Heliyon 10 (2024) e30529

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

N-glycosylation of immunoglobulin A in children and adults with type 1 diabetes mellitus

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ARTICLE INFO

Keywords:
Type 1 diabetes mellitus
Type 1 diabetes mellitus onset
Immunoglobulin a
N-glycosylation
IgA N-Glycosylation

ABSTRACT

Aims: To identify N-glycan structures on immunoglobulin A related to type 1 diabetes mellitus among children at the disease onset and adults with type 1 diabetes mellitus.
Methods: Human polyclonal IgA N-glycans were profiled using hydrophilic interaction ultra performance liquid chromatography in two cohorts. The first cohort consisted of 62 children at the onset of type 1 diabetes mellitus and 86 of their healthy siblings. The second cohort contained 84 adults with the disease and 84 controls. Associations between N-glycans and type 1 diabetes mellitus were tested using linear mixed model for the paediatric cohort, or general linear model for the adult cohort. False discovery rate was controlled by Benjamini-Hochberg method modified by Li and Ji.
Results: In children, an increase in a single oligomannose N-glycan was associated with type 1 diabetes mellitus ($B = 0.529$, $p = 0.0067$). N-glycome of the adults displayed increased branching ($B = 0.466$, $p = 0.0052$), trigalactosylation ($B = 0.466$, $p = 0.0052$), trisialylation ($B = 0.629$, $p < 0.001$), and mannosylation ($B = 0.604$, $p < 0.001$). The strongest association with the disease was a decrease in immunoglobulin A core fucosylation ($B = -0.900$, $p < 0.001$).
Conclusions: Changes in immunoglobulin N-glycosylation patterns in type 1 diabetes point to disruptions in immunoglobulin A catabolism and dysregulated inflammatory capabilities of the antibody, potentially impacting immune responses and inflammation.

Abbreviations: T1DM, type 1 diabetes mellitus; FcalphaR, Fcalpha receptor; MBL, mannose binding lectin; ACN, acetonitrile; HILIC, hydrophilic interaction liquid chromatography; UPLC, ultra performance liquid chromatography; GP, glycan peak; LB, low branching; HB, high branching; G0, agalactosylation; G1, monogalactosylation; G2, digalactosylation; G3, trigalactosylation; S1, monosialylation; S2, disialylation; S3, trisialylation; B, bisecting GlcNAc; CF, core fucosylation; HM, high mannosylation.

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https://doi.org/10.1016/j.heliyon.2024.e30529
Received 15 April 2024; Accepted 29 April 2024
Available online 3 May 2024

1. Introduction

Type 1 diabetes mellitus (T1DM) is a chronic autoimmune condition that primarily leads to the destruction of insulin-producing beta cells within the pancreas. This damage results in persisting hyperglycaemia due to low insulin levels, a characteristic feature of diabetes [1]. T1DM is often thought of as a disease arising during childhood, as it frequently manifests during the early years of life. However, the incidence is not exclusive to children, and adult-onset is also regularly reported, widening its scope and impact across the lifespan [2]. According to the International Diabetes Federation report for 2022 as many as 62 % of cases of newly diagnosed T1DM were people older than 20 years of age [3]. Although the incidence of T1DM is increasing worldwide, the exact causes of the disease still remain unclear [4]. Growing efforts to discover the cause, improve diagnosis and treatment options, but also reduce the risk of possible complications have led to the recognition of the potential of N-glycans in the field of diabetology.

N-glycosylation is a vital co- and post-translational protein modification with oligosaccharides, which has a significant impact on the structure and function of proteins and is crucial in processes such as protein folding, signal transduction, development, neurogenesis and immunity [5]. Due to its importance, N-glycosylation is strictly regulated by numerous enzymes and transcription factors [6]. This process is highly responsive to various cellular and environmental cues, reflecting the physiological state of the organism and its alterations over time [7]. Given its involvement in a range of physiological and pathological processes, glycosylation serves as a valuable indicator of an individual's current health status and potential risks for future diseases [8]. Increasing evidence indicates that protein N-glycosylation is integral to the regulation and maintenance of glucose homeostasis [9] and is intricately linked to nutrient sensing through the hexosamine biosynthetic pathway [10].

Previous research has shown that changes in protein glycosylation play a role in various pathological conditions, including autoimmune diseases [8], and among others are associated with T1DM [11–13]. A growing number of studies have revealed that N-glycosylation of various plasma proteins can change in children and adults with T1DM and that this change is related to the development of the disease itself, glycaemic regulation or the development of vascular complications [11–13]. It has also been demonstrated that the patterns of N-glycosylation of specific glycoproteins can be used to differentiate between types of diabetes [14].

One of the main components of the immune system, which is heavily glycosylated, but also understudies in the context of T1DM, is immunoglobulin A (IgA). IgA is the second most abundant antibody of human plasma that plays a key role in the defence of mucosal surfaces [15]. As an effector molecule of the immune system, it is involved in the initiation of pro-inflammatory as well as anti-inflammatory responses through interaction with Fcalpha receptor (FcalphaR), which is why this antibody and its disorders are implicated in various autoimmune diseases [16]. It is hypothesized that one of the main factors influencing these dual roles of IgA is its glycosylation, which in general is fundamental to the structure and function of immunoglobulins [17].

While the N-glycosylation of IgG has been well-studied over the past decade, the influence of different glycosylation sites on IgA's effector functions remains largely underexplored. For example, some of the few known functions are the role of oligomannose IgA glycans in the activation of the complement system via the mannan-binding lectin (MBL) pathway [18] or IgA clearance which is influenced by the presence of asialylated glycans [19]. Additionally, glycans might also affect IgA's activatory and immunosuppressive functions, which are also dependent on the subclass of Ig [20].

In the context of T1DM, previous studies on IgA in individuals with T1DM have shed light on the immune dynamics of the disease. A major focus in T1DM research has been the prevalence of selective IgA deficiency, which is significantly higher in T1DM patients (both children and adults) compared to the general population [21,22]. Recent research has also revealed disruptions in both systemic and intestinal IgA immune responses in T1DM individuals, indicating a systemic change in mucosal immunity [23]. This indicates the significant role of IgA in T1DM and emphasizes its importance in understanding and managing the disease. Additionally, research in T1DM has thoroughly explored immunoglobulin concentrations, including IgA, at various disease stages [21,24–26] and has reported altered IgA levels in saliva and serum. Children at the disease onset and adults with T1DM tend to exhibit elevated levels of serum IgA compared to those without the disease. These findings indicate aberrations in IgA glycoprotein metabolism and further research is needed to understand the mechanisms and clinical implications of these variations in T1DM patients. Abnormal concentrations of other immunoglobulins, including IgM, IgE, and IgG, have also been noted in T1DM patients, suggesting a broader dysregulation of immunoglobulin profiles at the disease onset. This complexity underscores the need for further research on immunoglobulins to enhance our understanding of the immune processes in autoimmune T1DM.

