A comprehensive glycome profiling of Huntington's disease transgenic mice

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Abstract

Background: Huntington's disease (HD) is an autosomal, dominantly inherited and progressive neurodegenerative disease, nosologically classified as the presence of intranuclear inclusion bodies and the loss of GABA-containing neurons in the neostriatum and subsequently in cerebellar cortex. Abnormal processing of neuronal proteins can result in the misfolding of proteins and altered post-translational modification of newly synthesized proteins. Total glycomics, namely, *N*-glycomics, *O*-glycomics, and glycosphingolipidomics (GSL-omics) of HD transgenic mice would be a hallmark for central nervous system disorders in order to discover disease specific biomarkers.

Methods: Glycoblotting method, a high throughput glycomic protocol, and matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS) were used to study the total glycome expression levels in the brain tissue (3 mice of each sex) and sera (5 mice of each sex) of HD transgenic and control mice. All experiments were performed twice and differences in the expression levels of major glycoforms were compared between HD transgenic and control mice.

Results: We estimated the structure and expression levels of 87 and 58 *N*-glycans in brain tissue and sera, respectively, of HD transgenic and control mice. The present results clearly indicated that the brain glycome and their expression levels are significantly gender specific when compared with those of other tissues and serum. Core-fucosylated and bisecting-GlcNAc types of *N*-glycans were found in increased levels in the brain tissue HD transgenic mice. Accordingly, core-fucosylated and sialic acid (particularly *N*-glycolylneuraminic acid, NeuGc) for biantennary type glycans were found in increased amounts in the sera of HD transgenic mice compared to

that of control mice. Core 3 type *O*-glycans were found in increased levels in male and in decreased levels in both the striatum and cortexes of female HD transgenic mice. Furthermore, serum levels of core 1 type *O*-glycans decreased and were undetected for core 2 type *O*-glycans for HD transgenic mice. In glycosphingolipids, GD1a in brain tissue and GM2-NeuGc serum levels were found to have increased and decreased, respectively, in HD transgenic mice compared to those of the control group mice.

Conclusion: Total glycome expression levels are significantly different between HD transgenic and control group mice.

General significance: Glycoblotting combined with MALDI-TOF/MS total glycomics warrants a comprehensive, effective, novel and versatile technique for qualitative and quantitative analysis of total glycome expression levels. Furthermore, glycome-focused studies of both environmentally and genetically rooted neurodegenerative diseases are promising candidates for the discovery of potential disease glyco-biomarkers in the post-genome era.

Key words: *Huntington's disease, Total glycomics, Transgenic mouse, Glycoblotting, Glyco-biomarker*

Abbreviations

HD, Huntington's disease; CDG, congenital disorders of glycosylation; CNS, central nervous system; GSL-omics, glycosphingolipidomics; PolyQ, polyglutamine; TCEP, Tris (2-carboxyethyl) phosphine hydrochloride; MeOH, methanol; ConA, Canavalia ensiformis; LCA, Lens culinaris; PHA-E₄, Phaseolus vulgaris; DSA, Datura stramonium; SSA, Sambucus sieboldiana; PNA, Arachis hypogaea; PAS, Periodic Acid-Schiff; MTT, 3-methyl-1-p-tolyltriazene; DTT,

dithiothreitol;PB, phosphate buffer;PBS, phosphate buffer saline;MALDI-TOF/MS, matrix-assisted laser desorption ionization-time of flight/mass spectrometry.

1. Introduction

Huntington's disease (HD), one of the trinucleotide repeat disorders, is nosologically classified as the presence of neuronal intranuclear inclusions (NIIs), dystrophic neuritis (DNs) and the loss of GABA-containing neurons in the neostriatum and, subsequently, in the cerebellar cortex [1]. The HD mutation is dominant and almost completely penetrates [2]. The increased numbers of CAG repeats in the HD gene are expressed as an elongated huntingtin (HTT) protein with 40 to 150 polyglutamine (polyQ) residues (CAG ranges for normal individuals are 9-35). The huntingtin, the function of which is unknown, is a 350 KDa protein encoded by the HD gene (initially labeled as IT15)[1-4]. It is associated with proteinaceous deposits that are found in patients' brain of which aggregated polyQ is a major component [5]. The disease is caused by an abnormal elongation of a CAG repeat sequence in exon 1 of the HD gene [6], which is sufficient to cause a progressive neurological phenotype in transgenic mice [7]. HD is an autosomal, dominantly inherited, and progressive neurodegenerative disorder and, accordingly, the hallmark for fatal genetic diseases [8-12].

