

Human plasma glycome in attention-deficit hyperactivity disorder and autism spectrum disorders

Nela Pivac^{1,#}, Ana Knežević^{2,#}, Olga Gornik², Maja Pučić³, Wilmar Igl⁴, Hilde Peeters⁵, An Crepel⁵, Jean Steyaert⁵, Mislav Novokmet³, Irma Redžić², Matea Nikolac¹, Vesna Novković Hercigonja⁶, Katarina Dodig Ćurković⁷, Mario Ćurković⁸, Gordana Nedić¹, Dorotea Muck-Seler¹, Fran Borovečki⁹, Igor Rudan^{10,11}, and Gordan Lauc^{2,3,*}

¹ Division of Molecular Medicine, Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb

² University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10000 Zagreb, Croatia

³ Genos Ltd., Glycobiology Division, Planinska 1, 10000 Zagreb, Croatia

⁴ Department of Genetics and Pathology, Rudbeck Laboratory; Uppsala University; Uppsala, Sweden

⁵ Center of Human Genetics, University Hospitals Leuven, Leuven, Belgium

⁶ Polyclinic Kocijan/Hercigonja, Lipovecka 17, 10000 Zagreb, Croatia

⁷ Clinical Hospital Osijek, Department of Child and Adolescent Psychiatry, J. Huttlera 4, 31000 Osijek, Croatia

⁸ Primary Care Unit Osijek, Park Kralja Petra Krešimira 4, 31000 Osijek, Croatia

⁹ University of Zagreb Medical School, 10000 Zagreb, Croatia

¹⁰ Croatian Centre for Global Health; University of Split Medical School; 21000 Split, Croatia

¹¹ Centre for Population Health Sciences, The University of Edinburgh Medical School, Teviot Place, Edinburgh, UK

These two authors contributed equally.

*Corresponding author: Professor Gordan Lauc, University of Zagreb, Faculty of Pharmacy and Biochemistry, Ante Kovačića 1, 10000 Zagreb, Croatia. Phone: +385 1 639 4467; Fax: +385 1 639 4400; email: glauc@pharma.hr

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Summary

Over a half of all proteins are glycosylated, and their proper glycosylation is essential for normal function. Unfortunately, due to structural complexity of nonlinear branched glycans and the absence of genetic template for their synthesis, the knowledge about glycans is lagging significantly behind the knowledge about proteins or DNA. Using a recently developed quantitative high throughput glycan analysis methods we quantified components of the plasma N-glycome in 99 children with attention-deficit hyperactivity disorder (ADHD), 81 child and 5 adults with autism spectrum disorder (ASD) and total of 340 matching healthy controls. No changes in plasma glycome were found to associate with ASD, but several highly significant associations were observed with ADHD. Further structural analysis of plasma glycans revealed that ADHD is associated with increased antennary fucosylation of biantennary glycans and decreased levels of some complex glycans with three or four antennas. The design of this study prevented any functional conclusions about the observed associations, but specific differences in glycosylation appears to be strongly associated with ADHD what warrants further studies into this direction.

Keywords

Attention deficit hyperactivity disorder (ADHD); autism spectrum disorder (ASD); plasma glycome; protein glycosylation; protein fucosylation; glycan branching

Introduction

Attention-deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs) are both highly heritable multifactorial and clinically heterogeneous childhood-onset neurodevelopmental disorders (1, 2). The hallmarks of ADHD are severe inattention, hyperactivity, and impulsivity while the characteristic symptoms of ASD include impaired communication and social interaction skills as well as repetitive and restricted behavior and interests (3). Several pieces of evidence point towards some shared heritability and thus genetic factors contributing to the occurrence of both ADHD and ASD (4). Children with ADHD and ASD frequently share symptoms of impaired communication skills, impaired social interactions (4), and develop difficulties in familial, educational and academic functioning (5, 6). The abnormal functioning of the dopaminergic and serotonergic systems (5, 7-9), among other neurotransmitters, were repeatedly found in ADHD and ASD, and therefore the candidate genes common for both disorders are dopaminergic transporter gene (DAT), dopaminergic receptor type 3 and type 4 genes (DRD3; DRD4), serotonin transporter (5HTT) gene, and genes for catechol-o-methyltransferase (COMT) and monoamine oxidase type A (MAO-A) (4), although genome wide association studies did not confirm with certainty all these candidates (10). Shared genetic basis is further suggested by the higher frequency of ADHD and ASD traits in children with a defined genetic diagnosis such as 22q11 deletion syndrome, XYY syndrome, microdeletions or duplications in the chromosome 15q13.2-q13.3 region (11, 12). However, the molecular agents, related to various biological processes and interactions between different risk factors responsible for development of ADHD and ASD remain elusive (6).

