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Glycosphingolipid-associated β-1,4 galactosyltransferase is elevated in patients with systemic lupus erythematosus

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ABSTRACT

Objective β-1,4 galactosyltransferase-V (β-1,4 GalT-V) is an enzyme that synthesises a glycosphingolipid known as lactosylceramide, which has been implicated in general inflammation and atherosclerosis. We asked if β-1,4 GalT-V was present at elevated levels in patients with SLE, a disease which is associated with increased risk of atherosclerosis.

Methods In this case–control observational study, serum samples were obtained from patients with SLE who are part of the Johns Hopkins Lupus Cohort. Control serum samples were obtained from healthy adult community members recruited from the Baltimore area. All serum samples (n=50 in the SLE group and n=50 in the healthy control group) were analysed with enzyme-linked immunoassays. These assays used antibodies raised against antigens that enabled us to measure the absorbance of oxidised phosphocholines per apolipoprotein B-100 (ox-PC/apoB) and the concentration of lipoprotein(a) (Lp(a)) and β-1,4 GalT-V.

Results Absorbance of ox-PC/apoB and concentrations of Lp(a) and β-1,4 GalT-V were significantly higher in the SLE serum samples as compared with the control serum ($p<0.0001$).

Conclusions We conclude that patients with SLE have elevated levels of β-1,4 GalT-V and ox-PC, which have previously been recognised as risk factors for atherosclerosis.

INTRODUCTION

The SLE-associated autoimmune response causes inflammation in the skin and joints, and also in organs such as the kidney, brain and heart.[1 2] There are many potential causes of SLE, and most cases feature interactions between genetic, hormonal and environmental risk factors.[3] SLE is associated with a 2.66-fold increased risk for atherosclerosis.[4] It has been shown that premenopausal women with SLE are at a significantly higher risk for vascular pathology and heart disease, when compared with young women without SLE.[5] Generalising this finding, it has been shown that SLE increases the risk for acute myocardial infarction in the general population, as evidenced by a population-based, case–control analysis using data from the UK-based General Practice Research Database.[6] Death in late-stage SLE is associated with abnormally high incidence of myocardial infarction, due to cardiac atherosclerosis.[7]

The elevated risk for cardiovascular events in SLE cannot be explained by the traditional Framingham risk factors, which include age, sex, total serum cholesterol, high-density lipoprotein (HDL) cholesterol, diastolic blood pressure, systolic blood pressure, left ventricular hypertrophy, diabetes mellitus and current cigarette smoking. Recent studies suggest that abnormal lipid profiles and immune response to lipids may have an important role in the increased risk for atherosclerosis in SLE.[8] As an example, lipoprotein(a) (Lp(a)) is a known causal factor in coronary heart disease. There are reports that have found patients with SLE to have elevated Lp(a) levels.[9] In addition, studies have also found that 45% of women with SLE have dysfunctional, proinflammatory HDL, which increases the risk of atherosclerosis in these patients.[10]

Atherosclerosis is an inflammatory disease and must be understood in the mechanistic context of pathways and mediators of inflammation.[11] On exposure to proinflammatory cytokines, vascular endothelial cells secrete tumour necrosis factor alpha (TNF-α).[12] When TNF-α is taken up by its receptors, which are expressed on the surface of endothelial cells, it activates β-1,4 galactosyltransferase-V (β-1,4 GalT-V).[13] β-1,4 GalT-V is a membrane-bound enzyme that transfers galactose residues from uridinediphosphate (UDP)-galactose onto a glycosphingolipid, glucosylceramide, to generate lactosylceramide (LacCer).[14]

In turn, LacCer activates nicotinamideadenine dinucleotide phosphateH (NADP(H)) oxidase to generate reactive oxygen species, for example, superoxides which initiate an elaborate signal transduction cascade, which ultimately induces the expression of a cell adhesion molecule, intercellular cell adhesion molecule (ICAM-1).[13] ICAM-1 serves as a receptor to capture circulating monocytes, leucocytes and neutrophils by binding to its ligand, Mac-1 (CD11b/CD18), expressed on the surface of these cells.[15] This cell–cell adhesion facilitates diapedesis, the transendothelial migration of monocytes and other leucocytes and neutrophils. Diapedesis is among the initial steps in inflammation as well as in atherosclerosis.