The atrophy of the neurons due to pediatric and adulthood diseases of the central nervous system leads to aberrant glycosylation and the biosynthesis pattern of the available glycans is altered. Aberrant glycosylation, in turn, impairs neurite growth and fasciculation, synapse formation and stabilization, and also causes psychomotor difficulties, mental retardation and other neuropathological symptoms that are highlighted by congenital disorders of glycosylation (CDG) [13]. Understanding the glycosylation pattern of *N*-, *O*-, and glycosphingolipids (GSL)-glycans, which is affected by either genetic or environmental cellular stressors, is a promising step

towards an easy prognosis of disease progression. Either serum or cerebrospinal fluids (CSF) specimens from human with neurodegenerative and aging are validated for biomarker detection [13-15]. Glycosylation is the most prevalent post-translational modifications (PTM) of proteins in higher organisms essential to modulate a wide range of protein and lipid functions within or on extracellular surfaces of the cell. Protein glycosylation is either *N*-linked (GlcNAc β -Asn) linkage with a minimal amino acid sequence of Asn-X-Ser/Thr, where X $\neq$ Pro) or *O*-linked (where GalNAc is added to Ser or Thr), and GSLs are synthesized by a sequential transfer of sugar residues to a ceramide lipid anchor. Interestingly, all share pivotal roles such as cellular differentiation, adhesion, immunity, signal transduction and growth control [16-18]. However, there is no comprehensive glycomics focusing on mammalian brain glycoconjugates towards the discovery of neurodegenerative biomarkers while a few *N*- and *O*-glycans specific to rat brain glycoproteins have been reported [19-21].

Glycomics is the systems-level study of the glycan repertoires (glycomes), which comprise the entire set of glycans produced in a single organism [22]. It has been an attractive area of research in the postgenome era emerging as a new paradigm for biomarker discovery [23-27]. However, the stereochemical and structural diversity of monosaccharides, the heterogeneous or highly complicated glycan structures of glycoproteins (microheterogeneity), the multivalent nature of glycan-protein interactions [27,28], and the lack of a direct genetic code for glycan complex modification (the non-templete dependent biosynthesis of glycans) [29-31] are the challenges that scientists are faced with. As the most structurally and functionally diverse class of molecules in nature to undergo versatile protein modifications, glycoconjugates play a role in tracing the cellular glycosylation patterns of multifunctional proteins [32] as well as finding potential biomarkers in the field of drug discovery [33,34].

Many efforts to identify glycosylation defects in cases of CDG. These include the study of conserved oligomeric Golgi (COG)-deficient patients and the increasing prevalence age and age-related diseases identified via glycoform characterization of serum, plasma and CSF by using ESI-MS, affinity chromatography, and 2-DE followed by MS/MALDI-MS/DNA sequencer adapted-fluorophore assisted carbohydrate electrophoresis (DSA-FACE) and HILIC-HPLC [13]. Moreover, glycosylation changes in non-invasive bodily fluids, inter alia, serum, urine, plasma glycomes and on individual glycoproteins have been identified as promising biomarkers for the detection of prostate cancer [28], hepatocarcinoma cancer (HCC) and cirrhosis of the liver [35,36], pancreatic cancer [37], ulcerative colitis [38], and bladder cancer [39]. Measuring the degree of alteration of specific glycans in serum/plasma or whole blood has helped to provide a novel, non-invasive method for determining prognosis in cases of cancer and the existence of specific altered glyco-phenotypes, which might represent risk factors for the sequela of certain diseases [40-44]. Huntingtin protein targeting experiments have also been performed to understand the pathogenesis of HD [2, 6]. Since oligosaccharides attached to the proteins play significant roles in tissue development and maintenance, the brain tissue of HD transgenic mice provides additional systems for such studies [6,19]. As a first initiative, we have estimated the glycan composition and quantified the total glycomes (*N*-, *O*-, and GSL-glycans) expressed in 12 weeks-old HD transgenic and WT mice via glycoblotting [45-47] assisted sample preparation, i.e., glycan enrichment, combined with MALDI-TOF/MS analysis.

