p < 0.05$. US: Ultrasound; GFR CKD-EPI: Glomerular Filtration Rate Chronic Kidney Disease Epidemiology Collaboration; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Non-HDL-C: non-HDL-cholesterol.

Table S2: Baseline characteristics of both the SLE and control group.

Variable	Control group (n= 73)	SLE group (n= 73)	*p*
Caucasian	71 (97.3 %)	70 (95.9 %)	> 0.999
Family history of premature CAD Family history of non-premature CAD	6 (8.2 %) 13 (17.8 %)	3 (4.1 %) 15 (20.5 %)	0.562
Family history of premature stroke Family history of non-premature stroke	5 (6.8 %) 16 (21.9 %)	6 (8.2 %) 10 (13.7 %)	0.426
Family history of thrombosis	3 (4.1 %)	1 (1.4 %)	0.620
Family history of cancer	39 (53.4 %)	38 (52.1 %)	0.868
Hyperuricemia	1 (1.4 %)	1 (1.4 %)	> 0.999
Menarche *(years)*	12.6 (1.7)	12.8 (1.6)	0.443
Pregnancies *(number)*	2 (0 to 3)	2 (1 to 3)	0.520
Miscarriages *(number)*	0 (0 to 1)	0 (0 to 1)	0.787

Variable	Control group (n= 73)	SLE group (n= 73)	*p*
Breastfeeding >2 months	31 (42.5 %)	28 (38.4 %)	0.613
Contraceptives	3 (4.1 %)	5 (6.8 %)	0.719
Ezetimibe	0 (0.0 %)	3 (4.1 %)	0.245
Systolic blood pressure *(mm Hg)*	122.3 (16.2)	124.1 (17.6)	0.514
Diastolic blood pressure *(mm Hg)*	76.5 (9.5)	78.0 (8.7)	0.323
Heart rate *(lpm)*	69.2 (9.2)	74.0 (11.3)	**0.006**
Sodium *(mmol/L)*	141 (140 to 143)	142 (140 to 143)	0.196
Potassium *(mmol/L)*	4.4 (0.2)	4.3 (0.4)	**0.033**
Chlorine *(mmol/L)*	102.5 (3.6)	102.2 (2.4)	0.693
Angiotensin-converting enzyme *(ukat/L)*	0.7 (0.5 to 0.8)	0.6 (0.4 to 0.9)	0.809
Creatine kinase *(µkat/L)*	1.3 (0.9 to 1.9)	1.3 (0.8 to 2.0)	0.441
Lactate dehydrogenase *(µkat/L)*	2.7 (2.4 to 3.1)	3.0 (2.6 to 3.5)	**0.001**
Aspartate amino transferase *(µkat/L)*	0.3 (0.3 to 0.4)	0.3 (0.3 to 0.4)	0.126
Alanine amino transferase *(µkat/L)*	0.3 (0.2 to 0.3)	0.3 (0.2 to 0.3)	0.681
Bilirubin *(µmol/L)*	8.0 (6.0 to 10.0)	6.0 (4.2 to 8.0)	**0.002**
Gamma-glutamyltransferase *(µkat/L)*	0.26 (0.18 to 0.38)	0.32 (0.21 to 0.48)	**0.028**
Alkaline phosphatase *(µkat/L)*	1.14 (0.9 to 1.3)	1.11 (0.9 to 1.3)	0.854
HDL-C/Total cholesterol	0.3 (0.1)	0.3 (0.1)	0.879
Magnesium *(mmol/L)*	0.9 (0.8 to 0.9)	0.8 (0.8 to 0.9)	**0.002**
Urates *(µmol/L)*	248 (221 to 291)	285.5 (225 to 321)	**0.016**
Urea *(mmol/L)*	5.4 (1.3)	6.2 (2.0)	**0.005**
Calcium *(mmol/L)*	2.4 (0.1)	2.4 (0.1)	0.376
Ferritin *(µg/L)*	59.0 (32.4 to 123.1)	48.1 (26.4 to 98.5)	0.207
Transferrin *(µmol/L)*	31.6 (29.6 to 34.5)	31.4 (29.0 to 35.5)	0.505
Transferrin saturation index *(%)*	25 (21 to 33)	22 (16 to 27)	**0.008**
Haptoglobin	1.2 (0.9 to 1.5)	1.4 (1.0 to 1.9)	**0.003**
Iron *(µmol/L)*	16 (13 to 20)	13 (10 to 17)	**0.003**
Pathological rheumatoid factor	8 (11.4 %)	12 (17.6 %)	0.300
Protein/creatinine *(g/mol)*	9.0 (6.8 to 10.6)	8.8 (0.3 to 18.8)	0.352
Thyrotropin *(mu.int./L)*	1.7 (1.3 to 2.6)	1.9 (1.4 to 3.1)	0.192
Thyroxine *(pmol/L)*	14.9 (13.9 to 16.5)	14.4 (12.4 to 16.5)	0.060
Immunoglobulin G *(mg/L)*	10391 (8940 to 12600)	12800 (10250 to 15075)	**< 0.001**
Immunoglobulin A *(mg/L)*	1781 (1350 to 2550)	2800 (1900 to 3587)	**< 0.001**
Immunoglobulin M *(mg/L)*	1020 (754 to 1480)	875 (527)	0.079
C3 complement *(mg/L)*	1069.3 (253.2)	1045.5 (230.4)	0.555
C4 complement *(mg/L)*	233.9 (54.4)	170.5 (83.2)	**< 0.001**