Once inside the subendothelial space, the monocytes differentiate into macrophages and internalise oxidised low-density lipoproteins (ox-LDL) trapped within the extracellular matrix. Increased concentrations of ox-LDL have been strongly implicated in the pathogenesis of atherosclerosis.[16] On internalisation of ox-LDL, macrophages release TNF-α,[17] which can further activate β-1,4 GalT-V.

Activated macrophages internalise the debris of scavenged, lysed lipoproteins and become foam cells that are loaded with cholesterol and lipid. Foam cells contribute to the formation of early atherosclerotic lesions, known as 'fatty streaks'. With continued endothelial injury and exposure to atherogenic lipoproteins and other risk mechanisms, these lesions progress to atherosclerotic plaques later in life.[18]

Lp(a) essentially consists of a little apoprotein a bound covalently to apolipoprotein B (apoB) of the LDL particle.[19] Variations in Apo(a) size are due to size polymorphism of kringle IV repeats in the LPA gene.[20 21] Lp(a) is synthesised and secreted by the liver. However, little is known about its catabolism. The plasma level of Lp(a) in man ranges from 0.2 to 200 mg/dL. Persons of African descent are known to have twofold to threefold higher level of Lp(a) than Asians or Caucasians.[22]

Because Lp(a) is structurally similar to plasminogen and tissue plasminogen activator, it can compete with plasminogen for its binding site, thus reducing fibrinolysis and enhancing clot formation. In addition, because Lp(a) increases the secretion of plasminogen activator, it can further enhance thrombogenesis.[22] Most importantly, Lp(a) transports atherogenic and proinflammatory molecules, for example, oxidised phospholipids implicated in cell proliferation,[23 24] and leads to migration of inflammatory cells, for example, neutrophils to the vessel walls.[25]

The present study examines the level of Lp(a), oxidised phosphocholines (ox-PC) and β-1,4 GalT-V in serum samples of patients with SLE. These two factors are associated with inflammation and atherosclerosis and may play a role in the increased risk for atherosclerosis in SLE.

METHODS

Patient population

All patients with SLE were part of the Hopkins Lupus Cohort Study. All patients gave written consent. The SLE serum samples (n=50) were obtained from 73% Caucasian and 25% African–American adults, with a mean age of 43 (SD of 12 years), from the Johns Hopkins Lupus Cohort. Patients with SLE were diagnosed using the Systemic Lupus International Collaborating Clinics classification criteria.[26 27] Out of the 50 adults, 48 were female and 2 were male. The recorded cardiovascular events included myocardial infarction, stroke and congestive heart failure. The samples were available from the Biobank and chosen without selection bias. The cohort (n=50) were, on average, 49 years of age (SD of 12), 74% were Caucasian American, 24% African–American and 2% of other ethnicity. Of the donors, 96% were female.

Healthy control serum samples (n=50) were obtained from 80% Caucasian American and 10% African–American adults and 10% other ethnicity, with a mean age of 29 (SD of 9 years). Of these donors, 100% were female. The controls were adult women (at least 18 years old) who had no history of autoimmune, cardiovascular, renal, hepatic, infectious, inflammatory and pain disorders, or who were pregnant or lactating. These were participants who responded to flyers placed in Baltimore and greater Baltimore area or who heard about the project from family or friends enrolled in the Johns Hopkins headache study. Those with chronic immune or inflammatory disorders, cerebrovascular disease, diabetes, headache, thyroid disorders, renal disorders or chronic pain were excluded.

SLE-specific information, for example, physician's global assessment, disease activity, medications and disease duration, was gathered and is summarised in table 1.

Measurement of serum levels of β-1,4 GalT-V

Of the serum samples (in triplicate), 10 µL was loaded onto 96-well, medium binding, clear flat-bottom polystyrene ELISA plates (Immulux, Chantilly, Virginia). The total volume was adjusted to 100 µL with bicarbonate buffer (50 mM Na_2CO_3-$NaHCO_3$). A synthetic β-1,4 GalT-V peptide (IGAQVYEQVLRSAYAKRNSSVNDc) served as a reference standard. After overnight incubation at 4°C, 200 µL of blocking buffer (1% bovine serum albumin/phosphate buffered saline) was added. Incubation continued overnight. Next, a mouse monoclonal antibody against β-1,4 GalT-V peptide was added and the plate was incubated for 1 hour at 37°C. We used horseradish peroxidase-labelled anti-IgG (Sigma, St Louis, Missouri) as the secondary antibody and incubated the plate for 1 hour at 37°C. Finally, 100 µL of 3, 3', 5, 5' tetramethyl benzidine solution was added. After 10 min of incubation at 37°C, absorbance was measured at 450 nm.