N-glycosylation of serum IgA has so far been poorly investigated in the context of diseases and inflammatory conditions. Given these insights, our study aimed to identify the changes in IgA N-glycans that are characteristic of the recent onset childhood T1DM and compare those to the ones identified in adults with T1DM. First, we hypothesized that IgA N-glycosylation differs between children newly diagnosed with T1DM and their healthy siblings, which could potentially contribute to T1DM risk assessment and understanding of the disease development. Second, we hypothesized that IgA N-glycosylation differs between adults affected with T1DM and healthy controls. Our approach involved comparing children diagnosed with T1DM and their healthy siblings, as well as adults with T1DM to their healthy controls, to identify T1DM-specific differences in IgA glycosylation patterns characteristic of various disease stages. To our knowledge, this is the first study of IgA N-glycosylation changes at the onset of T1DM and in adults.

2. Subjects

A total of 62 blood plasma samples of children and adolescents (median age 10 years, age range 1–16 years) with T1DM were collected through DanDiabKids registry within three months of diagnosis [27] and 86 samples of their healthy siblings (median age 11 years, age range 3–22 years) were used for the analysis of N-glycosylation of IgA. Blood plasma samples were taken from each subject

and clinical data (age, gender) was recorded. Since 1996, the registry in Denmark includes and monitors children newly diagnosed with type 1 diabetes before the age of 15, and from 2006 onwards, the registry includes children diagnosed with T1DM up to the age of 18 and is continuously synchronized with the Danish National Patient Register to verify the inclusion of all newly diagnosed patients [27]. For some affected children, multiple healthy siblings were included in the study (from 1 to 4 per affected person), but for most cases it was available a sample of only one healthy relative. The year of sampling ranged from 1997 to 2000, and the last data extraction and the disease status check was carried out in January 2019. Data on glycaemic control was unavailable for these subjects. After acquisition, the samples were stored at −20 °C.

The adult study cohort consisted of 84 individuals with T1DM recruited at Vuk Vrhovac Clinic for Diabetes, Endocrinology and Metabolic Diseases, Merkur University Hospital, Zagreb, Croatia and 84 matching healthy volunteers. All the participants were aged between 30 and 60 years (Table 1). Patient inclusion criteria were: T1DM diagnosed up to 35 years of age, positive relevant autoantibody status, active treatment with insulin for at least one year prior to sampling, with or without microvascular complications (complication status being stationary for at least 3 months before recruitment). The information on adult subjects included duration of the disease and their glycaemic control (HbA1c). Samples were acquired between year 2015 and 2017 and were stored at −20 °C.

All subjects or their legal guardians signed informed consent, whereafter ethical approvals from the local ethics committees were obtained. The study was conducted in accordance with the Declaration of Helsinki.

3. Materials and methods

3.1. Immunoglobulin A isolation from human plasma and serum

Isolation of IgA was performed as described previously [28]. In short, the steps included capturing human polyclonal IgA from either 40 μL of human plasma (children's samples) or human serum (adults' samples). This was achieved through affinity chromatography using the CaptureSelect™ IgA affinity matrix (Thermo Fischer Scientific, Massachusetts, USA). The IgA affinity matrix bead slurry (25 μL) was added to each well of a 96-well 0.7 μm Orochem filter plate (Orochem Technologies Inc., Illinois, USA), which was mounted on a vacuum manifold. Equilibration of the beads was achieved by washing the beads with water and four times with PBS. The samples (40 μL) were diluted sevenfold with PBS and added to the Orochem plate containing the equilibrated beads and incubated for 15 min on a plate shaker. After washing three times with PBS, the flowthrough was collected using a vacuum manifold and reapplied to the beads for another 15-min incubation to increase the isolation efficacy. Finally, the IgA was eluted using 200 μL of 0.1 M formic acid and neutralized with 34 μL of 1 M ammonium bicarbonate. Hundred μL of IgA eluate was aliquoted and dried using vacuum centrifugation for further analytical procedures.

3.2. N-glycan release from immunoglobulin A

Dried IgA samples were resuspended and denatured by the addition of 30 μl of 1.33 % (w/v) SDS (Sigma-Aldrich, St. Louis, MO, USA), incubated at 65 °C for 10 min. Ten μl of 4 % (v/v) Igepal-CA630 (Sigma-Aldrich, USA) were added to each sample, followed by a 15 min incubation on a plate shaker (GFL, Lauda-Königshofen, Germany) at room temperature. N-glycan release was achieved by the addition of 1.2 U of PNGase F (Promega, Madison, WI, USA) and overnight incubation at 37 °C.

3.3. Fluorescent labelling of released N-glycans and HILIC-SPE clean-up

The labelling solution was prepared by dissolving procainamide (38.3 mg/ml, Sigma-Aldrich, USA) and 2-picoline borane (44.8 mg/ml, Sigma-Aldrich, USA) in a mixture (70:30 v/v) of dimethyl sulfoxide (Sigma-Aldrich, USA) and glacial acetic acid (Sigma-Aldrich, USA). After the addition of the labelling mixture (25 μl), the samples were incubated at 65 °C for 2 h. Next, residual protein, enzyme, unbound dye, and the reducing agent were eliminated from the mixture utilizing hydrophilic interaction liquid chromatography solid-phase extraction (HILIC-SPE). Briefly, the 96-well 0.2 μm wwPTFE (Pall Corporation, New York, USA) filter plate was mounted on a vacuum manifold (Pall Corporation, USA), prewashed with 70 % ethanol (J.T. Baker, Phillipsburg, NJ, USA) and ultrapure water (Merck KGaA, Darmstadt, Germany), followed by equilibration with 96 % acetonitrile (ACN). The samples were brought to 96 % of ACN with 700 μl of ACN (VWR International, Radnor, PA, USA), and applied to the 0.2 μm wwPTFE (Pall Corporation, USA) filter plate. The wells were subsequently washed five times with 96 % ACN and labelled N-glycans were eluted with a total of 100 μl of

Table 1
Description of the cohorts used in the study.

Cohort	Subjects	Number of participants (m/f)	Median age (range)	Median T1DM duration (range)[a]	Median HbA1c (range)[a]
Recent T1DM onset in children	**Children with T1DM**	62 (34 m/28 f)	10 (1–16) years	≤3 months	*NA*
	Healthy siblings	86 (45 m/41 f)	11 (3–22) years	*NA*	*NA*
Adults with T1DM	**Adults with T1DM**	84 (42 m/42 f)	45 (30–60) years	16 (1–41) years	7.2 (4.7–13.4)
	Healthy volunteers	84 (42 m/42 f)	45 (30–60) years	*NA*	*NA*

[a] *NA* – not applicable.

ultra-pure water and stored at - 20 °C until further use.

3.4. Separation of N-glycans by hydrophilic interaction liquid chromatography

Fluorescently labelled N-glycans were separated by an Acquity UPLC (ultra performance liquid chromatography) H-Class instrument (Waters, Milford, USA) composed of a quaternary solvent manager, sample manager and a fluorescence detector unit with excitation and emission wavelengths set to 310 nm and 370 nm, respectively. The instrument was managed by Empower 3 software, build 3471 (Waters, Milford, USA). The separation was based on hydrophilic interaction liquid chromatography and was performed on glycan BEH amide 150 mm column (Waters, USA), with 100 mM ammonium formate (pH 4.4) and ACN (VWR International, USA) as solvents A and B, respectively. A linear gradient of 72%–59 % acetonitrile at flow rate 0.561 ml/min was applied in a 35 min analytical run.