Variable	Control group (n= 73)	SLE group (n= 73)	*p*
Hemoglobin *(g/L)*	136 (130 to 143)	131 (124 to 139)	**0.006**
Platelets *(x10⁹/L)*	259.4 (53.5)	237.1 (81.2)	0.053
Leukocytes *(x10⁹/L)*	6.0 (5.3 to 6.9)	5.8 (4.6 to 7.1)	0.295
Prothrombin time	0.9 (0.9 to 1.0)	0.9 (0.9 to 1.0)	0.660
Thromboplastin time	1.0 (0.9 to 1.1)	1.0 (0.9 to 1.2)	0.263
Fibrinogen *(g/L)*	3.2 (2.9 to 3.8)	3.2 (2.7 to 3.7)	0.668
Pathological D-dimer	1 (1.4 %)	7 (10.3 %)	**0.033**
Minimum ABI	1.1 (1.0 to 1.2)	1.1 (1.0 to 1.1)	0.880
Maximum ABI	1.2 (1.1 to 1.2)	1.1 (1.1 to 1.2)	0.333

Data are expressed as n (%) for qualitative variables and analyzed by chi² test or Fisher test; mean (standard deviation) for normally distributed quantitative variables and analyzed by analysis of variance (ANOVA); median (interquartile range) for non-normally distributed variables, and analyzed by nonparametric tests (Mann–Whitney U). Data highlighted in bold indicate $p < 0.05$. SLE: Systemic Lupus Erythematosus; CAD: coronary artery disease; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Non-HDL-C: non-HDL-cholesterol; ABI: ankle brachial index.

Table S3: Statistical analysis of the genetic variants of the *ZPR1, APOA5* and *GCKR* genes with carotid plaque.

Gene	Non-carotid plaque subjects (n= 112)	Carotid plaque subjects (n= 19)	*p*
ZPR1 (c.*724C>G) CC	77 (68.8 %)	15 (78.9 %)	NA
CG	34 (30.4 %)	4 (21.1 %)	
GG	1 (100 %)	0 (0 %)	
APOA5 (c.56C>G) CC	97 (86.6 %)	17 (89.5 %)	NA
CG	14 (12.5 %)	2 (10.5 %)	
GG	1 (100 %)	0 (0 %)	
GCKR (c.1337C>T) CC	41 (36.6 %)	4 (21.1 %)	0.381
CT	51 (45.5 %)	10 (52.6 %)	
TT	20 (17.9 %)	5 (26.3 %)	

Data are expressed as n (%); NA: not statistically applicable.