Measurement of serum levels of ox-PC

First, 50 µL of goat anti-human apoB-100 polyclonal antibody (Academy Bio-Medical, Houston, Texas) was loaded

Table 1 Summary of SLE-specific information at the time of sample collection

Factor	% positive or average+SD
Physician's global assessment (0–3)	0.6+0.6 (range 0–2)
SLE Disease Activity Index	1.9+2.5 (range 0–11)
On immunosuppressives	36%
On hydroxychloroquine	84%
On NSAIDs	32%
On statins	29%
Low C3	16%
Low C4	16%
On prednisone	36%
On prednisone >10 mg	6%
Anti-dsDNA-positive	24%
Lupus anticoagulant	28%
Disease duration (years)	10+7 (range 1–32)

dsDNA, double-stranded DNA; NSAIDs, non-steroidal anti-inflammatory drugs.

onto 96-well, medium binding, clear flat-bottom polystyrene ELISA plates (Immulux). The total volume was adjusted to 100 µL with tris-buffered saline-EDTA buffer. Human apoB-100 (Academy Bio-Medical) served as the standard. Following incubation at 4°C overnight and washing, 5 µL of serum samples was loaded onto the plates in triplicate. The total volume was adjusted to 100 µL with bicarbonate buffer (50 mM Na_2CO_3-$NaHCO_3$). After incubation for 1 hour at 37°C and washing, 50 µL (5 µg/mL) of antioxidised phosphocholine monoclonal antibody (E06; Avanti Polar Lipids, Alabaster, Alabama) was added. E06 is a widely accepted marker for ox-PC. Incubation continued overnight at 4°C. Next, IgM-alkaline phosphatase (Sigma Chemical Company, St Louis, Missouri) was added and the plate was incubated at 37°C for 1 hour. Finally, alkaline phosphatase-p-nitrophenyl phosphate was added and the plate was incubated for 1–2 hours until colour developed. Absorbance was measured at 450 nm.

Measurement of serum levels of Lp(a)

Of the serum samples (in triplicate), 5 µL was loaded onto 96-well, medium binding, clear flat-bottom polystyrene ELISA plates (Immulux). The total volume was adjusted to 100 µL with a bicarbonate buffer (50 mM Na_2CO_3-$NaHCO_3$). Human Lp(a) (Academy Bio-Medical) served as a reference standard. After overnight incubation at 4°C, 200 µL of blocking buffer (1% bovine serum albumin/phosphate buffered saline) was added and incubation continued overnight. The primary antibody, which was a sheep anti-human, Lp(a)-affinity purified antibody (Academy Bio-Medical), was added next. The plate was then incubated for 1 hour at 37°C. We used horseradish peroxidase-labelled anti-IgG (Sigma) as the secondary antibody and incubated the plate for 1 hour at 37°C. Finally, 100 µL of 3, 3', 5, 5' tetra-methyl benzidine solution was added. After 10 min of incubation at 37°C, absorbance was measured at 450 nm.

Measurement of serum levels of glucose and lipoproteins

The levels of glucose and lipoproteins were determined at Johns Hopkins University clinical laboratory facilities using standardised automated clinical diagnostic procedures, and are presented in table 2.

Statistical analysis

The main statistical analysis in this study involved the use of Welch's unpaired two-sample t-tests (one-tailed) to evaluate whether the mean β-1,4 GalT-V, Lp(a) and ox-PC values of the SLE group were significantly greater than those of the control group. The Welch's unpaired t-test allows for unequal variances in the two samples. The Welch's unpaired t-test requires the distribution of β-1,4 GalT-V, Lp(a) and ox-PC in both the SLE group and the control group to be normal. This requirement was not met, based on visual inspection of the relevant histograms. As a result, we used the logarithm (base 10) of the values of β-1,4 GalT-V, Lp(a) and ox-PC in the t-tests we performed.