The chromatography system was calibrated with an external standard of hydrolysed, procainamide labelled glucose oligomers from which the retention times of the individual glycans were converted to glucose units. These chromatograms were then compared and aligned to those previously identified [28] for structural assignment.

Data processing was performed manually, and each chromatogram was divided into 30 glycan peaks (GP1-GP30, Fig. 1). Each glycan peak was quantified as a percentage of the total integrated area. N-glycan structures pertaining to each GP are described in Supplementary Table A1. In addition to directly measured glycan traits, 12 derived traits that represent glycans with a similar structural feature, such as galactosylation, fucosylation, bisecting GlcNAc, and sialylation, were also calculated for IgA N-glycans (Supplementary Table A2).

3.5. Statistical analysis

Samples gathered from adults and children were analysed as two separate data sets. For each sample's chromatogram, areas under

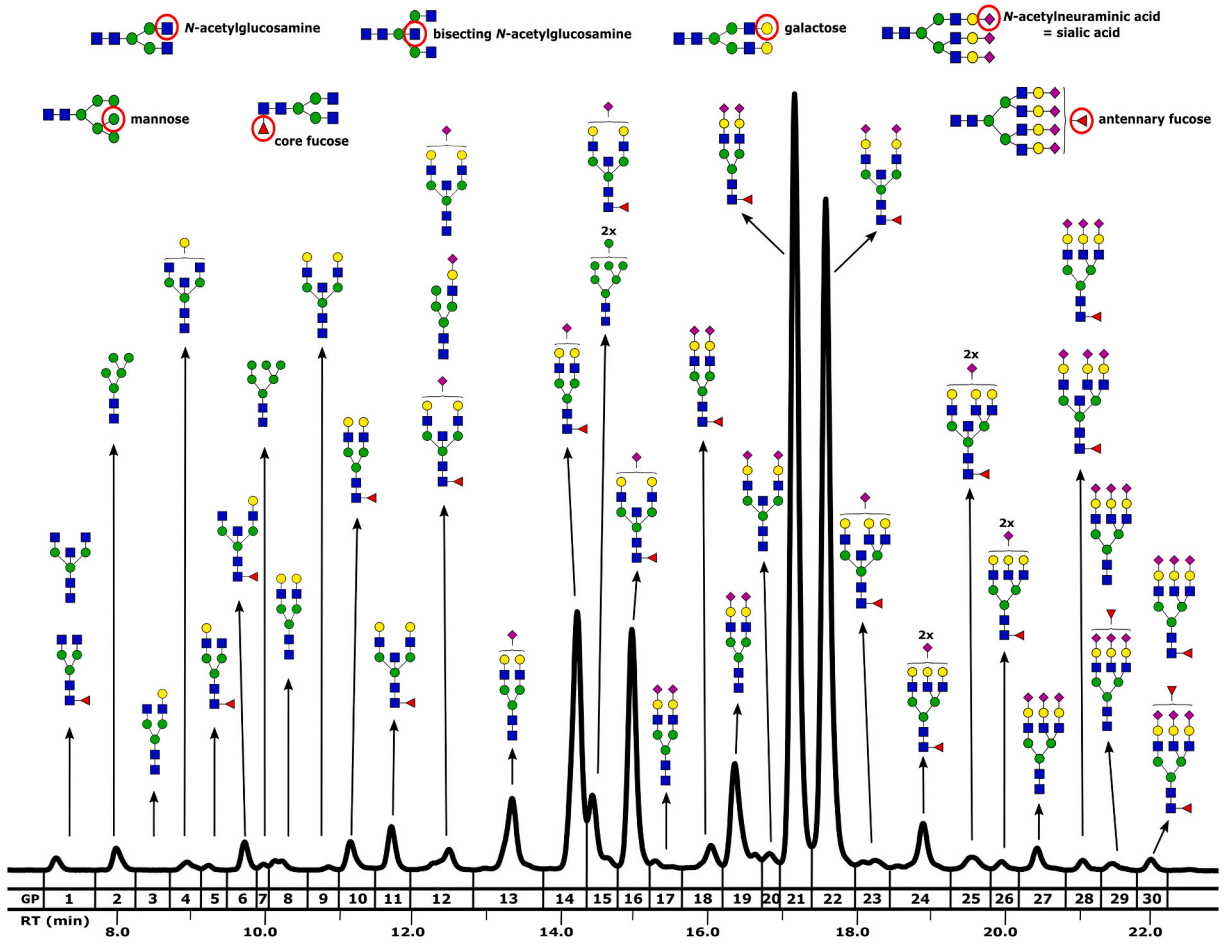

Fig. 1. HILIC-UPLC profile of the N-glycan structures released from the serum IgA of adult healthy control group. Only the most abundant glycoforms are shown for each glycan peak. GP: glycan peak; RT: retention time.

glycan peaks were normalized by the total chromatogram area. Obtained normalized areas were logit transformed and then batch effects were corrected using the ComBat method (R package sva). Batch-corrected normalized areas were back-transformed to the original scale and used for the calculation of the derived glycan traits.

Differences in glycan abundance in type 1 diabetes adult patients and healthy adults were analysed using the general linear model: the normalized area was modelled as a dependent variable while T1DM status, sex and age were modelled as independent variables. Effects of T1DM on glycan abundances in children were analysed with the mixed model (R package lme4) with the same dependent and independent variables, and family identifier modelled as a random intercept. Due to numerous models fitted and to control for the false discovery rate, estimated p values were adjusted using the Benjamini-Hochberg method modified by Li and Ji.

All statistical analysis was conducted in R programming software. Plots were generated using the ggplot2 R package.

4. Results

We aimed to analyse the N-glycan structures present on IgA in order to determine the differences in IgA N-glycosylation specific for T1DM in both paediatric and adult cohorts. Therefore, we profiled N-glycans of serum IgA in 62 children at the onset of T1DM and 86

Table 2
Association of IgA N-glycans with recent onset T1DM in children analysed by mixed linear model. B coefficient shows how much the expected value of the dependent variable (glycan trait) changes for a change in the independent variable (healthy, T1DM), while holding other variables constant.

