Effects of statins on the immunoglobulin G glycome

Toma Keser, Frano Vučković, Clara Barrios, Jonas Zierer, Annika Wahl, Akintunde O Akinkuolie, Jerko Štambuk, Natali Nakić, Tamara Pavić, Josipa Periša, Samia Mora, Christian Gieger, Cristina Menni, Tim D Spector, Olga Gornik, Gordan Lauc

PII: S0304-4165(17)30083-1
DOI: doi: 10.1016/j.bbagen.2017.02.029
Reference: BBAGEN 28791

To appear in: *BBA - General Subjects*

Received date: 22 November 2016
Revised date: 22 February 2017
Accepted date: 28 February 2017

Please cite this article as: Toma Keser, Frano Vučković, Clara Barrios, Jonas Zierer, Annika Wahl, Akintunde O Akinkuolie, Jerko Štambuk, Natali Nakić, Tamara Pavić, Josipa Periša, Samia Mora, Christian Gieger, Cristina Menni, Tim D Spector, Olga Gornik, Gordan Lauc, Effects of statins on the immunoglobulin G glycome, *BBA - General Subjects* (2017), doi: 10.1016/j.bbagen.2017.02.029

Effects of Statins on the Immunoglobulin G Glycome

Toma Keser[1], Frano Vučković[2], Clara Barrios[3,4] Jonas Zierer[3,5], Annika Wahl[6,7,8], Akintunde O Akinkuolie[9], Jerko Štambuk[2], Natali Nakić[10], Tamara Pavić[1], Josipa Periša[1], Samia Mora[9], Christian Gieger[6,7,8], Cristina Menni[3], Tim D Spector[3], Olga Gornik[1#], Gordan Lauc[1,2#*]

[1] Department of Biochemistry and Molecular Biology, University of Zagreb, Faculty of Pharmacy and Biochemistry, Zagreb, Croatia

[2] Genos Glycoscience Research Laboratory, Zagreb, Croatia

[3] Department of Twin Research and Genetic Epidemiology, King's College London, London, UK

[4] Department of Nephrology, Hospital del Mar, Institut Mar d'Investigacions Mediques, Barcelona, Spain

[5] Institute of Bioinformatics and Systems Biology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

[6] Research Unit of Molecular Epidemiology, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

[7] Institute of Epidemiology II, Helmholtz Zentrum München, German Research Center for Environmental Health, Neuherberg, Germany

[8] German Center for Diabetes Research (DZD e.V.), Neuherberg, Germany

[9] Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

[10] Laboratory of Prion Biology, Department of Neuroscience, Scuola Internazionale Superiore di Studi Avanzati (SISSA), Trieste, Italy

[#] Joint last authors

*Corresponding author: Gordan Lauc, PhD, Faculty of Pharmacy and Biochemistry, University of Zagreb, A. Kovačića 1, 10000 Zagreb, Croatia. Email: glauc@pharma.hr

ABSTRACT

Background: Statins are among the most widely prescribed medications worldwide and usually many individuals involved in clinical and population studies are on statin therapy. Immunoglobulin G (IgG) glycosylation has been associated with numerous cardiometabolic risk factors.

Methods: The aim of this study was to investigate the possible association of statin use with N-glycosylation of IgG. The association was analyzed in two large population cohorts (TwinsUK and KORA) using hydrophilic interaction liquid chromatography (HILIC-UPLC) in the TwinsUK cohort and reverse phase liquid chromatography coupled with electrospray mass spectrometry (LC-ESI-MS) in the KORA cohort. Afterwards we investigated the same association for only one statin (rosuvastatin) in a subset of individuals from the randomized double-blind placebo-controlled JUPITER study using LC-ESI-MS for IgG glycome and HILIC-UPLC for total plasma N-glycome.

Results: In the TwinsUK population, the use of statins was associated with higher levels of core-fucosylated biantennary glycan structure with bisecting N-acetylglucosamine (FA2B) and lower levels of core-fucosylated biantennary digalactosylated monosialylated glycan structure (FA2G2S1). The association between statin use and FA2B was replicated in the KORA cohort. In the JUPITER trial we found no statistically significant differences between the randomly allocated placebo and rosuvastatin groups.

Conclusions: In the TwinsUK and KORA cohorts, statin use was associated with a small increase of pro-inflammatory IgG glycan, although this finding was not confirmed in a subset of participants from the JUPITER trial.

General Significance: Even if the association between IgG N-glycome and statins exists, it is not large enough to pose a problem for glycomic studies.

Key words: N-glycosylation, immunoglobulin G, total plasma N-glycome, statin, rosuvastatin

Introduction

Glycosylation is a complex, highly specific and regulated co- and post-translational process that covalently links glycans (complex oligosaccharides) to proteins and lipids [1,2]. Structural variations in the attached glycans strongly affect structure and function of proteins. Nearly all human plasma proteins, with exception of albumin, are modified by glycans [3,4]. Structural differences in terminal glycan antennae is common and recent studies demonstrated significant variation in glycome composition both within and between individuals [5–7]. Glycosylation is not only age- and gender-specific but is also affected by many environmental factors such as smoking, diet and medication [6,8–10]. Furthermore, glycosylation patterns have been characterized in relation to cardiovascular disease risk [11,12]. In particular decrease in immunoglobulin G (IgG) galactosylation has been associated with adverse cardiometabolic risk factors, including: cholesterol, triglycerides, C-reactive protein (CRP), HbA1c, insulin, glucose, body mass index (BMI) and kidney disease [13–15].

Statins, also known as HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors, are among the most widely prescribed medications in the world. They are first-line drugs in the treatment of hypercholesterolemia and for cardiovascular prevention due to their efficacy in lowering LDL-cholesterol and

reducing cardiovascular morbidity and mortality [16,17]. However, more recent experimental and clinical investigations have revealed that statins are also potent anti-inflammatory agents that exert beneficial effects beyond LDL-cholesterol reduction [18,19].

Both glycans and statins are connected with inflammation processes. Glycosylation is changing in many inflammatory diseases [20] and minor changes in glycan composition can have a profound influence on IgG effector functions by modulating binding to Fc receptors, and can convert IgG from a pro-inflammatory into an anti-inflammatory agent [21–23]. Moreover, it has been shown that use of statins is associated with expression of some glycosyltransferases in leukocytes and the expression of those glycosyltransferases was also changed in coronary syndrome patients [12]. Furthermore, many individuals involved in clinical and population studies are on statin therapy. If use of statins influences glycome composition, it could bias the results of glycomic studies. Therfore, it would be biologically and clinically relevant to know how statins relate to IgG glycomes. In this study we analyzed the association of statins with N-glycosylation of IgG in two large population cohorts (TwinsUK and KORA). Afterwards we investigated the same association for only one statin (rosuvastatin) in a subset of samples from the randomized double-blind placebo-controlled JUPITER study.