We also used the same protocol to compare two subgroups within the SLE group: those who never tested positive for antiphospholipid antibodies (including lupus

Table 2 Glucose and lipid summary characteristics of SLE and control groups

	Control patients		Patients with SLE	
	Mean (SD)	Total (n)	Mean (SD)	Total (n)
Glucose (mg/dL)	90.0 (9.4)	50	88.25 (14.7)	48
Cholesterol (mg/dL)*	179.52 (36.5)	50	197.25 (35.5)	49
Triglycerides (mg/dL)	88.08 (44.4)	50	102.61 (47.40)	49
HDL cholesterol (mg/dL)	58.16 (16.6)	50	62.4 (18.6)	49
LDL cholesterol (mg/dL)	105.74 (34.7)	50	114.24 (36.6)	49

This table summarises the lipid and glucose data of SLE and control groups. The mean cholesterol concentration of the SLE group was significantly greater than that of the control group.
The asterisk represents p<0.05.
*Statistically significant at p=0.0161.
HDL, high-density lipoprotein; LDL, low-density lipoprotein.

anticoagulant antibodies, anticardiolipin antibodies and anti-beta 2 glycoprotein 1 antibodies) (n=38) and those who had tested positive for any of the aforementioned antibodies at some point in the past (n=12). As a note, some patients may have had a newly positive anti-beta 2 glycoprotein 1 lab value that we would not know about, since these antibodies were measured earlier than the β-1,4 GalT-V, Lp(a) and ox-PC levels.

Additionally, in order to investigate whether any of the aforementioned three parameters were associated with cardiovascular events in patients with SLE, we used the following analytical protocol. For each patient with SLE who had a cardiovascular event in their lifetime, we identified all the patients in the SLE group who did not have a cardiovascular event in their lifetime and were at an age within a 5-year window centred at the age of the patient with SLE who had a cardiovascular event. We then calculated the average values of β-1,4 GalT-V, Lp(a) and ox-PC in these patients. Also, we took the difference between these average values and the corresponding values for the patient with SLE who had a cardiovascular event. This yielded four different scores for each of the three parameters, because there were four patients with SLE who had a cardiovascular event in their lifetime. We analysed these scores using a one-sample t-test, with a hypothetical mean value of zero.

Finally, to control for race and age as potential confounding variables in our comparisons between the SLE group and the control group, as well as the comparisons between the subgroups within the SLE group (those with and without a history of antiphospholipid antibodies), we carried out multiple regression analysis. Two binary covariates were used for race: 'African–American' (0 if the person is not African–American, 1 if the person is African–American) and 'Other Race' (0 if the person is African–American or Caucasian American, 1 if the person identifies with another race). In the final model, the independent variables were 'African–American', 'Other Race', 'Age', and either 'Case Control Status (0 if person is in the control group, 1 if person is in the SLE group)' or antiphospholipid antibody status (0 if the person does not have a history of having antiphospholipid antibodies, 1 if the person does have a history of having antiphospholipid antibodies). The dependent variable was either log(Lp(a)), log(GalT-V) or log(ox-PC/apoB).

For all the statistical tests used in our analysis, statistical significance was defined by p<0.05.

Missing data

There were no missing data.

RESULTS

Serum concentration of β-1,4 GalT-V is higher in patients with SLE

The serum level of β-1,4 GalT-V had a mean (SD) of 1.9748 (0.7314) mg/dL in patients with SLE and 0.9915 (0.103) mg/dL in healthy controls. Given the sample sizes of each group (n=50 for the SLE group and n=50 for the control group), the SLE group had a greater serum level of β-1,4 GalT-V, with a statistically significant p value (<0.0001) (figure 1B).

Serum level of ox-PC/apoB is higher in patients with SLE

The serum absorbance of ox-PC/apoB had a mean (SD) of 0.357 (0.112) in patients with SLE and 0.242 (0.107) in healthy controls. The SLE group had a greater serum absorbance of ox-PC/apoB, with a statistically significant p value (<0.0001) (figure 1C).