Protein N-glycosylation is an ancient metabolic pathway invented before the diversification of Archea, Bacteria and Eukarya, which still exists in all three domains of life (13, 14). At least 2000 different glycan determinants are being added to the backbone of mammalian polypeptides (15) and the complexity of the glycoproteome is estimated to be several orders of magnitude greater than for the proteome itself (16). N-glycosylation is essential for multicellular life and its complete absence is embryonically lethal (17). Disregulation of glycosylation is associated with a wide range of diseases, including cancer, diabetes, cardiovascular, congenital, immunological and infectious disorders (18-20), but due to experimental difficulties in analyzing complex

glycan structures, the knowledge about glycan structure and function is still lagging behind the knowledge about other macromolecules (21). However, recent technological advances have allowed reliable, high-throughput quantification of N-glycans (22-24), which now permit large scale studies aimed at understanding of the role of protein glycosylation in complex diseases.

Variations in glycosylation are of great physiological significance since changes in glycans significantly change the structure and function of polypeptide parts of glycoproteins (25). Proper glycosylation of membrane receptors is particularly important as it modulates adaptive properties of the cell and affects communication between cells (26). Altered glycosylation and ensuing differences in function of various receptors could be a predisposing factor in different psychiatric disorders, and indeed some of them were reported to be associated with altered protein glycosylation (27-31). Here we report the first analysis of plasma N-glycans in patients with ADHD and ASD.

Experimental procedures

Sample population and study design

The study included 99 medication free children with ADHD (9.00 ± 2.78 years old), recruited sequentially from the Polyclinic Kocijan/Hercigonja, Zagreb, diagnosed using psychiatric interview (APA, 1994), according to the DSM-IV criteria, separate interviews with the children and their parents, psychological interview and psychological tests, physical examination, and the Conners' Rating Scale for Parents (CRS-P) -short version (32). Demographical data were uniform for all children: they were Caucasians of urban origin, attending kindergartens or primary schools, and were coming from the undisrupted families, with average intellectual abilities. Exclusion criteria were co-morbid depression, schizophrenia, oppositional defiant disorder, conduct disorder, borderline personality disorder and somatic disorders, all assessed by psychiatric interview based on DSM-IV criteria (APA 1994), or pharmacotherapy with carbamazepine, clomipramine, lamotrigine, or selective serotonin reuptake inhibitors.

Control group included 175 healthy children (10.94 ± 4.01), attending their regular physical exams, served as controls. Somatic or other psychiatric co-morbidities were excluded according to DSM-IV criteria and the Child Symptoms Inventory-4 (APA 1994). All parents signed written informed consent.

60 families with one or more children with ASD were included. These families were selected from the Autism Family Study by the Leuven Autism Research consortium (LAuRes). All patients have a clinical diagnosis of ASD according to DSM-IV criteria. All diagnoses were made by a child psychiatrist and a multidisciplinary team in the Expert Center for Autism in Leuven. All patients, siblings and parents have been examined by a clinical geneticist and patients with known underlying genetic conditions were excluded. Criterion for inclusion in this study was normal intelligence of all family members. All family members have a normal karyotype and FMR1 mutations and premutations were excluded. In total the sample includes 251 individuals (81 children and 5 adults with ASD and 165 individuals without ASD).

Blood samples (4 ml) were taken during routine laboratory tests from forearm vein into a plastic syringe with 1 ml of acid citrate dextrose anticoagulant at 08.00 a.m. after overnight fasting.

Local Ethics committees approved all protocols. The study was carried out in accordance with the Helsinki declaration, and the samples were coded in sequential way without recording any personal information about the patients.

Glycan release and labeling

Glycan release and labeling was performed as reported previously (33). Plasma proteins were immobilized in a block of SDS-polyacrylamide gel and N-glycans were released by digestion with recombinant N-glycosidase F. This was done in a 96 well microtitre plate to achieve the best throughput of sample preparation. After extraction, glycans were fluorescently labelled with 2-aminobenzamide.

Hydrophilic interaction high performance liquid chromatography (HILIC)

Released glycans were subjected to hydrophilic interaction high performance liquid chromatography (HILIC) on a 250 x 4.6 mm i.d. 5 μ m particle packed TSKgel Amide 80 column (Tosoh Bioscience, Stuttgart, Germany) at 30°C with 50 mM formic acid adjusted to pH 4.4 with ammonia solution as solvent A and acetonitrile as solvent B. 60 min runs were performed with fluorescence detector set with excitation and emission wavelengths of 330 and 420 nm, respectively. 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 (GU).