Material and methods

Study Subjects

This study was based on plasma samples obtained from three cohorts - the TwinsUK, the KORA and the JUPITER study. The demographic characteristics of the study populations are presented in Table 1. Written informed consent was obtained from all participants.

TwinsUK registry is a national register of adult twins. Twins were recruited as volunteers by successive media campaigns without selecting for particular diseases or traits [24]. Statin drug status was gathered for 2247 individuals (328 cases and 1919 controls) with glycan data available. Statins included atorvastatin, simvastatin, fluvastatin, pravastatin and rosuvastatin.

German population study KORA F4 [25] ("Kooperative Gesundheitsforschung in der Region Augsburg"), included 1665 samples with statin drug status (257 cases and 1408 controls) and available glycan data. Statins included atorvastatin, simvastatin, fluvastatin, pravastatin and lovastatin.

Justification for the Use of Statins in Primary Prevention: An Intervention Trial Evaluating Rosuvastatin trial (JUPITER; www.ClinicalTrials.gov; NCT00239681) [19] was a double-blind, placebo-controlled trial that evaluated rosuvastatin 20 mg daily versus placebo in the primary prevention of first major CVD events in healthy men ≥50 years and women ≥60 years with low-density lipoprotein cholesterol <130 mg/dL, but who were at increased risk of cardiovascular events on the basis of elevated high-sensitivity C-reactive protein (≥2 mg/L).

IgG isolation

Immunoglobulin G was isolated from plasma by affinity chromatography using 96-well protein G monolithic plates (BIA Separations, Ljubljana, Slovenia) as described previously [7]. Briefly, 100 µL of plasma was diluted 10× with PBS, filtered through 0.45 µm GHP filter plate (Pall Corporation, Ann Arbor, MI, USA), and then applied to the protein G plate and instantly washed. IgGs were eluted with 1 mL of 0.1 M formic acid (Merck, Darmstadt, Germany) and immediately neutralized with 1 M ammonium bicarbonate (Acros Organics, NJ, USA).

IgG N-glycans release and labeling

Isolated IgG samples were dried in a vacuum centrifuge. After drying, proteins were denatured with addition of 30 µL 1.33% SDS (w/v) (Invitrogen, Carlsbad, CA, USA) and by incubation at 60 °C for 10 min. Subsequently, 10 µL of 4 % Igepal-CA630 (Sigma-Aldrich, St. Louis, MO, USA) and 1.25 mU PNGase F (ProZyme, Hayward, CA, USA) in 10 µL 5× PBS were added. The samples were incubated overnight at 37 °C for N-glycan release. The released N-glycans were labeled with 2-aminobenzamide (2-AB). The labeling mixture was freshly prepared by dissolving 2-AB (19.2 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) and 2-picoline borane (44.8 mg/mL, Sigma-Aldrich, St. Louis, MO, USA) in DMSO (Sigma-Aldrich, St. Louis, MO, USA) and glacial acetic acid (Merck, Darmstadt, Germany) mixture (70:30, v/v). 25 µL of labeling mixture was added to each N-glycan sample in the 96-well plate and the plate was sealed using adhesive seal. Mixing was achieved by shaking for 10 min, followed by two hour incubation at 65 °C. Samples (in a volume of 75 µL) were brought to 80% ACN (v/v) by adding 300 µL of ACN (J.T. Baker, Phillipsburg, NJ, USA). Free label and reducing agent were removed from the samples using HILIC-SPE. 200 µL of 0.1 g/mL suspension of microcrystalline cellulose (Merck, Darmstadt, Germany) in water was applied to each well of a 0.45 µm GHP filter plate (Pall Corporation, Ann Arbor, MI, USA). Solvent was removed by application of vacuum using a vacuum manifold (Millipore Corporation, Billerica, MA, USA). All wells were prewashed using

5× 200 µL water, followed by equilibration using 3× 200 µL acetonitrile/water (80:20, v/v). The samples were loaded to the wells. The wells were subsequently washed 7× using 200 µL acetonitrile/water (80:20, v/v). Glycans were eluted 2× with 100 µL of water and combined eluates were stored at −20 °C until usage.

Total plasma proteins N-glycans release and labeling

Total plasma proteins glycans were prepared in the same way as the IgG glycans. The only difference is that, instead with dried IgG eluate, the preparation begun with 10 µl of blood plasma and with addition of 20 µL 2 % SDS (w/v) (Invitrogen, Carlsbad, CA, USA) to each sample before the incubation at 65 °C for 10 min.

Hydrophilic Interaction Chromatography (HILIC)-UPLC

Fluorescently labeled N-glycans were separated by hydrophilic interaction chromatography on Waters Acquity ultra-performance liquid chromatography (UPLC) instrument (Milford, MA, USA) consisting of a quaternary solvent manager, sample manager and a FLR fluorescence detector set with excitation and emission

wavelengths of 250 and 428 nm, respectively. The instrument was under the control of Empower 2 software, build 2145 (Waters, Milford, MA, USA). Labeled N-glycans were separated on a Waters bridged ethylene hybrid (BEH) Glycan chromatography column, 100 × 2.1 mm i.d. for IgG glycans and 150 × 2.1 mm i.d. for total plasma proteins glycans, 1.7 µm BEH particles, with 100 mM ammonium formate, pH 4.4, as solvent A and acetonitrile as solvent B. The separation method used a linear gradient of of 75-62% acetonitrile (v/v) at flow rate of 0.4 ml/min in a 25 min analytical run for IgG glycans and linear gradient of 70-53 % acetonitrile (v/v) at flow rate of 0.561 ml/min in a 24.81 min analytical run for total plasma proteins glycans. The separation temperature was 60°C for IgG glycans and 25°C for total plasma proteins glycans. Samples were maintained at 10 °C before injection. Data processing was performed using an automatic processing method with a traditional integration algorithm after which each chromatogram was manually corrected to maintain the same intervals of integration for all the samples. The chromatograms were all separated in the same manner into 24 peaks (GP1-24) for IgG glycans and 46 peaks (GP1-46) for total plasma proteins glycans and the amount of glycans in each peak was expressed as % of total integrated area. The system was calibrated using an external standard of hydrolyzed and 2-AB labeled glucose oligomers from which the retention times for the individual glycans were converted to glucose units [26]. Glycans were analyzed on the basis of their elution positions and measured in glucose units then compared to reference values in the “Glyco-Base” database (available at: http://glycobase.nibrt.ie) for structure assignment [27]. For IgG

glycans, in addition to 24 directly measured glycan traits, 52 derived traits were calculated. These derived traits average particular glycosylation features (galactosylation, fucosylation, bisecting GlcNAc, and sialylation) (Supplementary Table 1).