Glycoblotting [45-47] is a standardizable technology in high throughput glycomics. It was developed in our laboratory to facilitate the glycan enrichment process of highly complicated mixtures such as bodily fluids, cells, tissues, and organs of both human and knock-out mice. The purpose of this enrichment process was to assess changes in the *N*-, *O*-, GSL-glycans expression

levels of PTMs in various human diseases when compared with those of normal controls. This new operational method has been applied to large scale structural and functional analysis of protein glycosylation [48-51], diseases related to glycan biomarker exploration [36-39,52], and to reveal the trends and complex patterns of glycans in diverse taxonomic groups of different bird species [53]. It seems likely that MS-based glycome analysis is one of the rapid and sensitive methods to carry out large-scale analyses for the discovery of disease-relevant biomarkers [54]. Achievements stemming from a multitude of samples studies have encouraged us to extend our efforts to estimate the structural composition and total glycome expression levels in HD via glycoblotting-assisted MALDI-TOF/MS analysis and avidin-biotin complex lectin staining [55]. We were able to observe a difference in structure and in the amount of glycomes between HD transgenic and control group mice. As a touchstone of the neurodegenerative disease, total glycomics of HD can serve a blueprint to profile the glycomes of either environmentally or genetically based CNS disorders viz., Alzheimer's, and Parkinson's disease, spinocerebellar ataxia, and amyotrophic lateral sclerosis. Such an achievement would be a great step towards the discovery of new glyco-biomarkers for this particular class of diseases.

2. Material and Methods

2.1. Animal

Animal experiments were conducted under the guidelines and with the approval of the committee on animal experiments of Hokkaido University. B6CBA-Tg (HDexon1) 62Gpb/1J, aka R6/2, transgenic mice expressing the *N*-terminal region of a mutant human huntingtin gene [7] were purchased from Jackson Laboratory. R6/2 mice were bred by ovarian transplantation to B6CBAF1 (BCF1) mice by mating with a BCF1 male. The length of the trinucleotide repeat was monitored by PCR amplification and gel electrophoresis of the tail DNA of the offspring, and was determined to contain 120-150 repeats. Mice were group housed in individually ventilated shoebox cages with paper bedding and a filter top. Food and water were available *ad libitum*. The neurological phenotypes of the R6/2 mice appeared between 6-9 weeks of age and the mice died at 14-18 weeks. Even though the phenotype is very complex, there are a number of components of the motor disorders. These include resting tremors, movements resembling chorea, stereotypic involuntary movements, mild ataxia, dyskinesia of the limbs. As the disease progresses mice are frequently observed to eat without gaining weight or, in more severe cases, emaciated with an overall loss of muscle bulk [7]. In this study, we used 3 mice of each sex (HD transgenic and control mice) for brain tissues glycome analysis, 5 mice of each sex (HD transgenic and control mice) for serum glycome analysis and all experiments were performed twice.

2.2. Reagents and equipments

TCEP, trypsin, PAS kit, Mayer's heamatoxylin, DTT, ConA- and SSA-biotin conjugated lectins were purchased from Sigma-Aldrich. PNGase F was purchased from New England Biolabs® Inc.