Glycan trait	Description	B	p-value	Li Ji adjusted p-value	Significance[a]
GP1	FA2/A2B	0.040	0.7734	0.9482	
GP2	**M5**	**0.529**	**0.0004**	**0.0067**	+
GP3	A2G1	0.006	0.9696	0.9858	
GP4	A2BG1	−0.137	0.3456	0.8018	
GP5	FA2G1	−0.017	0.9159	0.9858	
GP6	FA2BG1	0.212	0.1564	0.5213	
GP7	M6	0.227	0.1731	0.5213	
GP8	A2G2	0.049	0.7596	0.9482	
GP9	A2BG2	0.024	0.8711	0.9858	
GP10	FA2G2	0.129	0.4120	0.8108	
GP11	FA2BG2	0.107	0.4631	0.8588	
GP12	FA2BG2S1/A1M1G1S1/A2BG2S1	0.325	0.0214	0.1348	lost
GP13	A2G2S1	0.136	0.4057	0.8108	
GP14	FA2G2S1	−0.004	0.9716	0.9858	
GP15	M8/FA2BG2S1	0.160	0.2456	0.6919	
GP16	FA2BG2S1/A1M1G1S1/A2BG2S1	0.045	0.7297	0.9482	
GP17	A2G2S2	−0.158	0.3267	0.8018	
GP18	FA2G2S2	0.016	0.9168	0.9858	
GP19	A2G2S2	−0.007	0.9649	0.9858	
GP20	A2BG2S2	−0.081	0.6068	0.9272	
GP21	FA2G2S2	−0.079	0.5538	0.9272	
GP22	FA2BG2S2	0.002	0.9858	0.9858	
GP23	FA3BG3S1	0.079	0.5883	0.9272	
GP24	FA3G3S2	−0.056	0.6807	0.9482	
GP25	FA3BG3S2	−0.212	0.1642	0.5213	
GP26	FA3G3S2	−0.136	0.2945	0.7791	
GP27	A3G3S3	−0.211	0.1406	0.5213	
GP28	FA3BG3S3/FA3G3S3	−0.031	0.8022	0.9564	
GP29	A3F1G3S3/A3G3S3	−0.246	0.1180	0.5213	
GP30	FA3F1G3S3/FA3G3S3	−0.378	0.0097	0.1232	lost
LB	Low branching	−0.055	0.6982	0.9482	
HB	High branching	−0.296	0.0243	0.1348	lost
G0	Agalactosylation	0.040	0.7734	0.9482	
G1	Monogalactosylation	0.057	0.7003	0.9482	
G2	Digalactosylation	−0.112	0.4186	0.8108	
G3	Trigalactosylation	−0.296	0.0243	0.1348	lost
S1	Monosialylation	0.237	0.1416	0.5213	
S2	Disialylation	−0.149	0.3585	0.8018	
S3	Trisialylation	−0.314	0.0261	0.1348	lost
B	Bisecting GlcNAc	0.071	0.5809	0.9272	
CF	Core fucosylation	−0.090	0.5626	0.9272	
HM	Oligomannose	0.376	0.0173	0.1348	lost

[a] In the column "Significance": + describes traits which remained significant after correction for multiple testing; 'lost' describes traits which lost significance after correction for multiple testing; blank cell describes traits which were not significant after testing. Description gives information about the glycan traits. For glycan peaks GP1-GP30 core structure consists of two N-acetylglucosamines and three mannose residues. Letters in this column detail sugar residues bound to the core structure and the number after it determines how many the residues are present in the glycan. A – N-acetylglucosamine, B – bisecting N-acetylglucosamine, F – fucose (F at the beginning denotes core fucose, F in the middle denots antennary fucose), G – galactose, M – mannose, S – sialic (N-acetylneuraminic) acid.

of their healthy siblings. In addition, we also examined N-glycans of serum IgA in 84 adults with T1DM and 84 healthy volunteers. The directly measured IgA N-glycan traits and derived glycan traits associated with T1DM are shown in Tables 2 and 3, respectively.

4.1. IgA N-glycosylation in children with recent onset T1DM

In children, only one glycan trait had significant association with T1DM: GP02 (Man5) was increased in T1DM. Two directly measured and four derived glycan traits lost significance after Li Ji correction for multiple testing (Table 2). The changes of the derived traits in question are a decrease in high branching, trigalactosylated and trisialylated glycans and an increase in high mannose glycans.

4.2. IgA N-glycosylation in adults with T1DM

In contrast to children, adults displayed increased number of significant associations of IgA N-glycan traits with T1DM as shown in Table 3. In total 13 directly measured and seven derived traits were significantly changed in adults with T1DM. For the directly measured N-glycan traits, the strongest observed associations with T1DM were the increase in GP17 and GP19 ($p\text{-value} = 4.35 \times 10^{-14}$ and $p\text{-value} = 1.60 \times 10^{-18}$, respectively), glycan peaks which consist of biantennary, disialylated A2G2S2 isomers. The most prominent association of highly branched structures was found for GP29 ($p\text{-value} = 1.98 \times 10^{-8}$) representing triantennary, trisialylated, antennary fucosylated A3F1G3S3 and triantennary, trisialylated A3G3S3 structures. Only three directly measured IgA N-glycan traits were decreased in adults (GP14, GP21 and GP25) and interestingly, all those glycan peaks consist of core fucosylated

Table 3
Association of IgA N-glycans with T1DM in adults analysed by general linear model. B coefficient shows how much the expected value of the dependent variable (glycan trait) changes for a change in the independent variable (healthy, T1DM), while holding other variables constant.

Glycan trait	Structure	B	p-value	Li Ji adjusted p-value	Significance[a]
GP1	FA2/A2B	0.251	0.0877	0.1501	
GP2	**M5**	**0.584**	$\mathbf{3.67 \times 10^{-5}}$	$\mathbf{1.92 \times 10^{-4}}$	+
GP3	A2G1	0.187	0.2157	0.3188	
GP4	**A2BG1**	**0.393**	**0.0079**	**0.0168**	+
GP5	**FA2G1**	**0.603**	$\mathbf{8.56 \times 10^{-5}}$	$\mathbf{3.61 \times 10^{-4}}$	+
GP6	FA2BG1	0.048	0.7373	0.7657	
GP7	**M6**	**0.562**	$\mathbf{7.45 \times 10^{-5}}$	$\mathbf{3.47 \times 10^{-4}}$	+
GP8	A2G2	0.060	0.6860	0.7657	
GP9	A2BG2	0.144	0.3303	0.4295	
GP10	FA2G2	0.053	0.7283	0.7657	
GP11	FA2BG2	−0.094	0.5332	0.6542	
GP12	FA2BG2S1/A1M1G1S/A2BG2S1	0.204	0.1666	0.2643	
GP13	**A2G2S1**	**0.443**	**0.0019**	**0.0050**	+
GP14	**FA2G2S1**	**−0.547**	$\mathbf{1.95 \times 10^{-4}}$	$\mathbf{6.89 \times 10^{-4}}$	+
GP15	M8/FA2BG2S1	−0.054	0.7219	0.7657	
GP16	FA2BG2S1/A1M1G1S/A2BG2S1	−0.037	0.7941	0.7941	
GP17	**A2G2S2**	**1.071**	$\mathbf{3.29 \times 10^{-15}}$	$\mathbf{4.35 \times 10^{-14}}$	+
GP18	FA2G2S2	0.311	0.0350	0.0679	lost
GP19	**A2G2S2**	**1.226**	$\mathbf{8.44 \times 10^{-20}}$	$\mathbf{1.60 \times 10^{-18}}$	+
GP20	A2BG2S2	0.066	0.6614	0.7657	
GP21	**FA2G2S2**	**−0.492**	$\mathbf{4.90 \times 10^{-4}}$	**0.0015**	+
GP22	FA2BG2S2	−0.176	0.2444	0.3381	
GP23	FA3BG3S1	0.053	0.7327	0.7657	
GP24	**FA3G3S2**	**0.354**	**0.0202**	**0.0411**	+
GP25	**FA3BG3S2**	**−0.484**	**0.0017**	**0.0048**	+
GP26	FA3G3S2	−0.190	0.2093	0.3188	
GP27	**A3G3S3**	**0.420**	**0.0042**	**0.0094**	+
GP28	FA3BG3S3/FA3G3S3	−0.225	0.1383	0.2277	
GP29	**A3F1G3S3/A3G3S3**	**0.879**	$\mathbf{2.41 \times 10^{-9}}$	$\mathbf{1.98 \times 10^{-8}}$	+
GP30	FA3F1G3S3/FA3G3S3	−0.049	0.7480	0.7657	
LB	**Low branching**	**−0.574**	$\mathbf{1.05 \times 10^{-4}}$	$\mathbf{4.05 \times 10^{-4}}$	+
HB	**High branching**	**0.466**	**0.0022**	**0.0052**	+
G0	Agalactosylation	0.251	0.0877	0.1501	
G1	Monogalactosylation	0.263	0.0733	0.1364	
G2	**Digalactosylation**	**−0.526**	$\mathbf{2.87 \times 10^{-4}}$	$\mathbf{9.34 \times 10^{-4}}$	+
G3	**Trigalactosylation**	**0.466**	**0.0022**	**0.0052**	+
S1	Monosialylation	−0.158	0.2911	0.3903	
S2	Disialylation	−0.172	0.2420	0.3381	
S3	**Trisialylation**	**0.629**	$\mathbf{2.99 \times 10^{-5}}$	$\mathbf{1.78 \times 10^{-4}}$	+
B	Bisecting GlcNAc	−0.108	0.4548	0.5743	
CF	**Core fucosylation**	**−0.900**	$\mathbf{1.20 \times 10^{-10}}$	$\mathbf{1.21 \times 10^{-9}}$	+
HM	**Oligomannose**	**0.604**	$\mathbf{1.57 \times 10^{-5}}$	$\mathbf{1.08 \times 10^{-4}}$	+