Serum concentration of Lp(a) is higher in patients with SLE

The serum level of Lp(a) had a mean (SD) of 18.988 (5.169) mg/dL in patients with SLE and 14.734 (1.399) mg/dL in healthy controls. The SLE group had a greater serum level of Lp(a) with a statistically significant p value (<0.0001) (figure 1A).

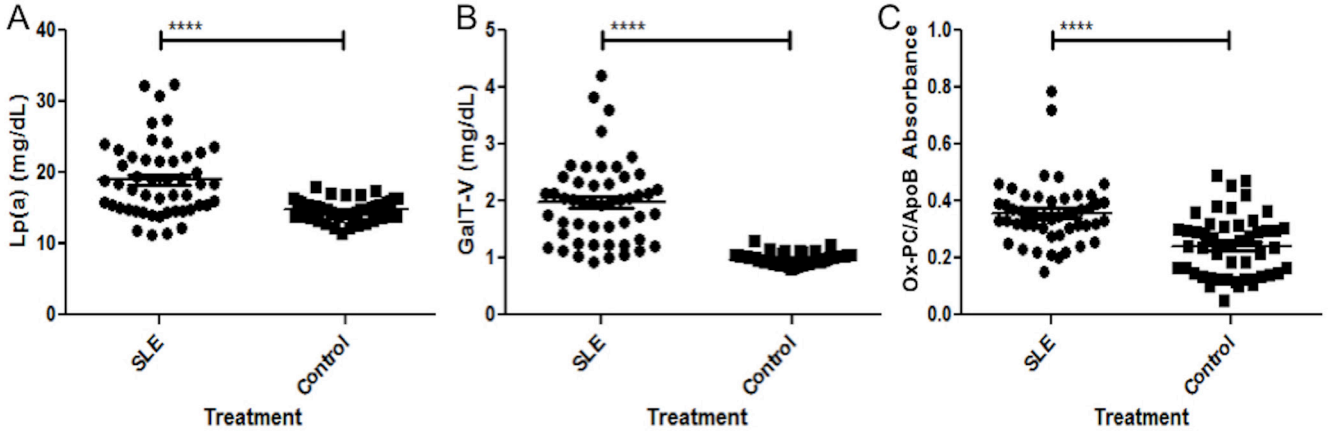

Figure 1 Levels of Lp(a), β-1,4 GalT-V and ox-PC/apoB-100 in SLE and control groups. This set of dot plots depicts the major finding that levels of (A) Lp(a), (B) β-1,4 GalT-V and (C) ox-PC/apoB-100 are significantly increased in the SLE group when compared with control. The four asterisks above each plot indicate p<0.0001. Each dot represents data from an individual patient. apoB, apolipoprotein B; β-1,4 GalT-V, β-1,4 galactosyltransferase-V; Lp(a), lipoprotein(a); ox-PC, oxidised phosphocholines.

Table 3 Results of the linear regression models for Lp(a), GalT-V and ox-PC in the control and SLE groups, accounting for race and age differences

	African–American coefficient (p value)	Other race coefficient (p value)	Age coefficient (p value)	Case–control status coefficient (p value)
Log(Lp(a))	0.021 (0.38)	−0.039 (0.29)	<0.0001 (0.95)	0.092 (<0.0001)
Log(Galt-V)	−0.010 (0.76)	−0.014 (0.78)	−0.002 (0.14)	0.297 (<0.0001)
Log(ox-PC/apoB)	0.029 (0.54)	0.099 (0.19)	−0.002 (0.27)	0.228 (<0.0001)

The dependent variables are specified in the left most column, and the independent variables (covariates) are specified in the first row. Each cell contains the coefficient and corresponding p value of the independent variable specified by the column headers, in the context of the linear regression model of the dependent variable specified by the row headers.
apoB, apolipoprotein B; GalT-V, galactosyltransferase-V; Lp(a), lipoprotein(a); ox-PC, oxidised phosphocholines.

Comparisons between patients with SLE with and without a history of antiphospholipid antibodies

We were not able to reject the null hypothesis that there is no difference between the mean values of β-1,4 GalT-V, Lp(a) and ox-PC/apoB between patients with SLE who have a history of antiphospholipid antibodies and patients with SLE who do not have a history of antiphospholipid antibodies. In other words, there is no significant difference in the mean values of β-1,4 GalT-V, Lp(a) and ox-PC/apoB between the two subgroups.