IgG tryptic digestion and purification

25 µg IgG was digested with 200 ng of trypsin at 37°C (Worthington, USA) overnight. Resulting tryptic glycopeptides were purified by reverse phase solid phase extraction using Chromabond C18ec beads (Marcherey-Nagel, Germany). C18 beads were activated with 80% ACN containing 0.1% trifluoroacetic acid (TFA) (Sigma-Aldich, USA) and conditioned with 0.1% TFA. Tryptic digests were diluted 10X with 0.1% TFA, loaded onto C18 beads, washed with 0.1% TFA and finally eluted with 20% ACN containing 0.1% TFA. Eluates containing tryptic glycopeptides were dried by vacuum centrifugation and dissolved with 20 µL of ultrapure water.

LC-ESI-MS/MS analysis of IgG tryptic glycopeptides

Tryptic digests were analyzed on nanoACUITY UPLC system (Waters, USA) coupled to micrOTOF-Q mass spectrometer (BrukerDaltonics, Bremen, Germany). 9 µL of eluates containing IgG tryptic glycopeptides was loaded into Acclaim PepMap100 C8 (5mm×300 µm i.d.) trap column and washed 1 min with 0.1% TFA (solvent A) at a flow rate of 40 µL/min. Separation was achieved on a Halo C18 nano-LC column (150mm×75 µm i.d., 2.7 µm HALO fused core particles) (Advanced Materials technology, USA) using a 3.5 min gradient at a flow rate of 1 µL/min from 18% to 25% solvent B (80% ACN). Column temperature was 30°C. Mass spectra were recorded from m/z 200 to 1900 with 2 averages at a frequency of 0,5 Hz. Quadrupole ion energy and collision energy of the MS were set at 4 eV. NanoACUITY UPLC system and the Bruker micrOTOF-Q were operated under HyStar software, version 3.2. In Caucasian populations, IgG2 and IgG3 tryptic Fc glycopeptides have identical peptide moieties and are therefore not distinguishable by this profiling method [28]. Data were extracted using an in-house Python script. Briefly, data were m/z recalibrated using a subset of hand-picked analytes having a high signal-to-noise ratio and the expected isotopic distribution. After recalibration, intensities for top four isopotologes were extracted using 10 ppm m/z window. Based on top signals, retention times were aligned towards the cohort median. After defining retention time bins for analytes of interest, all of the signals belonging to a single analyte for

every sample were summed up. In Caucasian populations, IgG2 and IgG3 have identical peptide moieties (E293EQFNSTFR301) of their tryptic Fc glycopeptides and were, therefore, not distinguished by the profiling method [23]. For statistical analysis we used the most prominent 20 glycopeptides that were present in subclasses IgG1 and IgG2/3 and the most prominent 10 glycopeptides that were present in IgG4. Derived traits that represent common biologically meaningful features (fucosylation, bisection, agalactosylation, monogalactosylation, digalactosylation and sialylation) shared among several measured glycans were calculated for each subclass group as described previously [29] (Supplementary Table 1).

Statistical Analysis

Statistical analysis was carried out using Stata version 12 and R (version 3.1.2) and visualized using the ggplot2 package.

Glycans were globally normalized and log transformed to account for the right-skewness of their distributions. To remove experimental biases, all measurements were adjusted for batch and run-day effects using ComBat (R-package sva). Derived glycan traits were calculated using normalized and batch-

corrected glycan measurements (exponential of batch corrected measurements). All variables were centered and scaled to have mean 0 and standard deviation 1. Outliers (more than 6SD from the mean) were excluded from the analysis.

Association analyses between statin use and glycan traits were performed using linear mixed regressions adjusting for age, sex, BMI in KORA cohort, and in the TwinsUK cohort, with additional adjustment for family relatedness as random effect. We used Bonferroni correction to account for multiple testing in the TwinsUK cohort ($P=0.05/77\approx6x10^{-4}$).

For the TwinsUK cohort, the Bonferroni-significant associations between statins and glycans were replicated in the previously excluded group of MZ discordant twins using the same model. If the regression coefficients were the same direction in both analyses, the results were combined using inverse-variance fixed effect meta-analysis.

For the JUPITER study the differences were calculated between glycan values after and before the randomized allocation of placebo or rosuvastatin. Differences between the placebo and rosuvastatin groups were tested using the Mann Whitney U test.

Results

Association of statins with IgG glycome in the TwinsUK cohort

IgG glycome composition and information about the use of statins was available for 2247 individuals from the TwinsUK cohort. The demographic characteristics of the study population are summarized in Table 1. Released and 2-AB labeled IgG glycans were analyzed by HILIC-UPLC. Chromatograms obtained for IgG glycans were separated in 24 glycan peaks, which correspond to different glycan structures (GP1-24, Figure 1). In addition to 24 directly measured glycan traits, 53 derived traits were calculated as described previously [7] (Supplementary Table 1). These derived traits represent common biologically meaningful features (fucosylation, bisection, agalactosylation, monogalactosylation, digalactosylation, sialylation, etc.) shared among several measured glycans.

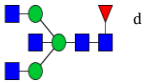
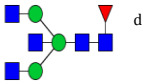
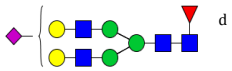
Table 1. The demographic characteristics of the study populations

	TwinsUK				KORA		JUPITER	
	No Discordant Twins (n=2181)		MZ Discordant Twins (n=66)					
	No statins	Statins	No statins	Statins	No Statins	Statins	Placebo	Rosuvastatin
N	1886	295	33	33	1408	257	47	50
Age (years)[a]	55 (±12)	63 (±8)	62 (±10)	62 (±10)	60 (± 9)	65 (±7)	67 (±5)	68 (±9)
Sex (% Female)	93%	90%	94%	94%	54%	43%	51%	50%
BMI (Kg/m2)[a]	26.5 (±4.8)	28.0 (±5.8)	27.6 (±5.4)	27.3 (±5.0)	28.0 (±4.8)	29.3 (±4.9)	27.8 (±4.6)	29.9 (±5.6)
Type 2 DM, N	28	27	2	3	97	52	1	0

[a] *Values are expressed as mean and standard deviation (in brackets)*

To find associations between statins use and IgG glycan traits, we performed linear regression analysis in the population excluding monozygotic (MZ) discordant twins. We then validated Bonferroni significant glycans in MZ twin pairs discordant for statins use. If the regression coefficients were in the same direction in both analyses, we combined the results using inverse-variance fixed effect meta-analysis. Table 2 presents the list of glycans significantly associated with statins use in the discovery cohort, the validation results in the MZ discordant cohort and the meta-analysis results. The use of statins was associated with a small increase in GP6 and $GP6^n$, which contain core-fucosylated biantennary glycan structure with bisecting N-acetylglucosamine (FA2B), together with biantennary monogalactosylated glycan structure (A2G1) in a much smaller amount, and a small decrease in GP18, which contains core-fucosylated biantennary digalactosylated monosialylated glycan structure (FA2G2S1), together with biantennary digalactosylated monosialylated glycan structure with bisecting N-acetylglucosamine (A2BG2S1) in a much smaller amount [7].