Table S4: Multivariate analysis of the risk factors for carotid plaque, including insulin resistance, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.677 (0.369 to 7.629)	0.503
GCKR (c.1337C>T) TT	Ref.	
CC	0.114 (0.016 to 0.831)	**0.032**
CT	0.731 (0.158 to 3.379)	0.688
Triglycerides *(mmol/L)*	12.864 (1.987 to 83.292)	**0.007**
Hypertension	3.244 (0.781 to 13.478)	0.105
Age	1.057 (0.987 to 1.132)	0.111
Insulin resistance	0.467 (0.061 to 3.597)	0.465
Nagelkerke R^2	40.8 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S5: Multivariate analysis of the risk factors for carotid plaque, including insulin resistance but not triglycerides, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.380 (0.320 to 5.942)	0.666
GCKR (c.1337C>T) TT	Ref.	
CC	0.190 (0.034 to 1.049)	**0.057**
CT	0.567 (0.135 to 2.386)	0.439
Hypertension	5.484 (1.408 to 21.360)	0.432
Age	1.025 (0.964 to 1.089)	0.432
Insulin resistance	4.853 (1.452 to 16.220)	**0.010**
Nagelkerke R^2	30.8 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S6: Multivariate analysis of the risk factors for carotid plaque, including triglycerides and the glucose index, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.693 (0.373 to 7.680)	0.495
GCKR (c.1337C>T) TT	Ref.	
CC	0.170 (0.029 to 0.985)	**0.048**
CT	0.578 (0.133 to 2.522)	0.466
Hypertension	4.334 (1.099 to 17.084)	**0.036**
Age	1.032 (0.969 to 1.099)	0.331
Triglycerides and glucose index	41.024 (3.892 to 432.438)	**0.002**
Nagelkerke R^2	35.9 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S7: Multivariate analysis of the risk factors for carotid plaque, including glucose, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.637 (0.354 to 7.575)	0.528
GCKR (c.1337C>T) TT	Ref.	
CC	0.112 (0.016 to 0.811)	**0.030**
CT	0.651 (0.143 to 2.971)	0.579
Triglycerides *(mmol/L)*	7.195 (2.159 to 23.981)	**0.001**
Glucose *(mmol/L)*	1.044 (0.745 to 1.465)	0.801
Hypertension	3.655 (0.882 to 15.149)	0.074
Age	1.049 (0.981 to 1.122)	0.163
Nagelkerke R^2	40.3 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S8: Multivariate analysis of the risk factors for carotid plaque, including severity by the SLICC/SDI index, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLICC/SDI= 0	1.564 (0.318 to 7.685)	0.582
SLICC/SDI≥ 1	1.633 (0.272 to 9.803)	0.592
GCKR (c.1337C>T) TT	Ref.	
CC	0.110 (0.015 to 0.821)	**0.031**
CT	0.665 (0.147 to 3.008)	0.597
Triglycerides *(mmol/L)*	7.556 (2.399 to 23.794)	**0.001**
Hypertension	3.555 (0.853 to 14.822)	0.082
Age	1.051 (0.983 to 1.123)	0.146
Nagelkerke R^2	40.3 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. SLICC/SDI: Systemic Lupus International Collaborating Clinics/ACR Damage Index. Data highlighted in bold indicate $p < 0.05$.

Table S9: Multivariate analysis of the risk factors for carotid plaque, including the cumulative dose of corticosteroids, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
ADC from 0 to 17 g	2.442 (0.488 to 12.205)	0.277
ADC > 17 g	0.852 (0.139 to 5.234)	0.862
GCKR (c.1337C>T) TT	Ref.	
CC	0.101 (0.013 to 0.754)	**0.025**
CT	0.584 (0.122 to 2.79)	0.500
Triglycerides *(mmol/L)*	9.553 (2.836 to 32.178)	**< 0.001**
Hypertension	4.306 (0.990 to 18.726)	0.052
Age	1.051 (0.982 to 1.125)	0.153
Nagelkerke R^2	42.2 %	

OR: odds ratio; CI: confidence interval. ADC: cumulative dose of corticosteroids. Data highlighted in bold indicate $p < 0.05$.

Table S10: Multivariate analysis of the risk factors for carotid plaque, including homocysteine, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.332 (0.268 to 6.607)	0.726
GCKR (c.1337C>T) TT	Ref.	
CC	0.101 (0.014 to 0.748)	**0.025**
CT	0.570 (0.124 to 2.616)	0.470
Triglycerides *(mmol/L)*	7.164 (2.239 to 22.926)	**0.001**
Hypertension	3.428 (0.809 to 14.520)	0.094
Age	1.034 (0.965 to 1.109)	0.342
Homocysteine	1.026 (0.889 to 1.183)	0.726
Nagelkerke R^2	38.9 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S11: Multivariate analysis of the risk factors for carotid plaque, including the dietary questionnaire, in both the SLE and control group.