[a] In the column "Significance": + describes traits which remained significant after correction for multiple testing; 'lost' describes traits which lost significance after correction for multiple testing; blank cell describes traits which were not significant after testing.

structures.

Accordingly, regarding the derived traits, the most pronounced change observed was a decrease in total core fucosylated N-glycans ($p\text{-value} = 1.21 \times 10^{-9}$). Other differences included a shift towards complex high branched structures and concomitant reduction in low branched structures, a decrease in digalactosylated and conversely an increase in trigalactosylated glycans and elevated levels of trisialylated and high mannose glycans in T1DM.

In addition, we assessed the correlation between N-glycosylation and disease duration in adult population using general linear model. After correction for multiple testing, no glycan traits exhibited significant association with the duration of T1DM.

4.3. Comparison of IgA N-glycosylation patterns in T1DM between adults with T1DM and children with recent onset T1DM

For a better comparison and understanding of the results, the directly measured and derived N-glycan traits of the adult and child populations are shown in detail in Figs. 2 and 3.

In children with T1DM, none of the derived properties reached the level of significance after correction for multiple testing, however, four derived properties were nominally significant. In contrast, statistically significant changes in seven derived IgA properties were observed in adults with T1DM. Comparison of the glycosylation patterns of the derived traits of IgA of the two populations is shown in Fig. 3. The traits present in adults, which were not nominally significant in the children's population, are a decrease in low branched structures and digalactosylated structures and a decrease in core fucosylated N-glycans in individuals with T1DM (Fig. 2, LB, G2, CF). In both populations, an increase in the derived trait describing oligomannose structures (Fig. 2, HM) was observed, although this property lost significance in children after correction. The other three traits describing highly branched glycans, trisialylated and trigalactosylated structures suggest changes in both populations, however, an inverse association of the mentioned properties with T1DM was observed in two populations (Fig. 2, HB, G3, S3). In children, these derived traits were nominally reduced, while in adults, a statistically significant increase in these traits was recorded compared to healthy controls. Due to the design of the experiment, it is not possible to directly compare the results obtained on the paediatric and adult population, but only their changes compared to the control groups.

Of the directly measured N-glycan traits, only GP2 (Man5) was increased with T1DM in children at the onset of the disease. The same change was observed in the adult population, in which GP7 (Man6) was also increased in T1DM. Comparison of glycosylation patterns of directly measured IgA traits of the two populations is shown in Fig. 3. As in the previous case, due to the design of the experiment, it is not possible to compare the data of the two populations directly; however, the changes in the charts suggest that

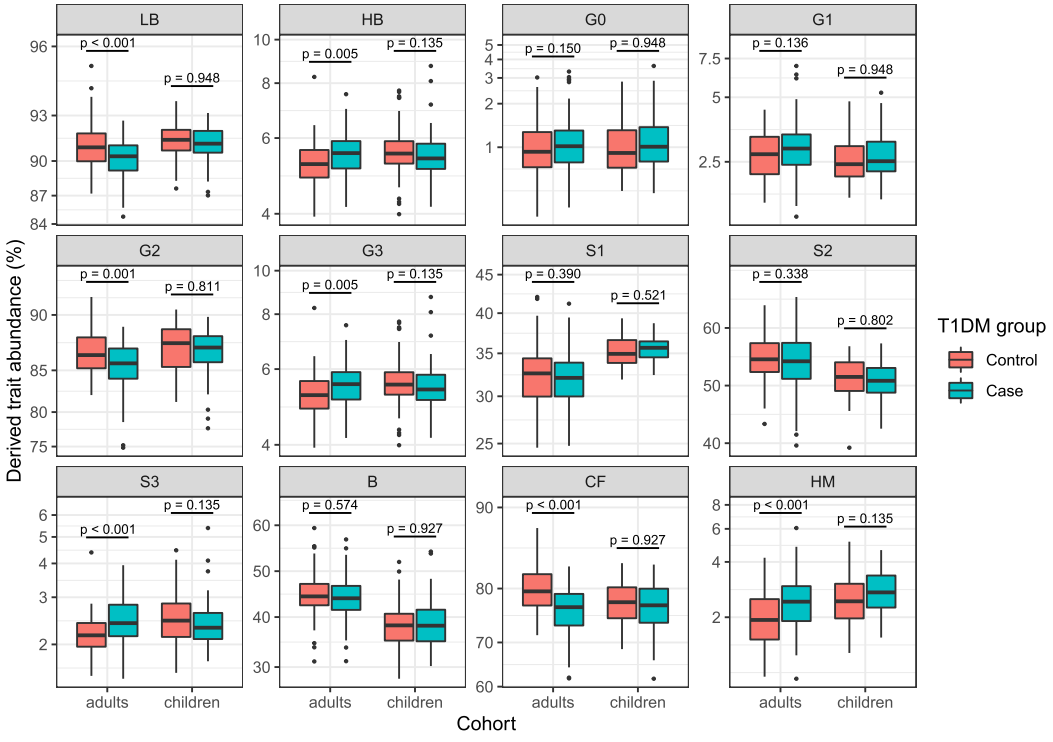

Fig. 2. Comparison of derived IgA N-glycan traits between children and adults. P-values are indicated above the respective boxplots. LB – low-branching glycans, HB – high-branching glycans, G0 – agalactosylation, G1 – monogalactosylation, G2 – digalactosylation, G3 – trigalactosylation, S1 – monosialylation, S2 – disialylation, S3 – trisialylation, B – glycans with bisecting GlcNAc, CF – core fucosylation, HM – high mannose glycans.