Table 2. IgG glycan traits significantly associated with statin use in the TwinsUK cohort ($P<6x10^{-4}$) and replicated in the KORA cohort ($P<0.05$)

Glycan trait	TwinsUK							KORA								
	Discovery			MZ discordant			Fixed effect meta-analysis	IgG1			IgG2/3			IgG4		
	Beta[e]	SE[f]	P value	Beta[e]	SE[f]	P value	Beta[e] [95%CI[g]]	P value	Beta[e]	SE[f]	P value	Beta[e]	SE[f]	P value	Beta[e]	SE[f]	P value
% of **FA2B**[d] glycan in **total** IgG glycans[a] 	0.036	0.009	4.26E-05	0.013	0.02	0.509	0.032 [0.02, 0.05]	5.50E-05	0.039	0.014	7.23E-03	0.039	0.014	6.55E-03	0.041	0.014	4.93E-03
% of **FA2B**[d] glycan in **neutral** IgG glycans[b] 	0.029	0.007	1.06E-04	0.013	0.017	0.438	0.026 [0.013, 0.04]	1.00E-04	0.031	0.015	3.60E-02	0.034	0.015	2.08E-02	0.038	0.015	9.19E-03
% of **FA2G2S1**[d] glycan in **total** IgG glycans[c] 	-0.031	0.009	3.39E-04	-0.267	0.02	0.189	-0.07 [-0.08, -0.05]	5.30E-18	not significant			not significant			not significant		

[a] *Corresponds to the main structure in GP6 in HILIC-UPLC measurement*

[b] *Corresponds to the main structure in GP6[n] in HILIC-UPLC measurement*

[c] *Corresponds to the main structure in GP18 in HILIC-UPLC measurement*

[d] *Structure abbreviations: all N-glycans have core sugar sequence consisting of two N-acetylglucosamines (GlcNAc) and three mannose residues; F indicates a core fucose α1–6 linked to the inner GlcNAc; Ax, number of antenna (GlcNAc) on trimannosyl core; A2, biantennary glycan with both GlcNAcs as β1–2 linked; B, bisecting GlcNAc linked β1–4 to β1–3 mannose; Gx, number of β1–4 linked galactose (G) on antenna; Sx, number (×) of sialic acids linked to galactose. Structural schemes: blue square, N-acetylglucosamine; red triangle, fucose; green circle, mannose; yellow circle, galactose; purple diamond, N-acetylneuraminic acid*

[e] *Regression Coefficient*

[f] *Standard Error*

[g] *Confidence Interval*

We also explored the association between individual statin use and IgG glycan traits for simvastatin (111 cases vs. 1919 controls) and atorvastatin (93 cases vs. 1919 controls) in the TwinsUK cohort. Association analysis was performed using linear mixed regressions analysis adjusting for age, sex, BMI and family relatedness as random effect. Although 31 glycan traits were nominally significantly associated with simvastatin use (including GP6, $GP6^n$ and GP18 with regression coefficients in the same direction as found for all statins together), none of them remained significant after correction for multiple testing (Supplementary Table 2). For atorvastatin no associations were found (Supplementary Table 2).

Replication in the KORA cohort

We aimed to replicate the associations found in the TwinsUK population in 1665 individuals from KORA cohort (257 cases and 1408 controls, Table 1). IgG Fc tryptic N-glycopeptides were measured by LC-ESI-MS, which enables glycosylation measurement of IgG Fc region at subclass-specific level. Linear regressions were applied to study the previously found significant associations between the use of statins and the glycan traits. Nominally statistically significant associations have been confirmed for two of three glycan traits (confirmed for FA2B in total and neutral IgG glycans, not confirmed for FA2G2S1) for each IgG subclass (IgG1, IgG2/3, IgG4) (Table

2). The regression coefficients were in the same direction, and of similar magnitude as found in the TwinsUK population.

Association of rosuvastatin with IgG Fc N-glycopeptides and total plasma proteins N-glycome in the JUPITER study

JUPITER is a randomized double-blind placebo-controlled study investigating the use of rosuvastatin versus placebo in the primary prevention of cardiovascular disease [19]. In a subset of JUPITER, we examined the effect of one year of rosuvastatin 20mg/day on the glycosylation of IgG Fc region at subclass-specific level (analysis of IgG tryptic glycopeptides by LC-ESI-MS). The analysis was performed on 97 individuals - 47 on placebo and 50 on rosuvastatin (three individuals in the placebo group were excluded from the analysis because of low signal intensity) (Table 1). Derived traits that represent common biologically meaningful features (fucosylation, bisection, agalactosylation, monogalactosylation, digalactosylation and sialylation) shared among several measured glycans were calculated for each subclass group as described previously [29] (Supplementary Table 1).

We investigated the differences between the placebo and the rosuvastatin group (Supplementary Figure 1). For each derived trait of each IgG subclass (IgG1, IgG2/3, IgG4), differences were calculated between glycan values after and before the placebo/rosuvastatin treatment. We found no statistically significant differences between the two groups (Supplementary Table 3). We also tried to replicate the associations found in the TwinsUK population (for glycan traits corresponding to FA2B and FA2G2S1). However, treatment with rosuvastatin had no effect on these glycan traits in any of the IgG subclasses.

To determine whether rosuvastatin induces a more general change in glycosylation of multiple proteins, we searched for associations between N-glycome of total plasma proteins (46 glycan peaks of enzymatically released, 2-AB labeled N-glycans obtained by HILIC-UPLC, Supplementary Figure 2) and the use of rosuvastatin in the same individuals from the JUPITER study (Supplementary Figure 3). We found no significant difference in N-glycosylation of plasma proteins between the studied groups (Supplementary Table 4).

Discussion

The aim of this study was to investigate the possible association of statin use with N-glycosylation of IgG in the TwinsUK and KORA populations. Afterwards we investigated the same association for only one statin (rosuvastatin) in a subset of participants from the randomized double-blind placebo-controlled JUPITER study.

In the TwinsUK population, three glycan traits (GP6, GP6^n and GP18) were found to be significantly associated with statin use in the discovery cohort, and they remained significant after the meta-analysis of the results from the discovery cohort and the validation results from the MZ discordant cohort. The use of statins was associated with higher levels of GP6 and GP6^n (which mostly contain core-fucosylated biantennary glycan structure with bisecting N-acetylglucosamine - FA2B) and lower levels of GP18 (which mostly contains core-fucosylated biantennary digalactosylated monosialylated glycan structure - FA2G2S1). The association between statin use and FA2B was replicated in the KORA cohort. IgG Fc tryptic N-glycopeptides with FA2B glycan were significantly associated with statin use for all three IgG subclasses (IgG1, IgG2/3 and IgG4), in both total and neutral glycans. The regression coefficients were in the same direction, and of similar magnitude as found in the TwinsUK population. The association between statin use and FA2G2S1 glycopeptides did not reach statistical significance.

