# LECTIN-BASED MICROARRAY AND MALDI-TOF-MS APPROACHES IN STUDY OF GLYCAN CHANGES IN ADHD

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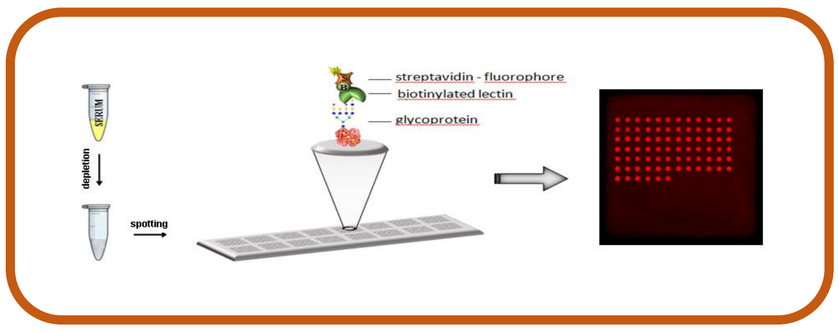
## Introduction & Goals

Changes in glycosylation are associated with the onset and development of many disorders so the glycosylation status of glycoproteins can significantly increase the informative value of protein biomarkers. Glycomic analysis is important in terms of the development of new approaches for research and diagnosis of many disorders including psychiatric ones[1]. We focused on glycomic analysis of serum samples from children patients with Attention-Deficit Hyperactivity Disorder (ADHD). ADHD is multifactorial and clinically heterogeneous childhood-onset neurodevelopmental psychiatric disorder affecting around 10% of children worldwide. The symptoms in general are inattentiveness, hyperactivity and impulsiveness. ADHD can be related to altered functioning of the dopaminergic and serotonergic systems including dopamine and serotonin receptors[2,3]. The goal of this study was to characterize profile of glycans in patients' serum samples and in matching healthy controls using lectin-based protein microarray and MALDI-TOF-MS.

## Methods & Methods

We compare the glycan profile of serum samples from patients with ADHD (n = 10) and the control group (n = 10) in 3 types of samples: whole serum samples, sera after depletion of abundant serum proteins (albumin and IgG), and IgG (isolated by Protein A). Lectin-based protein microarray enables high-throughput recognition of glycan – lectin interactions in the samples containing glycoproteins[4]. The glycoprotein samples were spotted into arrays on microarray slide and then incubated with a panel of biotinylated lectins. The detection was performed after incubation with fluorescent conjugate of streptavidin using microarray scanner. Mass spectrometry (MS) is a standard method used in glycomic analysis. Samples prepared according optimized protocol were analyzed by the MALDI-TOF MS (digestion by PNGase F, enrichment by PGC column, permethylation and MALDI-MS measurement.




- 10 patients' samples, 10 controls, 3 types of samples:
  - whole serum
  - serum after depletion of abundant proteins (albumin and IgG)
  - IgG (isolated by Protein A)
- lectin-based protein microarray - high-throughput glycoprofiling
- 10 patients' samples, 10 controls collection
- MALDI-TOF MS – identification of glycan structures

Bruker MALDI-TOF/TOF ultraflexExtreme MS reflectron positive ion mode

## Results

**Signals (relative intensities) from lectin-based microarray** analysis for all 3 types of samples (averages for all ADHD patients' and all controls' samples). Statistically significant differences (t-test) are highlighted (green: p<0.01, yellow: p<0.05).

| | | SNA | MAL II | MAL I | CONA | LCA | RCA | WGA | PHA-E | PHA-L | AAL | PhoSL |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Serum | ADHD | 36,99 | 0,90 | *0,00* | 21,68 | 3,23 | 15,68 | 1,57 | 2,01 | *0,26* | 5,01 | 2,98 |
| | Controls | 37,93 | 1,01 | *0,00* | 22,05 | 3,24 | 15,43 | 1,74 | 2,31 | *0,34* | 4,32 | 2,99 |
| Depleted serum | ADHD | 26,21 | 0,93 | *0,22* | 19,77 | 2,46 | 22,03 | 4,11 | 3,54 | *0,16* | 4,10 | 1,14 |
| | Controls | 25,83 | 1,14 | *0,29* | 20,75 | 2,77 | 21,25 | 4,35 | 4,22 | *0,17* | 3,75 | 1,12 |
| IgG | ADHD | 16,26 | *0,07* | *0,06* | 20,97 | 21,99 | 13,02 | 1,18 | 10,99 | 0,76 | 10,52 | 1,61 |
| | Controls | 21,75 | *0,10* | *0,08* | 19,51 | 20,33 | 13,19 | 1,03 | 9,92 | 0,70 | 8,80 | 1,79 |

**Signals (relative intensities) from MALDI-TOF MS** analysis for all 3 types of samples (averages for all ADHD patients' and all controls' samples). Statistically significant differences (t-test) are highlighted (green: p<0.01, yellow: p<0.05).