Patients with SLE who had a history of cardiovascular events versus those who did not

Using the one-sample t-test method described in the Methods section, we found that there was no significant association between β-1,4 GalT-V, Lp(a) and ox-PC values and the incidence of cardiovascular events in the SLE group. This finding will be analysed in the Discussion section.

Race and age

In order to assess if race and age were potentially confounding variables, we performed multiple regression analyses. The p values for case–control status in the multiple linear regression models for Lp(a), GalT-V and ox-PC/apoB were all statistically significant, while the p values for the race and age covariates were not statistically significant (table 3). Thus, we conclude that compared with the control group, the SLE group had greater serum levels of ox-PC/apoB, GalT-V and Lp(a). This finding is statistically significant and is not confounded by race or age.

The p values for antiphospholipid antibody status, race and age covariates in the multiple linear regression models for Lp(a), GalT-V and ox-PC/apoB were not statistically significant (p>0.05). We thus conclude that there is no significant difference in the serum levels of ox-PC/apoB, GalT-V and Lp(a) between the two SLE subgroups (those with and without a history of antiphospholipid antibodies). This finding is not confounded by race or age.

DISCUSSION

There are three major findings from this study. First, we observed that the level of Lp(a) was significantly greater in SLE serum compared with control serum, confirming previous findings.[9] Second, we observed that the level of ox-PC per unit apoB was significantly greater in the serum of patients with SLE. Apolipoprotein(a) associated with Lp(a) is the major carrier of ox-PC in plasma. We found Lp(a) to be enriched in ox-PC in this cohort of patients with SLE. Third, an unexpected result was that the β-1,4 GalT-V concentration was also significantly greater in SLE serum relative to control serum.

The following observations from our laboratory have led us to propose a pathway where ox-LDL may recruit β-1,4 GalT-V to induce inflammation and atherosclerosis relevant to the pathophysiology in SLE (summarised in figure 2): (1) Ox-LDL and/or oxidised phospholipids (present in ox-LDL) were shown to activate β-1,4 GalT-V, contributing to the generation of LacCer (a bonafide mitogenic agent), superoxide generation and cell proliferation via the mitogen activated protein kinase (MAP) kinase, alpha serine threonine protein kinase (AKT)-1 signalling pathway.[14 28] (2) Conversely, inhibition of β-1,4 GalT-V activity completely mitigated ox-LDL-induced cell proliferation in vitro in cultured human arterial smooth muscle cells and increased aortic intima-media thickening, cardiac hypertrophy and pulse wave velocity in vivo in ApoE−/− mouse model of atherosclerosis.[29] (3) β-1,4 GalT-V gene ablation in cultured human arterial endothelial cells rendered these cells insensitive to vascular endothelial growth factor (VEGF)-induced angiogenesis.[14] (4) Previously, we observed a strong correlation between ox-LDL and β-1,4 GalT-V in patients with hypercholesterolaemia. Collectively, our studies suggest an important role of β-1,4 GalT-V in proliferative disease and inflammatory disease, for example, SLE.

Several studies implicate reactive oxygen species with inflammation and tissue injury. However, recent studies in active and inactive SLE have shown differential production of reactive oxygen species in neutrophils in these patients.[30–32] In turn, this 'pro-oxidant environment' may well enhance lipid peroxidation in these patients, thus converting LDL to the oxidised variety.[30 33] As ox-LDL is taken up via multiple cognate receptors on the surface of endothelial cells, it activates β-1,4 GalT-V to generate LacCer to produce reactive oxygen species. As these molecules are membrane-permeable, they exit the cell and can oxidise circulating LDL and thus the cycle could