FA2B is one of the IgG's agalactosylated glycans. Glycans that lack terminal galactose activate complement and make IgG pro-inflammatory [30–32]. Also, FA2B is one of three glycans that change considerably with age (along with FA2G2 and FA2BG2) and the combination of these three glycans can explain up to 58% of variance in chronological age [33]. FA2B is increasing with age, and it is also increasing in many different pathological conditions (e.g. autoimmune diseases [34,35], kidney disease [14] and cancer [36]). In contrast, FA2G2S1, the major sialylated glycan in IgG glycome, is decreasing with age [33], and it was also reported to be decreased in patients with systemic lupus erythematosus [34] and kidney disease [14]. Terminal α2,6-sialylation of IgG glycans decreases the ability of IgG to bind Fcγ receptors (FcγRs), which increases expression of inhibitory FcγRIIB and is anti-inflammatory [21,37]. However, these findings have not been confirmed in all studies [22,38,39]. Taken together, these suggest that a pro-inflammatory pattern of IgG glycan was found in association with statins in both the TwinUK and KORA studies.

Glycosylation is known to be affected by factors such as: expression levels, localization and substrate affinities and specificities of the glyco-enzymes, as well as abundance and trafficking of glycoprotein substrates and activated sugar donors concentrations [40–43]. Therefore, the observed changes in N-glycan profiles may be

the result of slight alterations in the levels of glycosyltransferases and glycosidases, caused by statin use or some other underlying factor.

Patients who take statins usually have dyslipidemia and other CVD risk factors. Hence, there is a possibility that the associations that we found could be due to the connection between the IgG glycans and the lipid profile or other potential confounders, which was previously reported in some studies [33,44]. Therefore, to test whether the associations found between statin use and IgG glycosylation are the consequence of the direct influence of statin therapy, we investigated this association for randomly allocated rosuvastatin therapy versus placebo in the JUPITER trial. We found no statistically significant differences between the placebo and rosuvastatin groups after one year on study treatment. We also analyzed association between total plasma proteins N-glycome and rosuvastatin in the same individuals from the JUPITER study to determine whether rosuvastatin induces a more general change in glycosylation of multiple proteins. We found no difference in plasma glycosylation.

When we explored the association between individual statin use and IgG glycan traits in the TwinsUK cohort for simvastatin and atorvastatin, we found 31 nominally significantly associated glycan traits with simvastatin use and no glycan traits associated with atorvastatin use (Supplementary Table 2). Although none of

them remained significant after correction for multiple testing, the number of cases was too small (≈100) to detect a small effect size. Therefore, there is an indication that different statins may differently influence IgG N-gycome and that could be the reason we did not find any associations between rosuvastatin and IgG glycan traits. Another explanation might be that the association between statin use and IgG glycans we found in the TwinsUK and KORA cohorts was confounded by the lipid profile or other CVD risk factors, which drove the pro-inflammatory changes that we saw, and that those effects were eliminated in the randomized double-blind placebo-controlled JUPITER study.

Conclusion

In the TwinsUK and KORA cohorts, statin use was associated with a small increase of pro-inflammatory IgG glycan, although this finding was not confirmed in a subset of participants from the JUPITER trial. There are several possible explanations for this: (1) some statins, but not rosuvastatin, may influence the IgG glycome; (2) the association between statin use and IgG glycans may be confounded by the lipid profile or other indications for statin use (eg. CVD risk factors) which drove the pro-inflammatory changes that we saw in the TwinsUK and KORA cohorts but not in the randomized JUPITER study; (3) the number of individuals from the JUPITER that we included in the analysis was smaller than in other two cohorts (97

individuals from the JUPITER trial vs. 2247 individuals from the TwinsUK cohort and 1665 individuals from the KORA cohort), hence lack of association might also be due to power issues or due to lack of signal in the JUPITER trial, which enrolled individuals who had evidence of low-grade chronic inflammation. Although we cannot give a definite answer to the question whether statins have any influence on the composition of the IgG glycans, we can conclude that the magnitude of their effect on IgG N-glycome is too small to confound associations with N-glycome observed in clinical or epidemiological studies.

Acknowledgments

This work was supported by the University of Zagreb and the European Community's Seventh Framework Programme IntegraLife (contract #315997), HighGlycan (contract #278535) and HTP-GlycoMet (contract #324400) grants.

TwinsUK funding sources: The study also receives support from the Wellcome Trust European Community's Seventh Framework Programme (FP7/2007-2013 to TwinsUK); the MRC AimHY (MR/M016560/1) grant; the National Institute for Health Research (NIHR) Clinical Research Facility at Guy's & St Thomas' NHS Foundation Trust and NIHR Biomedical Research Centre based at Guy's and St Thomas' NHS Foundation Trust and King's College London.

The KORA study was initiated and financed by the Helmholtz Zentrum München – German Research Center for Environmental Health, which is funded by the German Federal Ministry of Education and Research (BMBF) and by the State of Bavaria. Furthermore, KORA research was supported within the Munich Center of Health Sciences (MC-Health), Ludwig-Maximilians-Universität, as part of LMUinnovativ. This study was supported by the German Center for Diabetes Research (DZD e.V.).

JUPITER funding sources: Research reported in this publication was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under Award Number R01HL117861 to Dr. Mora. Dr. Akinkoulie received support from the NIH T32 (HL007575). JUPITER was financially supported by AstraZeneca, who collected trial data and monitored sites but had no role in the design or conduct of the current study, including data analysis or interpretation, drafting or editing this report, or in preparation, review or the decision to submit the manuscript for publication.

References

[1] A. Kobata, A retrospective and prospective view of glycopathology., Glycoconj. J. 15 (1998) 323–31. http://www.ncbi.nlm.nih.gov/pubmed/9613818 (accessed February 8, 2016).

[2] R.G. Spiro, Protein glycosylation: nature, distribution, enzymatic formation, and disease implications of glycopeptide bonds., Glycobiology. 12 (2002) 43R–56R. http://www.ncbi.nlm.nih.gov/pubmed/12042244 (accessed February 8, 2016).

[3] Y. Miura, N. Hashii, H. Tsumoto, D. Takakura, Y. Ohta, Y. Abe, Y. Arai, N. Kawasaki, N. Hirose, T. Endo, Change in N-Glycosylation of Plasma Proteins in Japanese Semisupercentenarians., PLoS One. 10 (2015) e0142645. doi:10.1371/journal.pone.0142645.

[4] F. Clerc, K.R. Reiding, B.C. Jansen, G.S.M. Kammeijer, A. Bondt, M. Wuhrer, Human plasma protein N-glycosylation., Glycoconj. J. (2015). doi:10.1007/s10719-015-9626-2.