| | **m/z** | **1579,8** | **1661,8** | **1783,9** | **1835,9** | **1865,9** | **1988,0** | **2040,0** | **2070,0** | **2081,1** | **2111,1** | **2186,1** | **2192,1** | **2227,1** | **2244,1** | **2285,2** | **2315,2** | **2390,2** | **2396,2** | **2401,2** | **2431,2** | **2472,2** | **2489,3** | **2605,3** | **2646,3** | **2676,3** | **2792,4** | **2850,4** | **2880,4** | **2966,5** | **3054,5** | **3211,6** | **3241,6** | **3602,8** | **3776,9** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Structure | H5N2 | H3N4 | H6N2 | H3N4F1 | H4N4 | H7N2 | H4N4F1 | H5N4 | H3N5F1 | H4N5 | H5N3SA1 | H8N2 | H4N4SA1 | H5N4F1 | H4N5F1 | H5N5 | H6N3SA1 | H9N2 | H4N4F1SA1 | H5N4SA1 | H4N5SA1 | H5N5F1 | H5N4F1SA1 | H4N5F1SA1 | H5N5SA1 | H5N4SA2 | H5N5F1SA1 | H6N5SA1 | H5N4F1SA2 | H6N5F1SA1 | H5N5F1SA2 | H6N5SA2 | H6N5SA3 | H6N5F1SA3 |
| Serum | ADHD | 4,00 | - | 5,71 | 2,80 | - | 1,51 | 4,77 | 1,34 | - | - | 1,12 | 2,84 | 0,98 | 3,19 | 1,44 | - | 1,33 | 4,15 | - | 14,00 | 1,03 | 1,30 | 5,42 | - | 1,08 | 29,53 | 3,08 | 0,80 | 4,72 | 0,46 | 1,29 | 0,68 | 0,83 | 0,60 |
| | Controls | 3,21 | - | 5,61 | 1,79 | - | 1,47 | 3,08 | 1,34 | - | - | 1,11 | 2,97 | 0,96 | 2,52 | 1,24 | - | 1,45 | 3,16 | - | 14,66 | 0,79 | 1,52 | 6,35 | - | 0,79 | 32,34 | 4,49 | 0,77 | 4,37 | 0,49 | 1,22 | 0,87 | 0,94 | 0,50 |
| Depleted serum | ADHD | 5,89 | - | 7,12 | - | - | 2,68 | - | 2,25 | - | 1,80 | 2,07 | 4,03 | - | 2,17 | - | - | 2,36 | 6,33 | - | 10,64 | 4,35 | 1,96 | 4,32 | - | - | 28,82 | 2,19 | 1,68 | 4,79 | - | - | - | 2,44 | 2,11 |
| | Controls | 7,16 | - | 7,96 | - | - | 2,94 | - | 2,25 | - | 1,65 | 2,27 | 4,18 | - | 2,22 | - | - | 2,53 | 5,56 | - | 11,52 | 2,40 | 1,94 | 4,59 | - | - | 29,53 | 2,57 | 1,67 | 3,89 | - | - | - | 2,03 | 1,49 |
| IgG | ADHD | 0,70 | 0,55 | 1,19 | 20,82 | 0,93 | - | 32,61 | 1,00 | 3,47 | 0,59 | - | - | - | 16,66 | 5,97 | 0,46 | - | 0,42 | 0,89 | 0,75 | - | 1,87 | 5,45 | 0,50 | 0,76 | - | 3,04 | - | 0,60 | - | 0,75 | - | - | - |
| | Controls | 0,72 | 0,28 | 1,07 | 18,57 | 0,74 | - | 30,06 | 0,68 | 3,58 | 0,35 | - | - | - | 17,22 | 6,52 | 0,27 | - | 0,26 | 0,97 | 0,84 | - | 2,18 | 8,19 | 0,46 | 0,61 | - | 4,84 | - | 0,73 | - | 0,85 | - | - | - |

## Discussion & Conclusions

- in serum samples, statistically significant differences in signals between patient's and controls can indicate higher content of Fuc in patients' samples (AAL) and higher content of terminal GlcNac structures in controls (WGA) – increase of Fuc is in agreement with previously reported study[5] (in that work was plasma samples from ADHD patients analysed by chromatographic methods after glycan release)
- in depleted serum samples, statistically significant differences can indicate decrease of 2,3Sia (MAL-II), decrease of mannose structures (ConA) and decrease of tri-/tetraantennary complex type N-glycans in ADHD patients' samples (PHA-E) – the last is in agreement with study of Pivac et al.[5]
- in IgG samples, statistically significant differences show decrease of Sia and increase of Fuc in patients' samples
- results of MS analysis can indicate N-glycan structures which are possibly involved in these changes of glycosylation pattern
- conclusions drawn from the analysis of both methods (lectin-based microarray and MS) are mutually consistent, however, their interpretation and gluing is not an easy task and need further improvement in methodology of data processing and interpretation
- lectin-based protein microarray assay does not allow identification of glycan structures but is appropriate for rapid screening and detecting glycosylation changes this work outlines new approach for ADHD research enabling high-throughput screening of potential biomarkers
- we use this approach (combined analysis by lectin-based protein microarray and MS) also for other studies as eg glycoprofiling of samples of patients with colorectal and bladder cancers, glycan analysis of lung tissue regarding SARS-CoV-2 infection, or monitoring the glycostructure of therapeutic proteins

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