GLYCOSILATION PROFILE OF IMMUNOGLOBULIN G IN EARLY CHRONIC KIDNEY DISEASE

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Introduction and objectives

Epigenetic or post-transcriptional factors may be playing an important role in renal damage. Glycosylation is the most abundant and diverse form of post-transcriptional modification which participates in every physiological process.

Alternative immunoglobulin G (IgG) glycosylation acts as a switch between pro- and anti-inflammatory IgG functionality. Malfunction of this system is associated with different inflammatory and autoimmune diseases (*SLE, RA, Cancer, inflammatory bowel disease*).

To date no human studies investigated the role of the IgG glycosylation profiles on the onset of CKD.

Since the activation of inflammatory pathways and subsequent fibrosis are hallmark of renal injury, different IgG glycosylation profiles may provide an at risk phenotype to the developing of renal damage.

The aim of this study is to investigate the potential role of IgG glycosylation in kidney function, by analysing IgG glycome composition in a large population based cohort from the UK. As glycans are associated with many factors including genes, we validate our significant results in an independent population of identical twins discordant for renal diseases

Methods

-Study subjects were twins enrolled in the **TwinsUK registry**

-Estimate glomerular filtration rate (eGFR): Standard creatinine using the **CKD-EPI equation.** CKD was defined as eGFR <60 mL/min/1.73m^2. **Monozygotic** (MZ) pairs were considered **discordant** for renal function if one twin had eGFR≥90 and the other eGFR ≤90 mL/min/1.73m^2 and difference between eGFR was greater than 15mL/min/1.73m^2.

-Association analyses; random intercept **linear regressions** adjusting for age, sex, BMI, diabetes, hypertension and family relatedness. **Bonferroni** correction for multiple testing (76 glycans profiles) gave a significant threshold of (P<6.5x10^{-4}=0.05/76).

-UPLC analysis of the IgG glycome.

Figure (right) depicts an example of a UPLC chromatogram with graphical representation of glycan structures present in each chromatography peak (GP1–GP24).

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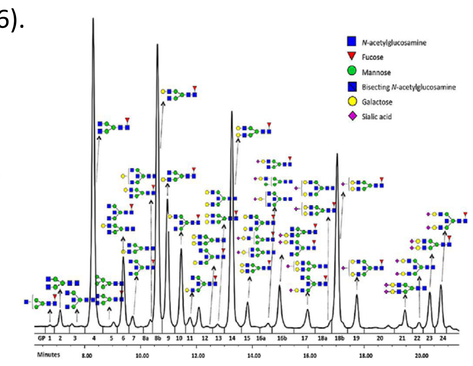

Results

Table 1: General characteristics of the study population

	Discovery Population	MZ Discordant Twins
Sample size,*n*	3212	62
Age,*yrs*	52.67±14.15	55.45 ± 12.2
MZ:DZ:singletons	506:1772:934	62:0:0
Female, *n (%)*	3050(94.9)	60(96.7)
BMI,*Kg/m^2*	25.95±4.65	25.64±5.65
Creatinine,*mg/mL*	0.83±0.15	0.75±0.10
eGFR, *mL/min/1.73m^2*	84.15±17.02	88.52±9.91
CKD (eGFR ≤ 60), *n (%)*	294(9.15)	1(1.6)
Type2 Diabetes, *n (%)*	72 (2.2)	4(6.4)
Hypertension,*n (%)*	705(21.9)	18(29.0)

Table 2: Glycan traits significantly associated with eGFR in the discovery, validation and meta-analysis.