[5] A. Knežević, O. Gornik, O. Polašek, M. Pučić, I. Redžić, M. Novokmet, P.M. Rudd, A.F. Wright, H. Campbell, I. Rudan, G. Lauc, Effects of aging, body mass index, plasma lipid profiles, and smoking on human plasma N-glycans., Glycobiology. 20 (2010) 959–69. doi:10.1093/glycob/cwq051.

[6] A. Knežević, O. Polašek, O. Gornik, I. Rudan, H. Campbell, C. Hayward, A. Wright, I. Kolčić, N. O'Donoghue, J. Bones, P.M. Rudd, G. Lauc, Variability, heritability and environmental determinants of human plasma N-glycome., J. Proteome Res. 8 (2009) 694–701. doi:10.1021/pr800737u.

[7] M. Pučić, A. Knežević, J. Vidić, B. Adamczyk, M. Novokmet, O. Polašek, O. Gornik, S. Šupraha-Goreta, M.R. Wormald, I. Redžić, H. Campbell, A. Wright, N.D. Hastie, J.F. Wilson, I. Rudan, M. Wuhrer, P.M. Rudd, D. Josić, G. Lauc, High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations, Mol Cell Proteomics. 10 (2011) M111.010090. doi:10.1074/mcp.M111.010090.

[8] J.E. Huffman, A. Knezevic, V. Vitart, J. Kattla, B. Adamczyk, M. Novokmet, W. Igl, M. Pucic, L. Zgaga, Å. Johannson, I. Redzic, O. Gornik, T. Zemunik, O. Polasek, I. Kolcic, M. Pehlic, C.A.M. Koeleman, S. Campbell, S.H. Wild, N.D. Hastie, H. Campbell, U. Gyllensten, M. Wuhrer, J.F. Wilson, C. Hayward, I. Rudan, P.M. Rudd, A.F. Wright, G. Lauc, Polymorphisms in B3GAT1, SLC9A9 and MGAT5 are associated with variation within the human plasma N-glycome of 3533 European adults., Hum. Mol. Genet. 20 (2011) 5000–11. doi:10.1093/hmg/ddr414.

[9] F. Dall'Olio, V. Vanhooren, C.C. Chen, P.E. Slagboom, M. Wuhrer, C. Franceschi, N-glycomic biomarkers of biological aging and longevity: a link with inflammaging., Ageing Res. Rev. 12 (2013) 685–98. doi:10.1016/j.arr.2012.02.002.

[10] R. Saldova, J.E. Huffman, B. Adamczyk, A. Mužinić, J.J. Kattla, M. Pučić, M. Novokmet, J.L. Abrahams, C. Hayward, I. Rudan, S.H. Wild, A.F. Wright, O. Polašek, G. Lauc, H. Campbell, J.F. Wilson, P.M. Rudd, Association of medication with the human plasma N-glycome., J. Proteome Res. 11 (2012) 1821–31. doi:10.1021/pr2010605.

[11] A.O. Akinkuolie, J.E. Buring, P.M. Ridker, S. Mora, A novel protein glycan biomarker and future cardiovascular disease events., J. Am. Heart Assoc. 3 (2014) e001221. doi:10.1161/JAHA.114.001221.

[12] I. Hadžibegović, Z. Vrselja, G. Lauc, G. Curić, Expression of leukocyte adhesion-related glycosyltransferase genes in acute coronary syndrome patients., Inflamm. Res. 63 (2014) 629–36. doi:10.1007/s00011-014-0735-3.

[13] J. Krištić, F. Vučković, C. Menni, L. Klarić, T. Keser, I. Beceheli, M. Pučić-Baković, M. Novokmet, M. Mangino, K. Thaqi, P. Rudan, N. Novokmet, J. Šarac, S. Missoni, I. Kolčić, O. Polašek, I. Rudan, H. Campbell, C. Hayward, Y. Aulchenko, A. Valdes, J.F. Wilson, O. Gornik, D. Primorac, V. Zoldoš, T. Spector, G. Lauc, Glycans are a novel biomarker of chronological and biological ages, Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 69 (2014). doi:10.1093/gerona/glt190.

[14] C. Barrios, J. Zierer, I. Gudelj, J. Štambuk, I. Ugrina, E. Rodríguez, M.J. Soler, T. Pavić, M. Šimurina, T. Keser, M. Pučić-Baković, M. Mangino, J. Pascual, T.D. Spector, G. Lauc, C. Menni, Glycosylation Profile of IgG in Moderate Kidney Dysfunction., J. Am. Soc. Nephrol. 27 (2016) 933–41. doi:10.1681/ASN.2015010109.

[15] M. Nikolac Perkovic, M. Pucic Bakovic, J. Kristic, M. Novokmet, J.E. Huffman, V. Vitart, C. Hayward, I. Rudan, J.F. Wilson, H. Campbell, O. Polasek, G. Lauc, N. Pivac, The association between galactosylation of immunoglobulin G and body mass index., Prog. Neuropsychopharmacol. Biol. Psychiatry. 48 (2014) 20–5. doi:10.1016/j.pnpbp.2013.08.014.

[16] D.J. Maron, S. Fazio, M.F. Linton, Current perspectives on statins., Circulation. 101 (2000) 207–13. http://www.ncbi.nlm.nih.gov/pubmed/10637210 (accessed June 21, 2016).

[17] S. Mora, R.J. Glynn, J. Hsia, J.G. MacFadyen, J. Genest, P.M. Ridker, Statins for the primary prevention of cardiovascular events in women with elevated high-sensitivity C-reactive protein or dyslipidemia., Circulation. 121 (2010) 1069–77. doi:10.1161/CIRCULATIONAHA.109.906479.

[18] D.J. Lefer, Statins as Potent Antiinflammatory Drugs, Circulation. 106 (2002) 2041–2042. doi:10.1161/01.CIR.0000033635.42612.88.

[19] P.M. Ridker, E. Danielson, F.A.H. Fonseca, J. Genest, A.M. Gotto, J.J.P. Kastelein, W. Koenig, P. Libby, A.J. Lorenzatti, J.G. MacFadyen, B.G. Nordestgaard, J. Shepherd, J.T. Willerson, R.J. Glynn, JUPITER Study Group, Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein., N. Engl. J. Med. 359 (2008) 2195–207. doi:10.1056/NEJMoa0807646.

[20] O. Gornik, G. Lauc, Glycosylation of serum proteins in inflammatory diseases., Dis. Markers. 25 (2008) 267–78. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3827815&tool=pmcentrez&rendertype=abstract.

[21] R.M. Anthony, T. Kobayashi, F. Wermeling, J. V Ravetch, Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway., Nature. 475 (2011) 110–3. doi:10.1038/nature10134.