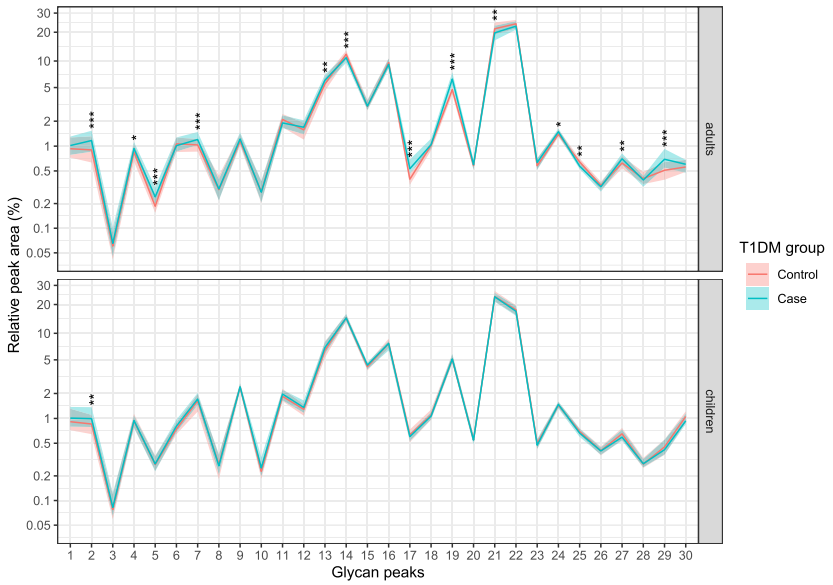

Fig. 3. Comparison between the profiles of directly measured IgA N-glycan abundances between children and adults. X-axis represents glycan peak (GP); Y-axis represents relative glycan abundance. Significance level is indicated by *.

several directly measured properties exhibit different behaviour in children compared to the adult population according to the respective ratio of glycan peak areas (Fig. 3, GP6, GP7, GP21, GP22, GP29 and GP30).

5. Discussion

This is the first study to analyse IgA N-glycosylation features in children and adults with T1DM in a case-control manner. Our results indicate that the N-glycome in children at the onset appeared mostly unaffected by the T1DM, with only one N-glycan trait (Man5) being elevated. On the other hand, in adults we observed a plethora of changes with T1DM and shifts toward more complex, tri-antennary structures.

Previously, it was shown that serum IgA levels are increased in both children and adults with T1DM [23,24]; this is thought to be a consequence of disrupted catabolism of IgA in T1DM, leading to its increased concentration. Additionally, it was shown that breakdown of IgA in liver is largely dependent on the glycosylation of this antibody and that asialoglycoprotein receptors (ASGPRs) in hepatocytes are crucial for recognition of properly glycosylated IgA which should bear exposed terminal galactose residues [19]. The N-glycosylation features observed in our study (Figs. 2 and 3) suggest that these indeed could be correlated, since we observed significant increases in complexity of glycan structures, including increase in trigalactosylation and trisialylation in adults with T1DM. In this case increased sialylation could act as an inhibitor and prevent binding to ASGPRs. Reduced availability of terminal galactoses would impede the catabolism of IgA in the liver. The ASGPR accounts for most of the catabolism of IgA and the clearance of IgA immune complexes. In normal IgA, an abundance of O-linked oligosaccharides seems to be associated with the rate of catabolic breakdown [29]. Due to the presence of O-glycans on IgA, the increase in N-glycosylation in the T1DM cases may not have a pronounced effect on this catabolic process, unless the largely sialylated glycoforms, with their negative charge, significantly inhibit receptor recognition [30].

The most prevalent post-translational glycan modification found on the glycoproteins of the immune system is core fucosylation, which is catalysed by an enzyme called core fucosyltransferase (FUT8). Core fucosylation plays a crucial role in eliciting an appropriate immune response [31]. In our adult cohort we observed significantly reduced core fucosylation on IgA in T1DM (Fig. 2). In contrast to IgA where the role of core fucosylation is not well understood, decreased core fucosylation of IgG leads to increased antibody-dependent cellular cytotoxicity and higher affinity to FcgamaRIII [32]. Similar changes could be possible with IgA as it was shown that it has both pro- and anti-inflammatory capabilities [33], however this remains to be explored in more detail. In addition, it was discovered that the Fcgamma receptor belongs to the same family of proteins as Fcalpha and could have related regulatory pathways [34].

Increased levels of high branched, trigalactosylated and trisialylated structures in adults with T1DM (Fig. 2) could suggest a shift towards the IgA1 subclass, which is reportedly the one bearing those glycans [17] and this increase in sialic acid residues indicates increased activity of sialyltransferases. Previously it was shown that IgA2 has proinflammatory action and that IgA1 without sialylated N-glycans has increased pro-inflammatory capacity [20]. Whether the increase in sialylation we observe is a just a consequence of pathophysiological changes in the system or an attempt to mitigate the inflammation remains to be investigated. The fact remains that T1DM is accompanied by a low degree of persisting inflammation [35] and that the IgA1 subclass, specifically when oversialylated has

a lower affinity toward Fcalpha receptors, thereby losing its proinflammatory capabilities. However, there is ongoing debate regarding the role of oligosaccharides in the recognition process by Fcalpha receptors. It was found that sialic acid slows down the binding of IgA to these receptors [36], suggesting that excessive sialylation could hinder the functional binding site due to conformational effects. Another possibility, as proposed in IgA nephropathy [37] and supported by the erythrocyte-macrophage model [30] is that an increase in sialic acid content could make IgA more negatively charged, thereby preventing its binding through electrostatic repulsion. Oversialylation could also promote inflammation, since it reduces interaction with FcalphaR through steric hindrance due to the fact that binding of monomeric IgA to FcalphaRI has been reported to trigger inhibitory signals via the γ chain ITAM as opposed to the aforementioned activatory ones [33]. Interestingly, in contrast to adults, in children with T1DM we observe nominal decrease in high branched, trigalactosylated and trisialylated N-glycans (Table 2), which is lost after correction for multiple testing. These changes could represent an insignificant shift of the balance towards the IgA2 subclass, which has a pro-inflammatory effect, in children at the onset of the disease, or they could be the result of reduced branching and sialylation of the IgA1 subclass, which would emphasize its pro-inflammatory properties. In both cases, the mentioned changes could contribute to the chronic systemic inflammation that occurs already at an early age in people with T1DM [35].

We also saw a decrease in low-branched and digalactosylated structures (Fig. 2) which are more attributed to IgA2, which has mostly simple high-mannose or biantennary glycans [17]. A previous study reported immune complexes containing IgA1 and IgA2 in type 2 diabetes mellitus, while IgA1-immune complexes were most frequently observed in type 1 diabetes mellitus [38]. IgA2 could then possibly remain available in the serum for a longer period of time, thus having a higher availability to bind to Fcalpha receptors and a more pronounced proinflammatory effect. Due to increased trisialylation which could be attributed to IgA1, IgA1 could have lower the affinity to Fcalpha receptors, enabling the IgA2 to bind to these receptors with less competition.