The chromatograms obtained were all separated in the same manner into 16 chromatographic areas (peaks) for initial profile and into 13 peaks for desialylated glycans, regarding the peak resolutions and similarity of glycan structures present as described before. The amounts of glycans present in each area were expressed as % of total integrated chromatogram (amount of total glycan structures / total serum N-glycome).

Weak anion exchange (WAX)-HPLC

Glycans were separated according to the number of sialic acids by weak anion exchange HPLC. The analysis was performed using a Prozyme GlycoSep C 75mm x 7.5mm column (Prozyme, Leandro, CA, USA) at 30°C with 20 % (v/v) acetonitrile in water as solvent A and 0.1 M acetic acid adjusted to pH 7.0 with ammonia solution in 20 % (v/v) acetonitrile as solvent B. Compounds were retained on the column according to their charge density, the higher charged compounds being retained the longest. A fetuin N-glycan standard was used for calibration.

Sialidase digestion

Aliquots of the 2AB-labeled glycan samples were dried down in 96 well PCR plates. To these, the following was added: 1 µl of 250 mM sodium phosphate incubation buffer (pH 6.0), 0.5 µl (0.0025 U) of ABS, *Arthrobacter ureafaciens* sialidase (releases α 2–3,6,8 sialic acid, Prozyme) and H₂O to make up to 5 µl. This was incubated overnight (16–18 h) at 37 °C and then passed through AcroPrep™ 96 Filter Plates, 350 µL well, 10K (Pall Corporation, Port Washington, NY, USA) before applying to the HPLC.

Fucosidase digestion

To examine differences in levels of structures present in peak DG7 between ADHD samples and controls, aliquots of the 2AB-labeled glycans were mixed together to make an ADHD glycan pool and controls glycan pool. These pooled samples were dry down and concentrated pool samples were applied on HILIC column. Peaks DG7 were collected from both glycan pools and dried down in PCR tubes before desired digestion. To these, the following was added: 1 µl of 250 mM sodium acetate incubation buffer (pH 5.0), 1 µl (0.003 mU) of AMF, *Almond meal* fucosidase (releases α 1–3,4 fucose, Prozyme) and H₂O to make up to 5 µl. This was incubated overnight (16–18 h) at 37 °C and then passed through AcroPrep™ 96 Filter Plates, 350 µL well, 10K (Pall Corporation, Port Washington, NY, USA) before applying to the HPLC.

Glycan structural features

Levels of glycans sharing the same structural features were approximated by adding the structures having same characteristic, from either HILIC, WAX or after sialidase treatment integrated glycan profiles (individual glycan structures present in each glycan group were

reported previously are shown in Supplementary Table 1). Glycans were quantified from WAX profiles according to the level of sialylation (monosialylated, disialylated, trisialylated, tetrasialylated). Glycan features were defined as: Core fucosylated glycans (FUC-C) = $DG6/(DG5+DG6)*100$; Antennary fucosylated glycans (FUC-A) = $DG7/(DG5+DG7)*100$; Biantennary glycans (BA) = $DG1+DG2+DG3+DG4+DG5+DG6+DG7$; Monosialylated biantennary glycans (BAMS) = $(GP7+GP8)/(DG5+DG6+DG7)*100$; Disialylated biantennary glycans BADS = $(GP9+GP10+GP11)/(DG5+DG6+DG7)*100$; Triantennary glycans (TRIA) = $DG8+DG9+DG10$; Tetraantennary glycans (TA) = $DG11+DG12+DG13$; Nongalactosylated glycans (G0) = $DG1+DG2$; Monogalactosylated glycans (G1) = $DG3+DG4$; Digalactosylated glycans (G2) = $DG5+DG6+DG7$; Trigalactosylated glycans (G3) = $GP12+GP13+GP14$; Tetragalactosylated glycans (G4) = $GP15+GP16$, Biantennary nongalactosylated glycan (A2) = $(GP1+DG1)/2$.