[22] P.N. Boyd, A.C. Lines, A.K. Patel, The effect of the removal of sialic acid, galactose and total carbohydrate on the functional activity of Campath-1H., Mol. Immunol. 32 (1995) 1311–8. http://www.ncbi.nlm.nih.gov/pubmed/8643100 (accessed February 9, 2016).

[23] Y. Mimura, P. Sondermann, R. Ghirlando, J. Lund, S.P. Young, M. Goodall, R. Jefferis, Role of oligosaccharide residues of IgG1-Fc in Fc gamma RIIb binding., J. Biol. Chem. 276 (2001) 45539–47. doi:10.1074/jbc.M107478200.

[24] A. Moayyeri, C.J. Hammond, A.M. Valdes, T.D. Spector, Cohort Profile: TwinsUK and healthy ageing twin study., Int. J. Epidemiol. 42 (2013) 76–85. doi:10.1093/ije/dyr207.

[25] H.-E. Wichmann, C. Gieger, T. Illig, MONICA/KORA Study Group, KORA-gen--resource for population genetics, controls and a broad spectrum of disease phenotypes., Gesundheitswes. (Bundesverband Der Ärzte Des Öffentlichen Gesundheitsdienstes. 67 Suppl 1 (2005) S26-30. doi:10.1055/s-2005-858226.

[26] L. Royle, C.M. Radcliffe, R.A. Dwek, P.M. Rudd, Detailed Structural Analysis of N-Glycans Released From Glycoproteins in SDS-PAGE Gel Bands Using HPLC Combined With Exoglycosidase Array Digestions, in: Glycobiol. Protoc., Humana Press, New Jersey, 2006: pp. 125–144. doi:10.1385/1-59745-167-3:125.

[27] M.P. Campbell, L. Royle, C.M. Radcliffe, R.A. Dwek, P.M. Rudd, GlycoBase and autoGU: tools for HPLC-based glycan analysis, Bioinformatics. 24 (2008) 1214–1216. doi:10.1093/bioinformatics/btn090.

[28] M. Balbín, A. Grubb, G.G. de Lange, R. Grubb, DNA sequences specific for Caucasian G3m(b) and (g) allotypes: allotyping at the genomic level., Immunogenetics. 39 (1994) 187–93. http://www.ncbi.nlm.nih.gov/pubmed/8276465 (accessed July 1, 2016).

[29] J.E. Huffman, M. Pučić-Baković, L. Klarić, R. Hennig, M.H.J. Selman, F. Vučković, M. Novokmet, J. Krištić, M. Borowiak, T. Muth, O. Polašek, G. Razdorov, O. Gornik, R. Plomp, E. Theodoratou, A.F. Wright, I. Rudan, C. Hayward, H. Campbell, A.M. Deelder, U. Reichl, Y.S. Aulchenko, E. Rapp, M. Wuhrer, G. Lauc, Comparative performance of four methods for high-throughput glycosylation analysis of immunoglobulin G in genetic and epidemiological research., Mol. Cell. Proteomics. 13 (2014) 1598–610. doi:10.1074/mcp.M113.037465.

[30] R. Malhotra, M.R. Wormald, P.M. Rudd, P.B. Fischer, R.A. Dwek, R.B. Sim, Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein., Nat. Med. 1 (1995) 237–43. http://www.ncbi.nlm.nih.gov/pubmed/7585040 (accessed February 9, 2016).

[31] C.M. Karsten, M.K. Pandey, J. Figge, R. Kilchenstein, P.R. Taylor, M. Rosas, J.U. McDonald, S.J. Orr, M. Berger, D. Petzold, V. Blanchard, A. Winkler, C. Hess, D.M. Reid, I. V Majoul, R.T. Strait, N.L. Harris, G. Köhl, E. Wex, R. Ludwig, D. Zillikens, F. Nimmerjahn, F.D. Finkelman, G.D. Brown, M. Ehlers, J. Köhl, Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIB and dectin-1., Nat. Med. 18 (2012) 1401–6. doi:10.1038/nm.2862.

[32] S. Mihai, F. Nimmerjahn, The role of Fc receptors and complement in autoimmunity., Autoimmun. Rev. 12 (2013) 657–60. doi:10.1016/j.autrev.2012.10.008.

[33] J. Krištić, F. Vučković, C. Menni, L. Klarić, T. Keser, I. Bečeheli, M. Pučić-Baković, M. Novokmet, M. Mangino, K. Thaqi, P. Rudan, N. Novokmet, J. Šarac, S. Missoni, I. Kolčić, O. Polašek, I. Rudan, H. Campbell, C. Hayward, Y. Aulchenko, A. Valdes, J.F. Wilson, O. Gornik, D. Primorac, V. Zoldoš, T. Spector, G. Lauc, Glycans are a novel biomarker of chronological and biological ages, Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 69 (2014) 779–789. doi:10.1093/gerona/glt190.

[34] F. Vučković, J. Krištić, I. Gudelj, M. Teruel, T. Keser, M. Pezer, M. Pučić-Baković, J. Štambuk, I. Trbojević-Akmačić, C. Barrios, T. Pavić, C. Menni, Y. Wang, Y. Zhou, L. Cui, H. Song, Q. Zeng, X. Guo, B.A. Pons-Estel, P. McKeigue, A. Leslie Patrick, O. Gornik, T.D. Spector, M. Harjaček, M. Alarcon-Riquelme, M. Molokhia, W. Wang, G. Lauc, Association of systemic lupus erythematosus with decreased immunosuppressive potential of the IgG glycome., Arthritis Rheumatol. (Hoboken, N.J.). 67 (2015) 2978–89. doi:10.1002/art.39273.

[35] I. Trbojević Akmačić, N.T. Ventham, E. Theodoratou, F. Vučković, N.A. Kennedy, J. Krištić, E.R. Nimmo, R. Kalla, H. Drummond, J. Štambuk, M.G. Dunlop, M. Novokmet, Y. Aulchenko, O. Gornik, H. Campbell, M. Pučić Baković, J. Satsangi, G. Lauc, Inflammatory bowel disease associates with proinflammatory potential of the immunoglobulin G glycome., Inflamm. Bowel Dis. 21 (2015) 1237–47. doi:10.1097/MIB.0000000000000372.

[36] E. Theodoratou, K. Thaçi, F. Agakov, M.N. Timofeeva, J. Štambuk, M. Pučić-Baković, F. Vučković, P. Orchard, A. Agakova, F.V.N. Din, E. Brown, P.M. Rudd, S.M. Farrington, M.G. Dunlop, H. Campbell, G. Lauc, Glycosylation of plasma IgG in colorectal cancer prognosis., Sci. Rep. 6 (2016) 28098. doi:10.1038/srep28098.

[37] I. Quast, C.W. Keller, M.A. Maurer, J.P. Giddens, B. Tackenberg, L.-X. Wang, C. Münz, F. Nimmerjahn, M.C. Dalakas, J.D. Lünemann, Sialylation of IgG Fc domain impairs complement-dependent cytotoxicity., J. Clin. Invest. 125 (2015) 4160–70. doi:10.1172/JCI82695.