Variable	*OR (95 % CI)*	*p*
SLE	1.608 (0.346 to 7.464)	0.544
GCKR (c.1337C>T) TT	Ref.	
CC	0.113 (0.015 to 0.827)	**0.032**
CT	0.677 (0.143 to 3.210)	0.624
Triglycerides *(mmol/L)*	7.560 (2.397 to 23.844)	**0.001**
Hypertension	3.563 (0.867 to 14.635)	0.078
Age	1.050 (0.982 to 1.122)	0.156
Dietary questionnaire *(score)*	1.013 (0.750 to 1.367)	0.935
Nagelkerke R^2	39.9 %	

SLE: systemic lupus erythematosus; *OR*: odds ratio; CI: confidence interval. Data highlighted in bold indicate $p < 0.05$.

Table S12: Statistical analysis of the allelic variants of the *GCKR* gene and lipid profile adjusted for lipid-lowering drugs.

Variable	Allelic variant *GCKR* (c.1337C>T) gene	Non LLT (n= 105)	LLT (n= 41)	*p* LLT	*p* *GCKR* gene	*p* interaction (LLT and *GCKR* gene)
TC *(mmol/L)*	CC	5.2 (0.8)	5.1 (0.9)	0.056	0.267	0.293
	CT	5.2 (0.9)	4.5 (0.7)			
	TT	5.2 (1.3)	4.9 (0.5)			
TG *(mmol/L)*	CC	1.0 (0.5)	1.6 (0.9)	**< 0.001**	**0.028**	0.354
	CT	0.9 (0.4)	1.2 (0.4)			
	TT	0.9 (0.5)	1.2 (0.4)			
HDL-C *(mmol/L)*	CC	1.6 (0.6)	1.6 (0.4)	0.216	0.393	0.415
	CT	1.8 (0.4)	1.8 (0.4)			
	TT	1.7 (0.5)	1.6 (0.3)			
LDL-C *(mmol/L)*	CC	3.1 (0.7)	2.7 (0.8)	**0.004**	0.87	0.182
	CT	2.9 (0.6)	2.2 (0.5)			
	TT	2.9 (0.7)	2.7 (0.3)			
Non-HDL-C *(mmol/L)*	CC	3.5 (0.8)	3.5 (1.0)	0.208	0.109	0.145
	CT	3.4 (0.8)	2.8 (0.6)			
	TT	3.3 (0.9)	3.3 (0.8)			
Apo B *(g/L)*	CC	1.0 (0.2)	1.0 (0.3)	0.260	0.099	0.178
	CT	0.9 (0.2)	0.9 (0.1)			
	TT	1.0 (0.3)	0.9 (0.2)			

Data are expressed as mean (standard deviation) and analyzed by analysis of covariance (ANCOVA) and adjusted for lipid-lowering drugs. Data highlighted in bold indicate $p < 0.05$. LLT: Lipid-lowering treatment; TC: total cholesterol; TG: triglycerides; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; Non-HDL-C: non-HDL-cholesterol; Apo B: apolipoprotein B.

Table S13: Statistical analysis of the allelic variants of the *GCKR* gene and carotid plaque adjusted for lipid-lowering drugs.

Variable	**Allelic variant *GCKR* (c.1337C>T) gene**	**Non LLT (n= 105)**	**LLT (n= 41)**	***p* LLT**	***p* *GCKR* gene**	***p* interaction (LLT and *GCKR* gene)**
Carotid plaque (present)	CC	0 (0 %)	4 (28.6 %)	**0.044**	0.868	0.754
	CT	5 (10.9 %)	5 (33.3 %)			
	TT	1 (6.3 %)	4 (44.4 %)			

Data are expressed as n (%) and analyzed by multivariate logistic regression. Data highlighted in bold indicate $p < 0.05$. LLT: Lipid-lowering treatment.