Statistical Analysis

Associations between ADHD and N-glycans were analyzed using a standard linear fixed effects model (*i.e.* linear regression) with N-glycans as the criterion and ADHD as the predictor. Additionally, sex and age were used as covariates. The analysis was performed using the *lm()* and *anova()* functions of the statistical analysis system R (Version 2.7.2) (34)

Associations between ASD and N-glycans were calculated using a linear mixed effects model with N-glycans as the criterion and ASD as the predictor (fixed effect). Additionally, sex and age were used as covariates (both fixed effects). The pedigree structure (ie father, mother, child) was modeled as a random effect. We used a linear mixed effects model because a standard linear fixed effects model is not adequate for this data structure and will give progressive results, ie to low p-values. The sample is clustered in families, and therefore each individual of two related individuals contains less “information” than a sample consisting of two unrelated individuals. The analysis was performed using the statistical analysis program SOLAR (<http://solar.sfbgenetics.org/>) (35)

Multiple tests on associations between mental disorders and plasma N-glycans were performed. To account for false positive results because of α inflation, we performed a Bonferroni adjustment per disorder. Therefore, we used an α error threshold of $0.05/46 =$

0.001. Since we are aware of the fact that this method is conservative if applied to correlated traits such as plasma N-glycans, as the method is based on the assumption of independent traits, we have taken this into account in the discussion.

Results

Using a recently developed method for high throughput quantitative analysis of glycans we analyzed plasma glycome in 99 medication free children with ADHD (9.00 - 2.78 years old) and 175 healthy control children (10.94 - 4.01). Three HPLC analyses were performed for each sample resulting in 33 directly quantified groups of glycans. The exact composition of the each glycan group is shown in Supplementary Table 1. Further 13 glycosylation features were derived from the measured values as described in the Materials and methods section, resulting in the total number of 46 analyzed glycan variables. Basic descriptive parameters for these 46 glycan features are shown in Table 1.

Some glycans significantly change with age (36), thus associations between ADHD and N-glycans were analyzed using a standard linear fixed effects model with N-glycans as the criterion, ADHD as the predictor and sex and age as covariates. After correction for multiple testing ($0.05/46 = 0.001$) four associations of glycan levels were found to be significant, while further five were suggestively close to statistical significance (Table 1).

The most prominent changes in plasma glycans in ADHD were the increases in glycan groups GP11 and DG7 and a decrease in GP12 (Figure 1). Examples of control and ADHD chromatograms are presented in the Supplementary Figure 1. While GP11 partly overlapped with GP10 and could not have been collected after chromatographic separation, DG7 group was clearly separated from other peaks what enabled us to collect it for more detailed structural analysis. Main glycan structures present in GP7 are M7 (oligomannose glycan composed of two core N-acetylglucosamine and seven mannose residues) and A2FG2 (complex biantennary glycan with two core N-acetylglucosamine, three mannose, two antennary N-acetylglucosamine, one antennary fucose and two antennary galactose residues). To determine which of the two structures is associated with ADHD, we pooled desialylated labeled glycans from 16 ADHD and 16 control samples, separated glycans by HPLC HILIC chromatography and collected eluates corresponding to DG7 peaks. These two eluates (ADHD and control) were then treated with a enzyme (α -1-3,4 *almond meal* fucosidase), which removes antennary fucose. Defucosylated ADHD and control glycan pools were then reanalyzed by HPLC to

separate A2G2 (A2FG2 after the removal of fucose) from M7. Results presented in Fig 2 clearly demonstrate that while M7 is the major component of DG7 in controls, A2FG2 is the major component of DG7 in ADHD samples, and that it is the increase in A2FG2 which associates DG7 with ADHD. Confirmation for this observation was provided by treating the same pair of samples with the enzyme $\alpha(1-2,3,6)$ *Jack bean* mannosidase which specifically cleaves mannose residues. This experiment (data not shown) showed that while in control samples the major glycan structure in DG7 is M7, in ADHD A2FG2 is increased and becomes the main glycan structure in DG7. This conclusion was further supported by digestion of the complete plasma glycome with $\alpha(1-2,3,6)$ *Jack bean* mannosidase which confirmed that there are no changes in the proportion of oligomannose structures (including M7) in ADHD (Supplementary Figure 2)

The same type of glycan analyses were also performed on 81 autistic children and their 168 healthy siblings and parents. Basic descriptive parameters for the 46 measured glycan features are presented in Table 2. Since this study sample was clustered in families, a linear mixed effects model was used to calculate statistical significance of the observed differences. No significant associations between glycan levels and ASD were observed.