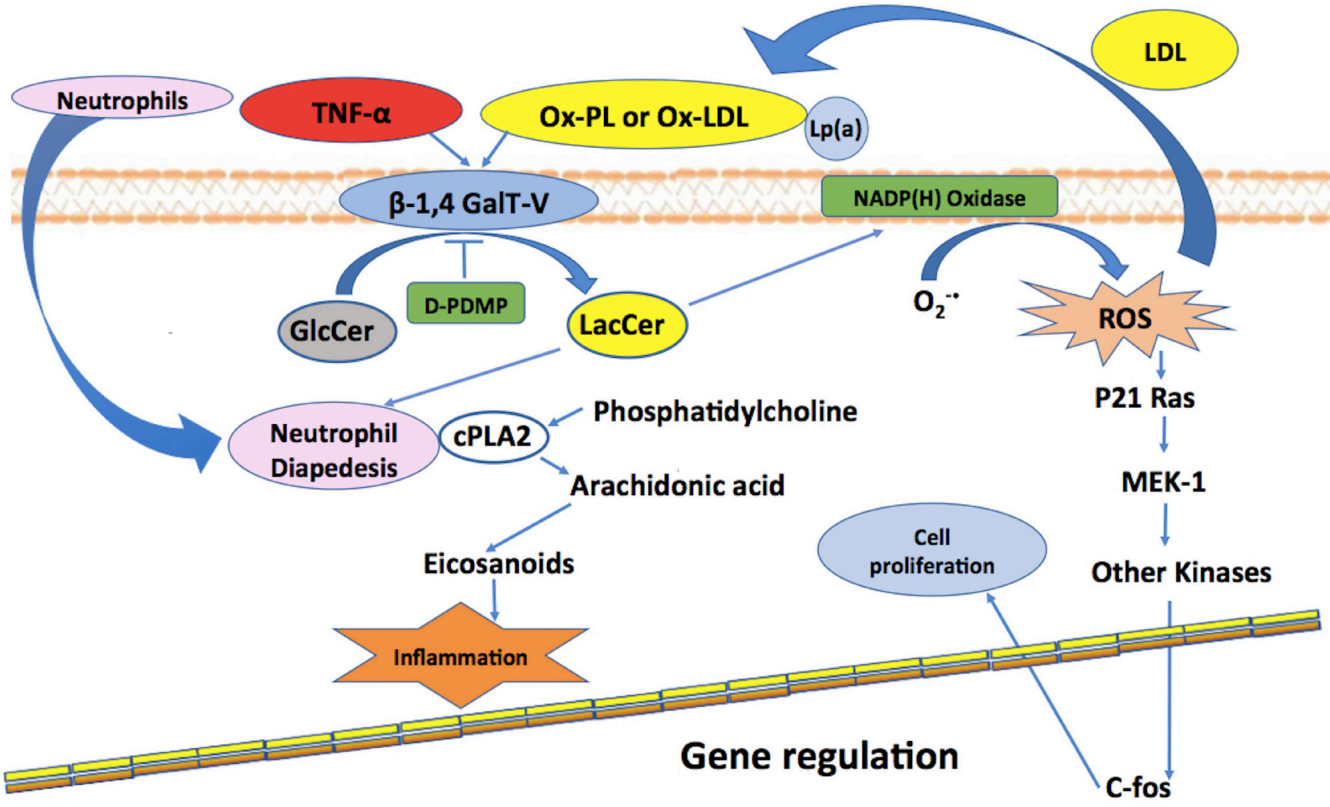

Figure 2 GalT-V topology map. This signal topology map highlights the function of β-1,4 GalT-V, a key enzyme that is implicated in the inflammatory processes of atherosclerosis pathophysiology. SLE-related inflammation results in elevated levels of ROS, which oxidises LDL to produce ox-LDL. Neutrophils consume ox-LDL and release the inflammatory cytokine known as TNF-α. Both ox-LDL and TNF-α activate LacCer synthase, formally known as β-1,4 GalT-V. β-1,4 GalT-V produces LacCer, which initiates signalling pathways that lead to increased neutrophil diapedesis. Once inside the endothelial cell, neutrophils are exposed to LacCer directly. Within neutrophils, LacCer activates cPLA2, which catalyses the production of arachidonic acid. Arachidonic acid is a precursor in the synthesis of inflammatory molecules such as eicosanoids. LacCer contributes further to inflammation and cell proliferation by increasing ROS production via activation of NADPH oxidase. D-PDMP inhibits β-1,4 GalT-V, thus working against the positive feedback loop of LDL oxidation. β-1,4 GalT-V, β-1,4 galactosyltransferase-V; cPLA2, cytosolic phospholipase 2; D-PDMP, D-1-phenyl-2-decanoylamino-3morpholino-1-propanol; GlcCer, Glucosylceramide; LacCer, lactosylceramide; Lp(a), lipoprotein(a); MEK, mitogen activates protein kinase; NADP(H), nicotinamide adenine dinucleotide phosphate H; ox-LDL, oxidised low-density lipoprotein; ox-PL, oxidised phospholpipids; ROS, reactive oxygen species; TNF-α, tumour necrosis factor alpha.

repeat itself. In our study and previous studies in patients with SLE, the level of ox-PC molecules per apoB protein was significantly greater in the serum of patients with SLE as compared with control serum (table 3), and the present report shows increased β-1,4 GalT-V in SLE serum compared with control, contributing to inflammation.