On the other hand, in adults with T1DM there was a significant increase in high mannose structures, which were also nominally present in children with T1DM. These glycans were found on IgA1 but were more abundant on IgA2 which acts as proinflammatory agent [20]. These structures could be relevant to the systemic inflammation of T1DM, inducing it through the MBL pathway. High mannose structures also potentially disrupt the catabolism, since there is more agalactosylated glycans which do not have terminal galactose required for breakdown recognition. In addition, the only IgA N-glycan trait that was significant in children was the increase in Man5 (Table 2, Fig. 3), which was also observed in adults where we also see an increase in Man6 (Table 3, Fig. 3). Increased levels of mannosylation on IgA could cause MBL recognition and binding, thus initiating an inflammation cascade through the lectin pathway [18]. Furthermore, it was shown that the concentration of MBL is increased in T1DM [39] which could serve to further stimulate the ongoing inflammation due to overly mannosylated IgA N-glycans. It is hypothesized that MBL levels and detrimental complement activation increase as a consequence of diabetes. In our recent paper on children at the onset of T1DM, we showed that Man5 from IgG N-glycans was significantly increased in T1DM, and that the derived trait describing high-mannose structures differed most significantly among all tested derived traits between studied groups for total plasma N-glycans [13].

Interestingly, IgA levels in children with T1DM are also reportedly increased [24], despite having almost normal N-glycosylation, and despite the fact that their IgA does not show an increase in complex abundantly sialylated N-glycans, which could be more difficult to catabolise in hepatocytes. The above suggests that N-glycosylation of IgA does not significantly reflect pathophysiological changes at the onset of T1DM at an early age, but potentially plays a role in or is a consequence of long-term inflammatory processes accompanying T1DM in the adulthood.

It is worth noting that the differences between IgA N-glycoprofiles of children and adults could be a direct result of two different cohort types used in the experiment. For the paediatric population, by using healthy siblings as a control group, we aimed to control the influence of genetic and environmental factors, focusing more explicitly on the impact of the disease onset itself on IgA N-glycoprofile. It is also possible that some of the healthy siblings could potentially develop T1DM which could hinder the identification of N-glycans characteristic for T1DM onset. In the case of the adult population, such cohort was not available, but a regular cohort with independent case and control samples. Hence the changes specific for T1DM in adults also reflect environmental and genetic factors, which could contribute to the variety of N-glycan changes observed in adult T1DM.

6. Conclusions

In conclusion, our study has revealed changes in IgA N-glycosylation patterns in individuals with T1DM, which may have implications in the immune responses and inflammatory processes. In T1DM, changes in IgA N-glycosylation differed substantially between children and adult subjects. Adults exhibited an increase in complex glycan structures, while IgA N-glycome of children at the onset displayed only one significant association with T1DM, suggesting that IgA N-glycosylation is not strongly associated with the onset of the disease. The changes in IgA glycosylation patterns observed in both children and adults with T1DM highlight IgA as a potential target for future research on managing the condition and its associated inflammation. This study emphasizes the importance of research on N-glycans as modulators of immune processes and the insufficiently investigated role of IgA in T1DM.

Acknowledgments

This work was supported by the European Regional Development Fund grant ‘CardioMetabolic’ agreement [KK.01.2.1.02.0321]. The funding sources had no involvement in the study. We thank all the participants for contributing to this research.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

CRediT authorship contribution statement

Matej Nemčić: Data curation, Investigation, Methodology, Validation, Visualization, Writing – original draft. **Sofia Shkunnikova:** Data curation, Investigation, Validation, Writing – original draft. **Domagoj Kifer:** Formal analysis, Visualization, Writing – review & editing. **Branimir Plavša:** Resources, Writing – review & editing. **Marijana Vučić Lovrenčić:** Resources, Writing – review & editing. **Grant Morahan:** Resources, Writing – review & editing. **Lea Duvnjak:** Resources, Writing – review & editing. **Flemming Pociot:** Resources, Writing – review & editing. **Olga Gornik:** Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

The authors declare they have no known competing financial interests or personal relationships that could influence the work reported in this research paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30529.