Discussion

We have performed the first analysis of human plasma N-glycome in patients with ADHD and ASD. While we have found no differences between children with ASD and their healthy siblings, several significant associations between plasma glycans and ADHD were identified (Table 1). By performing exoglycosidase sequencing, we have convincingly demonstrated that the observed association between ADHD and increased DG7 (and consequently also increased FUC-A, which is calculated from DG7) derived from the increase in relative levels of biantennary glycans with antennary fucose (A2FG2). In addition, ADHD was associated with a regular pattern of changes in GP11, GP12, GP14, GP16, tetrasialo-glycans, and trigalactosylated glycans. Two of these changes (GP11 and GP12) were statistically highly significant, while the remaining four were only slightly below the pre-set significance level of $p < 0.001$ (p values between 0.0012 and 0.0024). Since the Bonferroni correction is highly conservative, these results suggest that the levels of GP14, GP16, tetrasialo glycans, and trigalactosylated glycans are also altered in ADHD. All these changes were consistent with the association between ADHD and the decrease in branching of plasma glycans. The only group of glycans which was found to be increased in ADHD was GP11 (which is composed of mostly biantennary glycans), while all glycan groups which were decreased in ADHD consisted of tri- and tetraantennary glycans.

Glycan branching is very important for the regulation of adaptive properties of the cell membrane (26) and it was reported that the increased branching promote order in the cell membrane and prolong membrane receptor half-life (37). In addition, exact structure of glycans attached to individual membrane receptors significantly affect their functional properties (25), thus it is easily imaginable that differences in glycosylation efficiency could result in altered neural signaling and represent a predisposition for the development of ADHD. It is important to note that we have analyzed levels of individual glycans in plasma. Each glycan can originate from a number of different proteins, and since glycosylation is cell- and protein-specific, relatively small difference in plasma levels could have important local functional consequences.

Recently we completed the first comprehensive population study of human plasma N-glycome which revealed variability that by far exceeds the variability of proteins and DNA (33). However, within a single individual composition of plasma glycome is remarkably stable (38) and environmental factors have rather limited impact on the majority of glycans (36). Specific altered glyco-phenotypes that can be associated with specific pathologies were also identified to exist in a population (39). Here we have described a new glyco-phenotype composed of increased A2FG2 and decreased tri- and tetraantennary glycans which associates with ADHD, while plasma N-glycome was not found to be significantly altered in ASD. We would speculate that the decrease in glycan branching could be one of the predisposing factors for ADHD since it was already reported to be associated with the activity of membrane receptors (26). If this proves to be correct, this would open a new direction for the development of potential therapeutics for ADHD.

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Conflict of interest

None declared.

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Figure and table captions

Figure 1. Levels of glycan groups DG7, GP11 and GP12 in ADHD and controls. Plasma glycome of ADHD patients and controls was separated into 13 desialylated chromatographic peaks (DG series) and 16 sialylated chromatographic peaks (GP series) and expressed as percentages of the total plasma glycome. The majority of peaks did not differ between groups, but statistically significant differences were observed for DG7, GP11 and GP12. Each individual is represented as a black dot and median level is marked with a horizontal line.

Figure 2. Structural analysis of glycans associated with ADHD. A) Glycan peak DG7 isolated by HPLC from the control glycan pool. This peak consists of two structures: A2FG2 and M7, which both elute at 26 min. B) Analysis of DG7 from control glycan pool after digestion with $\alpha(1-3,4)$ fucosidase which converts A2FG2 to A2G2. The new peak eluted at 23.75 min, contrary to M7 structure which remained on the original of DG7 peak. C) Analysis of DG7 from ADHD glycan pool after digestion with $\alpha(1-3,4)$ fucosidase. Comparison of panels B and C clearly reveals that in ADHD there is an increase of relative ratio of A2FG2 compared to M7.

Table 1. Descriptive parameters of plasma glycome in children with ADHD and controls.

Glycan structures present in each glycan peaks (GP1-16, DG1-13) are shown in Supplementary table 1, and the way other glycans traits were calculated is described in the Materials and Methods section. Associations between ADHD and N-glycans were analyzed using a standard linear fixed effects model (ie linear regression) with N-glycans as the criterion and ADHD as the predictor. Additionally, sex and age were used as covariates. Associations with statistical significance below the pre-set level of $p < 0,001$ are shown in bold.

Table 2. Descriptive parameters of plasma glycome in children with ASD and controls. Glycan structures present in each glycan peaks (GP1-16, DG1-13) are shown in Supplementary table 1, and the way other glycans traits were calculated is described in the Materials and Methods section. Associations between ASD and N-glycans were calculated using a linear mixed effects model with N-glycans as the criterion and ASD as the predictor (fixed effect). Additionally, sex and age were used as covariates (both fixed effects).