		Discovery		MZ Discordant	Fixed effect meta-analysis	
Glycan	Description	ß[95%CI]	p	ß[95%CI]	ß[95%CI]	p
GP18	The percentage of FA2G2S1 glycan in total IgG glycans	1.48 [0.89;2.07]	8.60 x 10^{-7}	0.59 [-2.23;3.41]	4.23 [2.38;7.52]	9.51 x10^{-7}
GP14	The percentage of FA2G2 glycan in total IgG glycans	1.46 [0.85;2.07]	2.92 x10^{-6}	1.33 [-1.81;4.48]	4.29 [2.35;7.81]	2.04 x10^{-6}
GP6^n	The percentage of FA2B glycan in total neutral IgG glycans (GP^n)	-1.39 [-1.98;-0.80]	3.56 x10^{-6}	-0.84 [-3.44;1.76]	0.26 [0.14;0.45]	3.16 x10^{-6}
GP14^n	The percentage of FA2G2 glycan in total neutral IgG glycans (GP^n)	1.29 [0.68;1.90]	3.06 x10^{-5}	1.99 [-1.70;5.67]	3.70 [2.03;6.73]	1.82 x10^{-5}
FBS1/FS1	Ratio of fucosylatedmonosialylated structures with and without bisecting GlcNAc	-1.12 [-1.65;-0.59]	3.48 x10^{-5}	-0.58 [-3.16;1.99]	0.33 [0.20;0.56]	3.42 x10^{-5}
FBS1/(FS1+FBS1)	The incidence of bisecting GlcNAc in all fucosylatedmonosialylated structures in total IgG glycans	-1.10 [-1.63;-0.57]	4.63 x10^{-5}	-0.60 [-3.14;1.95]	0.34 [0.20;0.57]	4.46 x10^{-5}
G2^n	The percentage of digalactosylated structures in total neutral IgG glycans	1.20 [0.60;1.80]	8.81 x10^{-5}	1.98 [-1.83;5.78]	3.38 [1.87;6.10]	5.53 x10^{-5}
GP6	The percentage of FA2B glycan in total IgG glycans	-1.14 [-1.71;-0.57]	8.90x10^{-5}	-1.01 [-3.78;1.76]	0.32 [0.18;0.56]	6.84 x10^{-5}
FBS^{total}/FS^{total}	Ratio of all fucosylatedsialylated structures with and without bisecting GlcNAc	-1.07 [-1.60;-0.54]	8.21 x10^{-5}	-0.30 [-2.84;2.23]	0.36 [0.21;0.60]	9.52 x10^{-5}
G0^n	The percentage of agalactosylated structures in total neutral IgG glycans	-1.16 [-1.76;-0.56]	1.52 x10^{-4}	-1.20 [-4.71;2.31]	0.31 [0.17;0.57]	1.20 x10^{-4}
GP2^n	The percentage of A2 glycan in total neutral IgG glycans (GP^n)	-0.91 [-1.42;-0.40]	5.02 x10^{-4}	-2.00 [-4.66;0.67]	0.39 [0.23;0.64]	2.20 x10^{-4}
GP2	The percentage of A2 glycan in total IgG glycans	-0.90 [-1.42;-0.38]	6.28 x10^{-4}	-2.33 [-5.13;0.47]	0.39 [0.23;0.64]	2.55 x10^{-4}
FGS/(F+FG+FGS)	The percentage of sialylation of all fucosylated structures without bisecting GlcNAc in total IgG glycans	1.01 [0.46;1.56]	2.96 x10^{-4}	0.57 [-2.21;3.35]	2.71 [1.58;4.64]	2.85 x10^{-4}
FG2^n/(BG2^n + FBG2^n)	Ratio of fucosylated digalactosylated non-bisecting GlcNAc structures and all digalactosylated structures with bisecting GlcNAc	0.91 [0.38;1.44]	7.32 x10^{-4}	0.93 [-1.59;3.44]	2.49 [1.48;4.19]	5.54 x10^{-4}

Table 2: The linear regression in the discovery population identified 14 glycans significantly associated with eGFR; 6 glycans positively associated while 8 were negatively associated. The regression coefficients were in the same direction in an independent groups of MZ twins discordant for renal disease. The 14 significant glycan traits fall into three particular glycosylation features: galactosylation, sialylation and the level of bisecting N-acetylglucosamine (GlcNAc) and Core fucosylation of the IgG