References

[1] K.M. Gillespie, Type 1 diabetes: pathogenesis and prevention, C. Can. Med. Assoc. J. = J. l'Association Medicale Can. 175 (2006) 165–170.
[2] D.M. Maahs, N.A. West, J.M. Lawrence, E.J. Mayer-Davis, Epidemiology of type 1 diabetes, Endocrinol, Metab. Clin. North Am. 39 (2010) 481–497.
[3] G.D. Ogle, F. Wang, G.A. Gregory, J. Maniam, Type 1 Diabetes estimates in children and adults, Int. Diabetes Fed. Reports 102 (2022) 1–15.
[4] M. Mobasseri, M. Shirmohammadi, T. Amiri, N. Vahed, H.H. Fard, M. Ghojazadeh, Prevalence and incidence of type 1 diabetes in the world: a systematic review and meta-analysis, Heal. Promot. Perspect. 10 (2020) 98–115.
[5] P. Stanley, N-glycans, encycl, Cell Biol. 1–6 (2) (2022) 487–494. Second Ed.
[6] G. Lauc, I. Rudan, H. Campbell, P.M. Rudd, Complex genetic regulation of protein glycosylation, Mol. Biosyst. 6 (2010) 329–335.
[7] A. Varki, Biological roles of glycans, Glycobiology 27 (2017) 3–49.
[8] C. Reily, T.J. Stewart, M.B. Renfrow, J. Novak, Glycosylation in health and disease, Nat. Rev. Nephrol. 15 (2019) 346–366.
[9] T. Štambuk, M. Klasić, V. Zoldoš, G. Lauc, N-glycans as functional effectors of genetic and epigenetic disease risk, Mol. Aspect. Med. 79 (2021) 100891.
[10] M.C. Ryczko, J. Pawling, R. Chen, A.M. Abdel Rahman, K. Yau, J.K. Copeland, C. Zhang, A. Surendra, D.S. Guttman, D. Figeys, J.W. Dennis, Metabolic reprogramming by hexosamine biosynthetic and golgi N-glycan branching pathways, Sci. Rep. 61 (6) (2016) 1–15, 2016.
[11] D.C.W. Poland, C.G. Schalkwijk, C.D.A. Stehouwer, C.A.M. Koeleman, B. Van Het Hof, W. Van Dijk, Increased α3-fucosylation of α1-acid glycoprotein in Type I diabetic patients is related to vascular function, Glycoconj. J. 18 (2001) 261–268.
[12] M.L. Bermingham, M. Colombo, S.J. McGurnaghan, L.A.K. Blackbourn, F. Vučković, M.P. Baković, I. Trbojević-Akmačić, G. Lauc, F. Agakov, A.S. Agakova, C. Hayward, L. Klarić, C.N.A. Palmer, J.R. Petrie, J. Chalmers, A. Collier, F. Green, R.S. Lindsay, S. Macrury, J.A. McKnight, et al., N-glycan profile and kidney disease in type 1 diabetes, Diabetes Care 41 (2018) 79–87.
[13] N. Rudman, D. Kifer, S. Kaur, V. Simunović, A. Cvetko, F. Pociot, G. Morahan, O. Gornik, Children at onset of type 1 diabetes show altered N-glycosylation of plasma proteins and IgG, Diabetologia 65 (2022) 1315–1327.
[14] A. Juszczak, T. Pavić, F. Vučković, A.J. Bennett, N. Shah, E. Pape Medvidović, C.J. Groves, M. Šekerija, K. Chandler, C. Burrows, N. Rojnić Putarek, M. Vučić Lovrenčić, J. Ćuća Knežević, T.J. James, A.L. Gloyn, G. Lauc, M.I. McCarthy, K.R. Owen, O. Gornik, Plasma fucosylated glycans and C-reactive protein as biomarkers of HNF1A-MODY in young adult-onset nonautoimmune diabetes, Diabetes Care 42 (2019) 17–26.
[15] M.A. Kerr, The structure and function of human IgA, Biochem. J. 271 (1990) 285–296.
[16] A. Breedveld, M. Van Egmond, IgA and FcαRI: pathological roles and therapeutic opportunities, Front. Immunol. 10 (2019) 553.
[17] L. Ding, X. Chen, H. Cheng, T. Zhang, Z. Li, Advances in IgA glycosylation and its correlation with diseases, Front. Chem. 10 (2022).
[18] A. Roos, L.H. Bouwman, D.J. van Gijlswijk-Janssen, M.C. Faber-Krol, G.L. Stahl, M.R. Daha, Human IgA activates the complement system via the mannan-binding lectin pathway, J. Immunol. 167 (2001) 2861–2868.
[19] A. Rifai, K. Fadden, S.L. Morrison, K.R. Chintalacharuvu, The N-glycans determine the differential blood clearance and hepatic uptake of human immunoglobulin (Ig)A1 and IgA2 isotypes, J. Exp. Med. 191 (2000) 2171–2181.
[20] U. Steffen, C.A. Koeleman, M.V. Sokolova, H. Bang, A. Kleyer, J. Rech, H. Unterweger, M. Schicht, F. Garreis, J. Hahn, F.T. Andes, F. Hartmann, M. Hahn, A. Mahajan, F. Paulsen, M. Hoffmann, G. Lochnit, L.E. Muñoz, M. Wuhrer, D. Falck, et al., IgA subclasses have different effector functions associated with distinct glycosylation profiles, Nat. Commun. 11 (2020) 1–12.
[21] O. Boyarchuk, L. Dobrovolska, H. Svystunovych, Selective immunoglobulin A deficiency in children with diabetes mellitus: data from a medical center in Ukraine, PLoS One 17 (2022) e0277273.
[22] F.H. Abouali, D.S. Sheha, H.M. Elsayed, N.A. Ibrahim, S.T. Kamal, Y.S. Sharawy, IgA deficiency in type 1 diabetes mellitus patients, QJM An Int. J. Med. 113 (2020).
[23] J. Huang, G. Huang, X. Li, F. Hu, Z. Xie, Y. Xiao, S. Luo, C. Chao, K. Guo, F.S. Wong, Z. Zhou, L. Wen, Altered systemic and intestinal IgA immune responses in individuals with type 1 diabetes, J. Clin. Endocrinol. Metab. 105 (2020) e4616–e4625.
[24] J. Svensson, S. Eising, H.B. Mortensen, M. Christiansen, I. Laursen, Ø. Lernmark, A. Nilsson, L.B. Simonsen, B. Carstensen, F. Pociot, J. Johannesen, High levels of immunoglobulin E and a continuous increase in immunoglobulin G and immunoglobulin M by age in children with newly diagnosed type 1 diabetes, Hum. Immunol. 73 (2012) 17–25.
[25] B.A.M. Ali, M.A. Sayed, H.M. Moness, M.M. Mahmoud, Assessment of serum levels of immunoglobulin a and immunoglobulin g in children newly diagnosed with type 1 diabetes mellitus (T1D), Curr. Pediatr. Res. 21 (2017) 627–631.
[26] A. Ahmadiafshar, M.R. Mohsenifard, S. Mazloomzadeh, Evaluation of serum & salivary IgA in patients with type 1 diabetes, PLoS One 10 (2015) e0122757.

[27] J. Svensson, C.S. Cerqueira, P. Kjærsgaard, L. Lyngsøe, T. Hertel, M. Madsen, H. Mortensen, J. Johannesen, Danish registry of childhood and adolescent diabetes, Clin. Epidemiol. 8 (2016) 679–683.
[28] T. Štambuk, D. Kifer, L. Smirčić-Duvnjak, M.V. Lovrenčić, O. Gornik, Associations between plasma protein, IgG and IgA N-glycosylation and metabolic health markers in pregnancy and gestational diabetes, PLoS One 18 (2023) e0284838.
[29] Y. Pilatte, J. Bignon, C.R. Lambré, Sialic acids as important molecules in the regulation of the immune system: pathophysiological implications of sialidases in immunity, Glycobiology 3 (1993) 201–218.
[30] R. Schauer, A.K. Shukla, C. Schröder, E. Müller, The anti-recognition function of sialic acids: studies with erythrocytes and macrophages, Pure Appl. Chem. 56 (1984) 907–921.
[31] Y. Sun, X. Li, T. Wang, W. Li, Core fucosylation regulates the function of pre-BCR, BCR and IgG in humoral immunity, Front. Immunol. 13 (2022).
[32] J. Golay, A.E. Andrea, I. Cattaneo, Role of fc core fucosylation in the effector function of IgG1 antibodies, Front. Immunol. 13 (2022) 929895.
[33] P. de Sousa-Pereira, J.M. Woof, IgA: structure, function, and developability, Antibodies 8 (2019).
[34] M. Daëron, Fc receptors and fc receptor-like molecules within the immunoreceptor family, Encycl. Immunobiol. 2 (2016) 360.
[35] M. Clark, C.J. Kroger, R.M. Tisch, Type 1 diabetes: a chronic anti-self-inflammatory response, Front. Immunol. 8 (2017).
[36] C. Basset, V. Durand, N. Mimassi, Y.L. Pennec, P. Youinou, M. Dueymes, Enhanced sialyltransferase activity in B lymphocytes from patients with primary Sjögren's syndrome, Scand. J. Immunol. 51 (2000) 307–311.
[37] J.C.K. Leung, P.Y.K. Poon, K.N. Lai, Increased sialylation of polymeric immunoglobulin A1: mechanism of selective glomerular deposition in immunoglobulin A nephropathy? J. Lab. Clin. Med. 133 (1999) 152–160.
[38] G. Triolo, E. Giardina, G. Seddio, C. Terrizzi, Analysis of IgA1 and IgA2 subclass distribution in IgA- containing circulating immune complexes in patients with diabetes mellitus, Boll. Ist. Sieroter. Milan. 66 (1987) 116–119.
[39] T.K. Hansen, S. Thiel, S.T. Knudsen, C.H. Gravholt, J.S. Christiansen, C.E. Mogensen, P.L. Poulsen, Elevated levels of mannan-binding lectin in patients with type 1 diabetes, J. Clin. Endocrinol. Metab. 88 (2003) 4857–4861.