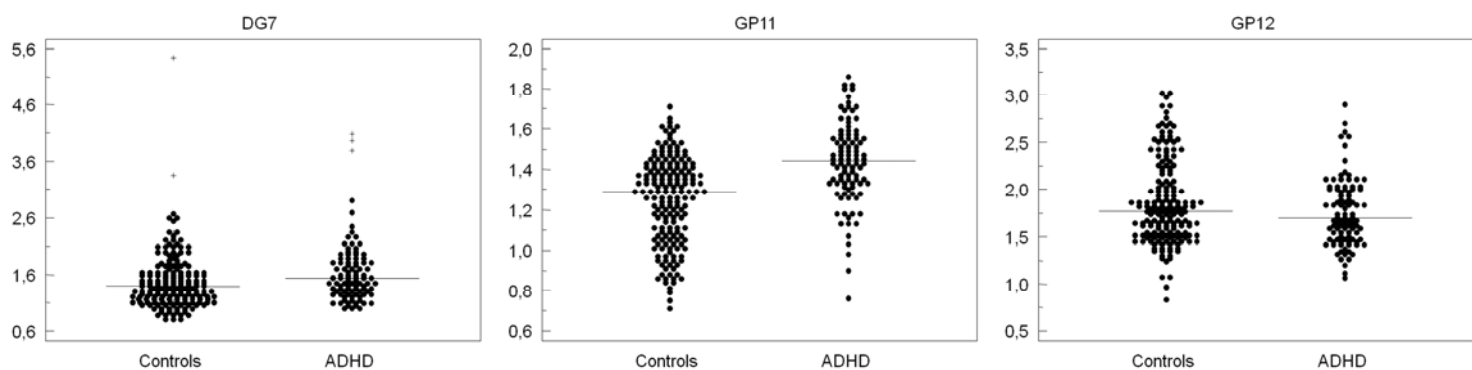


Figure 1. Levels of glycan groups DG7, GP11 and GP12 in ADHD and controls. Plasma glycome of ADHD patients and controls was separated into 13 desialylated chromatographic peaks (DG series) and 16 sialylated chromatographic peaks (GP series) and expressed as percentages of the total plasma glycome. The majority of peaks did not differ between groups, but statistically significant differences were observed for DG7, GP11 and GP12. Each individual is represented as a black dot and median level is marked with a horizontal line

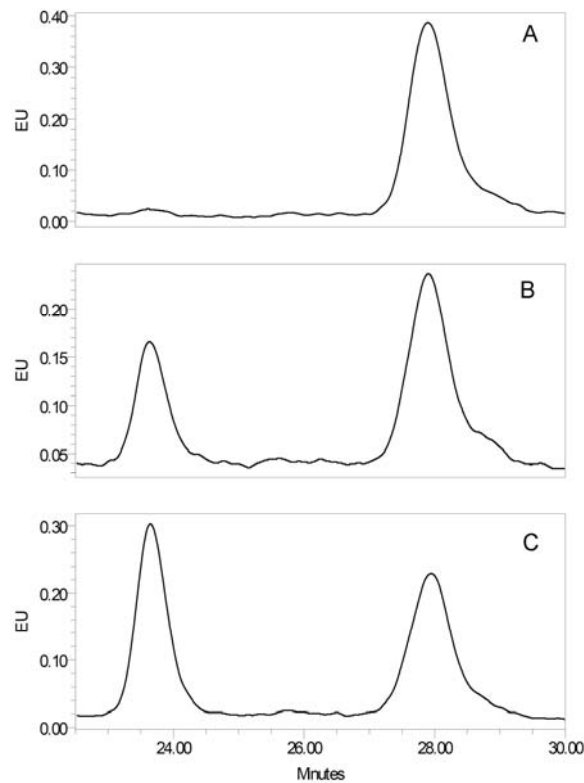


Figure 2. Structural analysis of glycans associated with ADHD. A) Glycan peak DG7 isolated by HPLC from the control glycan pool. This peak consists of two structures: A2FG2 and M7, which both elute at 26 min. B) Analysis of DG7 from control glycan pool after digestion with $\alpha(1-3,4)$ fucosidase which converts A2FG2 to A2G2. The new peak eluted at 23.75 min, contrary to M7 structure which remained on the original of DG7 peak. C) Analysis of DG7 from ADHD glycan pool after digestion with $\alpha(1-3,4)$ fucosidase. Comparison of panels B and C clearly reveals that in ADHD there is an increase of relative ratio of A2FG2 compared to M7.