Figure 1: Correlation of IgG glycosylation and eGFR in the discovery and MZ discordant populations

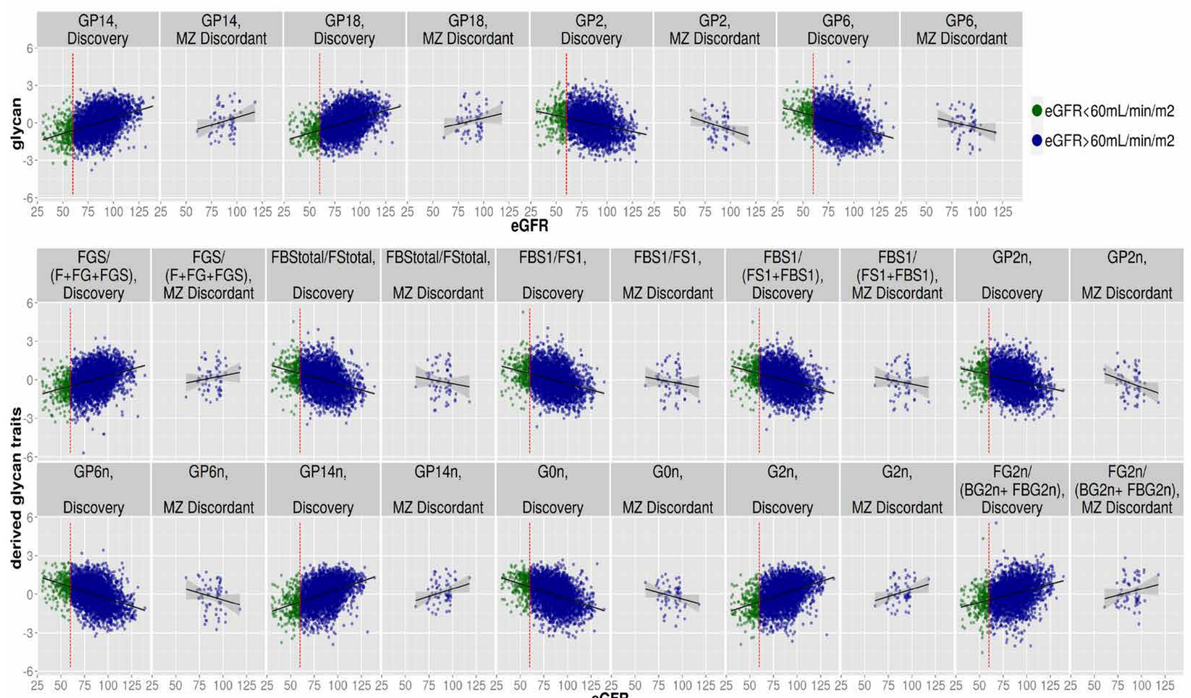

Figure 2: IgG glycans profile in 8 pair twins discordant for renal function. **Extreme phenotype**