The role of LacCer in contributing to inflammation by way of increased cell adhesion between endothelial cells and neutrophils has also been documented. LacCer is present in all cells, but is highly enriched in neutrophils.[34] LacCer raises the activity of cytosolic phospholipase A2 in human neutrophils.[35] This enzyme cleaves arachidonic acid located at carbon-2 position in phosphatidylcholine. In turn, arachidonic acid is a precursor in the synthesis of inflammatory molecules such as eicosanoids and prostaglandins (figure 2). In neutrophils, LacCer treatment also increased the expression of platelet endothelial cell adhesion molecule (PECAM-1),[36] as well as Mac-1,[35 36] which serves as a ligand to bind to ICAM-1 localised on the surface of human endothelial cells. Activation of NADP(H) oxidase by LacCer occurs by inducing the migration of p47phox and p67phox from the cytosol to the plasma membrane,[37] accompanied with the infiltration/migration of neutrophils from the circulation into the dermis in the skin and increased expression of TSG-6 (TNF-stimulated gene-6) protein. This caused skin inflammation and hair discolouration in ApoE−/− mice.[29 38] Conversely, treatment with an inhibitor of β-1,4 GalT-V not only decreased the activity of β-1,4 GalT-V and mass of LacCer but also oxidised PC level in a dose-dependent and time-dependent manner,[29] and reversal of skin arterial stiffness and skin phenotype.[38] Previously, we have shown that in cultured human arterial smooth muscle cells and endothelial cells, oxidised PC and TNF-α can dose and time dependently increase the activity of β-1,4 GalT-V, generating LacCer and increasing superoxide generation to induce the expression of cell adhesion molecules, ICAM-1 and PECAM-1.[13 39] Collectively,

we hypothesise that β-1,4 GalT-V activation would be expected to exacerbate inflammation via LacCer signalling, especially considering the finding in this study that β-1,4 GalT-V level is significantly elevated in patients with SLE, relative to healthy controls. The increased number of monocytes in the subendothelial space would consume the increased amount of ox-LDL present, becoming foam cells, which contribute to the development of atherosclerotic plaques (figure 2).

There are limitations to this study. First, the patients with SLE and healthy controls were not selected randomly from the general population. Second, the patients with SLE may have had other comorbidities which we did not inspect. These two points limit the generalisability of our findings and may have introduced confounding variables to our data. Thus, we cannot use these data to claim that elevated β-1,4 GalT-V levels increase the risk for cardiovascular events in patients with SLE and/or disease specificity with regard to SLE. Lastly, linear regression analysis data revealed that our data were statistically significant after adjusting for age. However, since our sample size is small, our conclusions about confounders will require further investigation.

In summary, this is the first report where increased levels of β-1,4 GalT-V have been associated with SLE along with traditional markers of SLE such as ox-LDL and Lp(a). Our study suggests that increased β-1,4 GalT-V levels may be a novel target for therapeutic drugs, which could reduce the inflammation and atherosclerosis associated with SLE. Findings from this study support the value of future research in this area.

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Contributors VS analysed the data, communicated with the authors, and wrote and edited the manuscript. MAP planned and provided indepth clinical data on serum samples from patients with SLE and critiqued the manuscript. BLP provided clinical data and serum samples from normal subjects. SRJ designed and conceived the study and critiqued the manuscript and statistical analysis. SC conceived and designed the study, conducted the ELISA, and edited and wrote the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

Ethics approval The Johns Hopkins Institutional Review Board approval was obtained for this study. The Hopkins Lupus Cohort Study is approved by the Johns Hopkins University School of Medicine Institutional Review Board on a yearly basis.

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