Table1

	ADHD				Controls				Linear Regression with ADHD as a predictor	
	N	Median	Percentiles		N	Median	Percentiles		P	β
			25 th	75 th			25 th	75 th		
Age	99	9.00	7.00	11.00	173	11.00	6.00	15.00		
Glycan feature										
GP1	96	0.07	0.05	0.09	175	0.06	0.04	0.08	4.04E-01	0.12
GP2	96	3.39	2.71	4.02	175	3.17	2.57	3.79	7.15E-01	-0.05
GP3	96	1.66	1.52	1.86	175	1.65	1.47	1.84	9.69E-01	0.01
GP4	96	5.65	4.84	6.53	175	5.70	4.84	6.22	9.92E-01	0.00
GP5	96	2.21	2.01	2.41	175	2.12	1.92	2.39	7.91E-03	0.37
GP6	96	4.47	3.86	5.05	175	4.28	3.83	4.80	3.80E-02	0.30
GP7	96	8.97	8.48	9.57	175	8.82	8.34	9.60	1.76E-01	0.19
GP8	96	10.32	9.72	10.84	175	10.29	9.57	10.98	3.98E-01	-0.12
GP9	96	39.80	37.74	41.85	175	39.28	37.83	41.58	3.33E-01	0.13
GP10	96	6.06	5.54	6.69	175	6.48	5.90	7.01	3.18E-01	-0.14
GP11	96	1.45	1.33	1.56	175	1.29	1.08	1.41	1.22E-13	0.99
GP12	96	1.70	1.48	2.01	175	1.77	1.53	2.17	2.84E-06	-0.53
GP13	96	6.02	5.06	6.95	175	5.98	5.03	6.72	6.05E-01	-0.07
GP14	96	5.27	4.53	6.24	175	6.04	5.21	6.87	2.44E-03	-0.42
GP15	96	0.84	0.69	1.03	175	0.76	0.59	0.95	7.94E-01	0.03
GP16	96	1.49	1.29	1.75	175	1.51	1.25	1.89	2.36E-03	-0.38
DG1	96	0.08	0.07	0.11	175	0.09	0.07	0.11	3.08E-01	-0.14
DG2	96	3.67	2.94	4.20	175	3.36	2.76	3.96	8.58E-01	0.02
DG3	96	2.32	2.11	2.58	175	2.29	2.07	2.53	4.62E-01	0.10
DG4	96	6.73	5.84	7.71	175	6.72	5.86	7.37	9.25E-01	0.01
DG5	96	50.54	48.60	52.95	175	50.68	48.22	53.20	1.66E-01	0.19
DG6	96	14.54	12.70	16.03	175	14.73	13.28	16.52	2.41E-01	-0.17
DG7	96	1.54	1.31	1.87	175	1.39	1.16	1.65	1.02E-04	0.53
DG8	96	11.65	10.25	13.08	175	11.69	10.53	13.08	2.80E-01	-0.15
DG9	96	2.80	2.32	3.64	175	3.33	2.58	4.13	1.29E-02	-0.35
DG10	96	0.98	0.89	1.06	175	0.93	0.85	1.02	1.63E-02	0.34
DG11	96	2.83	2.49	3.43	175	2.72	2.32	3.30	7.92E-01	-0.03
DG12	96	0.80	0.61	1.01	175	0.83	0.65	1.03	1.90E-01	-0.19
DG13	96	0.72	0.54	0.80	175	0.63	0.50	0.75	2.40E-01	0.16
Monosialo	88	21.68	20.40	22.76	175	21.59	20.06	23.41	7.31E-01	0.05
Disialo	88	60.77	59.22	61.92	175	59.54	58.27	61.26	3.45E-02	0.27
Trisialo	88	15.07	13.42	16.71	175	15.48	14.07	17.00	6.95E-02	-0.25
Tetrasialo	88	2.72	2.34	3.13	175	2.79	2.34	3.51	1.24E-03	-0.40
FUC-C	96	22.38	19.14	24.59	175	22.41	20.15	24.98	2.01E-01	-0.18
FUC-A	96	2.98	2.49	3.48	175	2.69	2.30	3.18	9.56E-04	0.46
BAMS	96	28.75	27.62	30.09	175	28.80	27.40	30.27	7.95E-01	-0.04
BADS	96	70.63	68.65	72.71	175	70.46	68.65	72.48	5.18E-01	0.09
BA	96	80.00	78.19	81.71	175	79.60	77.91	81.37	4.07E-02	0.27
TRIA	96	15.46	14.25	16.91	175	16.12	14.93	17.21	1.52E-02	-0.33
TA	96	4.37	3.99	5.05	175	4.23	3.59	5.01	5.93E-01	-0.07
G0	96	3.75	3.00	4.28	175	3.43	2.86	4.07	8.89E-01	0.02
G1	96	9.09	8.20	10.01	175	9.00	8.19	9.73	8.59E-01	0.03
G2	96	67.11	65.22	68.60	175	67.16	65.20	68.74	8.71E-02	0.22
G3	96	13.17	11.58	14.70	175	13.86	12.54	15.31	1.21E-03	-0.42
G4	96	2.38	1.97	2.73	175	2.25	1.91	2.83	4.18E-02	-0.25
A2	96	0.08	0.06	0.11	175	0.07	0.06	0.10	8.73E-01	0.02

Table 2.