(Ipswich, Massachusetts, USA). Ammonium carbamate (99%) and 1-propansulfonic acid-2-hydroxyl-3-myristamido (PHM) were obtained from Tokyo Kasei (Chuo-ku, Tokyo, Japan). Chitooligosaccharide mixture was from Seikagaku Biobusiness (Tokyo, Japan). BlotGlyco[®]H bead was purchased from Sumitomo Bakelite (Tokyo, Japan). MTT, *O*-benzylhydroxylamine hydrochloride (BOA) and disialylated biantennary oligosaccharide (A2; (Hex)₂ (HexNAc)₂ (NeuAc)₂ + (Man)₃ (GlcNAc)₂) were purchased from Tokyo Chemical Industry Co., Ltd. Somnopentyl[®] (Pentobarbital sodium) and Agarose LO3 were purchased from Kyoritsu Seiyaku (Tokyo) and Takara Bio, Inc (Otsu), respectively, of Japan. DSA-, LCA-, PHA-E₄-, and PNA-biotin-conjugated lectins were from J-OIL MILLS, Inc (Chuo-ku, Tokyo, Japan). VECTASTAIN[®] Elite ABC and ImmPACT DAB (3, 3-diaminobenzidine tetrahydrochloride) Peroxidase Substrate Kits were from Vector Laboratories (Burlingame, CA, USA). Immunopen, other reagents and solvents were obtained from Wako Pure Chemicals (Tokyo, Japan) and Kanto Chemical Co., Inc., unless otherwise stated.

MultiScreen Solvinert filter plates and Mass PREP[™] HILIC μ Elution plates were purchased from Millipore and Waters, respectively. The ozone generator used (NG81-NO3, T-153) was from Nigorikawakogyo, Japan. Slicer VT1000S was obtained from Leica Microsystems Co. Ltd. Our fluorescence digital microscope (BA210EINT) was obtained from Shimadzu (Tokyo, Japan), and the automated glycan-processing machine (Sweetblot) was obtained from Systems Instruments Corporation (SIC). All mass measurements were performed using MALDI-TOF/MS (Ultraflex III, Bruker Daltonics, Germany).

2.3. Perfusion of mice

For lectin staining, each mouse was injected intraperitoneally with 0.1 mL Somnopentyl® for using a 26G½ inch needle. The mice were subsequently observed until they fell asleep to avoid vasoconstriction reflex and ensure that the each mouse survived. Each mouse was fixed on the surgical table with a plastic vat and the abdominal skin was cut with scissors and stripped. The abdominal wall, diaphragm, and costal bone were cut to expose the heart. After the peristaltic pump was switched on and a steady flow was confirmed, the right atrial appendage was cut and the needle was quickly inserted into the left ventricle. Perfusion was performed for 10 mins with a fixing solution (4% paraformaldehyde in 0.1 M phosphate buffer, PB) by a peristaltic pump adjusted to a flow rate of 10 mL/min, until the internal thoracic artery and liver were whitish in color, and the body became rigid. After the neck, head skin and cranial bone were cut, the brain was removed, placed in a tube containing a fixing solution and stored at 4°C. For glycoblotting, perfusion was done with PBS instead of fixing solution. For some experiments using unperfused brain tissues, tissues were recovered from the mice immediately following euthanasia by cervical dislocation. The brain tissues for glycoblotting were stored at -80°C until they were used [55].

2.4. Embedding, slicing and lectin staining (avidin-biotin complex method)

The desired portion of the perfused mouse brain tissues (striatum and cortex) were prepared and placed on a glass dish. A 2% agarose LO3/0.1 M PB was poured onto the brain tissue and the gel was allowed to solidify. Tissue slicing was carried out using a LECIA VT1000S slicer (Speed, 7; Frequency, 6.5; and Feed, 40 µM) after which the slices were stored at 4°C in a 24-wells dish containing 0.1 M PB/0.1% NaN₃. The tissue slices were then washed with PBS and air dried. Each slide was marked with an Immunopento to minimize the wastage of antibody and to ensure an efficient immunoreaction. Tissue sections were suspended in a 700 µL biotin-labeled lectin