[38] I.K. Campbell, S. Miescher, D.R. Branch, P.J. Mott, A.H. Lazarus, D. Han, E. Maraskovsky, A.W. Zuercher, A. Neschadim, D. Leontyev, B.S. McKenzie, F. Käsermann, Therapeutic effect of IVIG on inflammatory arthritis in mice is dependent on the Fc portion and independent of sialylation or basophils., J. Immunol. 192 (2014) 5031–8. doi:10.4049/jimmunol.1301611.

[39] A.C. Issekutz, D. Rowter, S. Miescher, F. Käsermann, Intravenous IgG (IVIG) and subcutaneous IgG (SCIG) preparations have comparable inhibitory effect on T cell activation, which is not dependent on IgG sialylation, monocytes or B cells., Clin. Immunol. 160 (2015) 123–32. doi:10.1016/j.clim.2015.05.003.

[40] K. Mariño, R. Saldova, B. Adamczyk, P.M. Rudd, Changes in Serum N-Glycosylation Profiles: Functional Significance and Potential for Diagnostics, in: A. Pilar Rauter (Ed.), Carbohydr. Chem., 37th ed., The Royal Society of Chemistry, Cambridge, UK, 2012: pp. 57–93. doi:10.1039/9781849732765-00057.

[41] K.W. Moremen, M. Tiemeyer, A. V Nairn, Vertebrate protein glycosylation: diversity, synthesis and function., Nat. Rev. Mol. Cell Biol. 13 (2012) 448–62. doi:10.1038/nrm3383.

[42] F. Dall'Olio, N. Malagolini, M. Trinchera, M. Chiricolo, Mechanisms of cancer-associated glycosylation changes., Front. Biosci. (Landmark Ed. 17 (2012) 670–99. http://www.ncbi.nlm.nih.gov/pubmed/22201768 (accessed January 20, 2017).

[43] K.J. Yarema, C.R. Bertozzi, Characterizing glycosylation pathways., Genome Biol. 2 (2001) REVIEWS0004. http://www.ncbi.nlm.nih.gov/pubmed/11387039 (accessed January 20, 2017).

[44] C. Menni, T. Keser, M. Mangino, J.T. Bell, I. Erte, I. Akmačić, F. Vučković, M. Pučić Baković, O. Gornik, M.I. McCarthy, V. Zoldoš, T.D. Spector, G. Lauc, A.M. Valdes, Glycosylation of immunoglobulin G: Role of genetic and epigenetic influences, PLoS One. 8 (2013).

Figure legends

Figure 1. Chromatogram of 2-AB labeled N-linked glycans released from IgG and separated by HILIC-UPLC. The integration areas, together with glycan structures present in each peak are given. Peaks are numbered from GP1-GP24.

Supplementary files

Supplementary Figure 1. The differences in derived IgG glycan traits (for each IgG subclass) between the placebo (red, 0) and the rosuvastatin (blue, 1) group. For each group there are two time points - before (left) and after (right) the placebo/rosuvastatin treatment.

Supplementary Figure 2. Chromatogram of 2-AB labeled N-linked glycans released from total plasma proteins and separated by HILIC-UPLC. The integration areas, together with glycan structures present in each peak are given. Peaks are numbered from PGP1-PGP46.

Supplementary Figure 3. . The differences in total plasma N-glycome (glycan peaks PGP1-46 obtained by HILIC-UPLC) between the placebo (red, 0) and the rosuvastatin (blue, 1) group. For each group there are two time points - before (left) and after (right) the placebo/rosuvastatin treatment.

Supplementary Table 1. Description of IgG glycan traits (directly measured and derived traits) measured by HILIC-UPLC and LC-ESI-MS (with mass list).

Supplementary Table 2. Association analysis between individual statin use (for simvastatin and atorvastatin) and IgG glycan traits in the TwinsUK cohort . P values and regression coefficients (beta) were obtained from linear mixed regressions analysis adjusting for age, sex, BMI and family relatedness as random effect.

Supplementary Table 3. P values for JUPITER study IgG glycans obtained from Mann Whitney U test. For each glycan trait for each IgG subclass (IgG1, IgG2/3, IgG4) differences were calculated between glycan values after and before the placebo/rosuvastatin treatment and then the placebo and the rosuvastatin groups were compared.

Supplementary Table 4. P values for JUPITER study total plasma N-glycans obtained from Mann Whitney U test. For each glycan trait differences were calculated between glycan values after and before the placebo/rosuvastatin treatment and then the placebo and the rosuvastatin groups were compared.

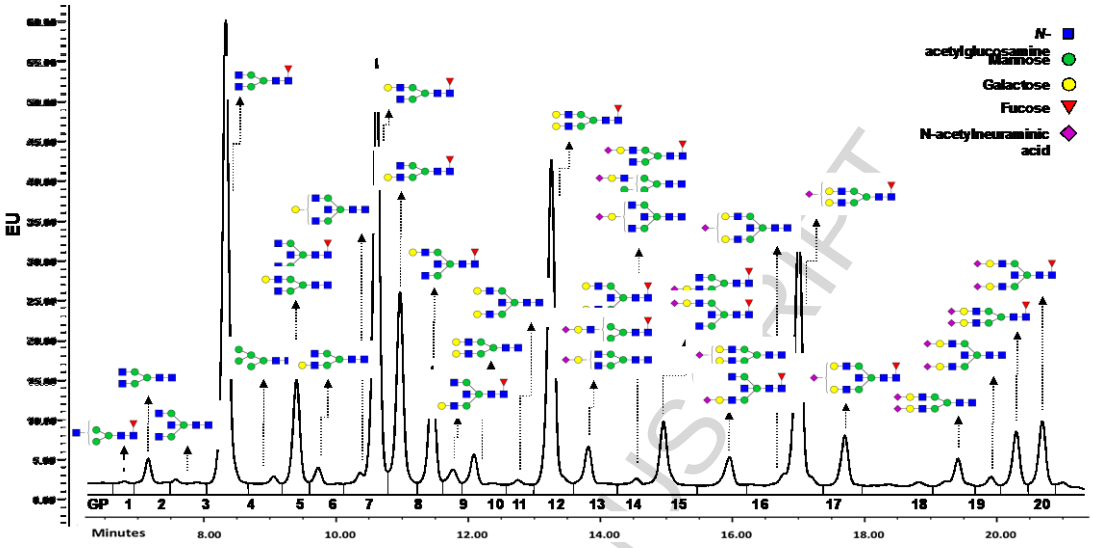


Figure 1

Highlights

- In the first cohort 3 glycan traits were significantly associated with statin use
- 2 of them were replicated in the second cohort
- In a rosuvastatin clinical trial no significant differences were found
- Statin use does not pose a problem for glycomic studies