	ASD				Controls				Linear Regression with ASD as predictor p
	N	Median	Percentiles		N Valid	Median	Percentiles		
			25	75			25	75	
Age	78	11.00	9.00	14.00	48	12.50	9.25	15.75	
Glycan feature									
GP1	78	0.06	0.05	0.07	48	0.05	0.04	0.07	5.03E-01
GP2	78	2.93	2.53	3.43	48	2.55	2.09	3.19	3.76E-01
GP3	78	1.66	1.55	1.84	48	1.61	1.40	1.82	6.39E-02
GP4	78	5.50	4.89	5.95	48	5.21	4.55	6.03	7.81E-01
GP5	78	2.34	2.20	2.49	48	2.29	2.13	2.52	6.90E-02
GP6	78	4.16	3.77	4.48	48	4.27	3.74	4.80	7.22E-01
GP7	78	9.65	9.14	10.35	48	9.57	9.03	10.36	6.87E-01
GP8	78	10.53	9.85	11.08	48	10.78	10.01	11.24	8.88E-01
GP9	78	40.28	38.37	41.58	48	40.38	38.25	41.64	9.87E-01
GP10	78	6.62	5.81	7.25	48	6.58	6.04	7.02	5.86E-01
GP11	78	1.62	1.48	1.76	48	1.59	1.41	1.74	1.00E+00
GP12	78	1.56	1.42	1.66	48	1.56	1.42	1.71	8.71E-01
GP13	78	5.52	4.97	6.14	48	5.76	5.01	6.62	6.22E-01
GP14	78	5.38	4.63	6.47	48	5.56	4.93	6.08	6.79E-01
GP15	78	0.76	0.60	0.87	48	0.77	0.65	0.91	6.05E-01
GP16	78	1.30	1.16	1.47	48	1.27	1.12	1.49	5.41E-01
DG1	78	0.07	0.05	0.10	48	0.06	0.04	0.09	9.72E-01
DG2	78	3.53	3.08	4.20	48	3.25	2.76	3.69	7.49E-01
DG3	78	2.50	2.28	2.71	48	2.44	2.09	2.69	1.06E-01
DG4	78	6.48	5.80	6.99	48	6.27	5.21	6.91	7.64E-01
DG5	78	51.71	50.20	53.22	48	51.52	50.04	53.05	9.60E-01
DG6	78	14.80	13.75	16.06	48	14.88	13.84	15.95	7.76E-01
DG7	78	1.50	1.37	1.71	48	1.54	1.37	1.73	3.38E-01
DG8	78	10.74	9.93	11.95	48	11.09	10.12	12.33	7.17E-01
DG9	78	3.03	2.37	3.94	48	3.32	2.69	3.75	3.84E-01
DG10	78	0.98	0.90	1.06	48	0.98	0.92	1.03	4.64E-01
DG11	78	2.65	2.29	2.93	48	2.59	2.36	2.98	9.46E-01
DG12	78	0.76	0.61	0.96	48	0.79	0.69	0.96	4.89E-01
DG13	78	0.75	0.62	0.91	48	0.77	0.66	0.93	9.63E-01
Monosialo	78	22.15	21.04	23.79	48	22.29	20.82	23.60	6.27E-01
Disialo	78	60.14	58.94	61.34	48	59.94	58.74	61.11	6.05E-01
Trisialo	78	13.55	12.64	15.06	48	13.72	12.66	16.07	6.78E-01
Tetrasialo	78	3.47	3.10	4.00	48	3.52	3.05	3.89	1.67E-01
FUC-C	78	22.27	20.45	23.94	48	22.56	20.82	24.05	8.52E-01
FUC-A	78	2.84	2.56	3.28	48	2.88	2.64	3.31	3.38E-01
BAMS	78	29.73	28.20	31.21	48	29.76	28.50	31.04	6.80E-01
BADS	78	70.78	69.21	72.46	48	71.41	68.68	72.38	5.99E-01
BA	78	80.60	79.29	82.17	48	80.73	78.23	81.73	4.32E-01
TRIA	78	15.18	13.93	16.28	48	15.17	14.40	16.96	3.02E-01
TA	78	4.16	3.72	4.65	48	4.27	3.85	4.66	9.95E-01
A2	78	0.07	0.05	0.09	48	0.06	0.04	0.08	8.13E-01