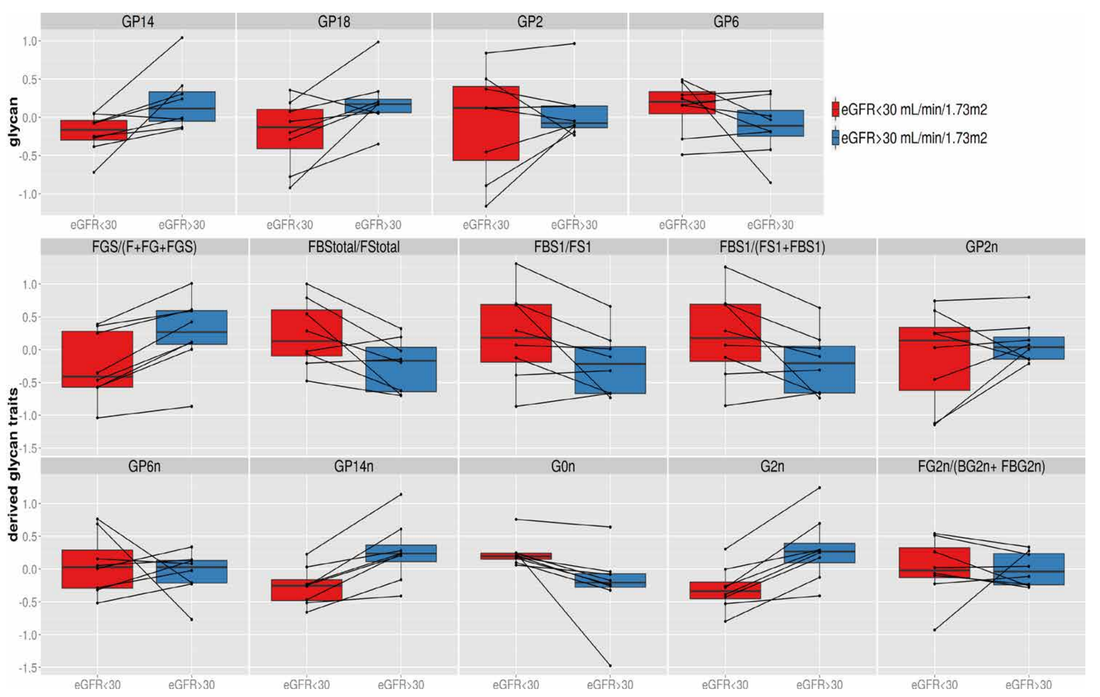

Table and figure 2:

Subjects with agalactosylated glycans (GP2, GP2^n, GP6 and G0^n) had higher risk of CKD and lower with galactosylated IgG (GP14 and G2^n). Lack of terminal galactose activates complement cascade and makes IgG pro-inflammatory, while the addition of galactose decreases its inflammatory potential.

For sialylation, the major sialylated glycan, (GP18) and the percentage of sialylated structures without bisecting *N*-GlcNAc represented by ratio FGS/(F+FG+FGS), increased with eGFR. These sialylated glycan traits displayed a protective independent risk for CKD.

The level of bisecting GlcNAc in sialylated IgG glycans represented by three ratios, FBS^{total}/FS^{total}, FBS1/FS1 and FBS1/(FS1+FBS1, as well as in digalactosylated neutral gG glycans (FG2^n/(BG2^n+FBG2^n)) were found to be inversely associated with eGFR.

We observed a decreased risk of CKD when sialylated and core fucosylated glycans did not have bisecting GlcNAc; and contrary lower eGFR if those glycans contained bisecting GlcNAc (FBS^{total}/FS^{total}, FBS1/FS1 and FBS1/(FS1+FBS1)). The presence of bisecting GlcNAc was always associated with higher risk of CKD. Core fucosylation modulates the antibody-dependent cellular cytotoxicity (ADCC). IgG-containing glycans that lack core fucose have 100-fold higher affinity to the FcγRIIIa and are therefore much more efficient than fucosylated glycoforms.

Figure 3: Associations in an independent population with more severe renal phenotype. IgG glycans profiles followed the same pattern than the discovery population with the worsening of the renal function.

Conclusions

Our results highlight the promising role of glycomics in renal studies. Uncovering this relationship by extending the research with clinical subsets and longitudinal data would help to identify further novel markers potentially useful to detect at risk patients, at early stages of CKD. These results open new avenues to our understanding of renal damage and encourage further studies in populations with more severe CKD, as well as studies comparing patients with autoimmune CKD with patients with due to other aetiologies. Moreover, this would help to identify, potential therapeutic targets.

Supportive bibliographic references: *Moayyeri, A et al . TwinsUK cohort. International journal of epidemiology. 2013. Gornik, O et al. Biochimica et biophysica acta 2012*

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