solutions (30 µg/mL, except ConA was 10 µg/mL) and incubated for 1 hr on ice. Each slice was then washed three times with PBS over a period of 5 mins and was incubated with ABC reagent (700 µL, VECASTAIN[®] EliteABC kit standard) for 30 mins. PBS wash was repeated as described above. The tissue was incubated with ImmPACT DAB peroxidase substrate (700 µL) for 2 mins followed by washing in two changes of water in 5 min intervals. Following counter staining with Mayer's haematoxyline for one minute, the tissue was washed with tap water for 5 mins. Next, the tissue was dehydrated by passing through 70, 80, 90 and 100% ethanol and xylene washes, successively and, in 5 mins intervals for each concentration. DAB-labeled slides were mounted in a non-aqueous mounting medium (Entellan New, Merck Millipore, Germany) using a light microscope. Both the striatum and cortex were viewed at 40X, 100X, and 400X [55]. PAS staining was performed based on the manufacture's protocol. Briefly, tissue sections were deparaffinized and hydrated in deionized water. The slides contained tissue sections were immersed in periodic acid solution for 5 mins at room temperature. The slides were rinsed in several changes of distilled water and were immersed in Schiff's reagent for 15 mins at room temperature. After the slides were washed in running tap water for 5 mins, the slides were counter stained in Hematoxylin solution for 90 seconds. The slides were rinsed in running tap water, dehydrated, and cleared. Finally, the slides were mounted in xylene-based mounting medium in a same manner as described above.

2.5. Mouse serum preparation

Blood was collected from the heart of 12-week-old mice (n=5 of each sex of HD transgenic and control mice). Briefly, the mice were restrained and the heart was opened after the abdominal bone, diaphragm, and costal bone were cut. About 400-500 µL of blood was collected using 26G½ syringes while the heart was beating. The blood was allowed to clot at 4°C for 16 hours.

Then, the clotted blood was centrifuged at 5,000 rpm for 5 mins. The supernatant (serum) was carefully transferred to a new Eppendorf tube and stored at -80°C until the day of use.

2.6. Releasing *N*-glycans from brain tissue glycoproteins

Prefused mouse brain tissue (n=3 of each sex for HD transgenic and control mice), sub-fragmented into 2-3 mm squares with razor, was homogenized in a lysis buffer (2% SDS, 5 mM TCEP in 0.1 M Tris-acetate, pH 7.4, 100 mg/mL) using a Polytron at 25,000 rpm over a 30 second period three times on ice. The lysates were centrifuged at 20,400 g for 20 mins at 4°C. The amount of protein was quantified from 5 µL of the homogenates via the Bicinchoninic acid (BCA) method using a Pierce[®] BCA protein assay kit (prod# 23227, Thermo Scientific, USA). Ice-cold acetone (in an amount four times the volume of homogenates) was added and incubated over night at -20°C in order to precipitate proteins. The precipitate was collected by centrifugation at 20,400 g for 20 mins at 4°C and washed with 500 µL ice-cold acetonitrile. Next, precipitate was centrifuged as described above and air dried for 5 mins. The precipitate was dissolved in freshly prepared ammonium bicarbonate (ABC, 50 µL, 200 mM) containing 0.1% 1-propanesulfonicacid-2-hydroxyl-3-meristamido (PHM) in 10 mM ABC and the resulting solution was incubated at 37°C for 10 mins in a rotary shaker. Proteins were reduced by 120 mM 1, 4-dithiothriitol (DTT) at 60°C for 30 mins and were alkylated using 123 mM iodoacetamide (IAA) at room temperature in the dark for 1 hour. The mixture was digested using 10 µL trypsin (40 U/µL in 1 mM HCl) at 37°C overnight followed by heat-inactivation of the enzyme at 90°C for 10 mins. After cooling to room temperature, 2 U PNGase F was added and incubated at 37°C overnight. The sample was dried by SpeedVac and stored at -30°C until the day of use. Protein concentration was adjusted to 100 µg using de-ionized water and applied to glycoblotting, as described below.

2.7. *N*-glycans release from mouse serum glycoproteins

Mouse serum (n=5 of each sex of HD transgenic and control mice) was pretreated for the release of *N*-glycans and followed by glycoblotting in order to enrich the *N*-glycans prior to MALDI-TOF/MS and TOF/TOF analysis. Briefly, 10 μ L of mouse serum was diluted six times in freshly prepared ABC (0.33 M)-PHM (0.4% in 10 mM ABC)-DTT mixture (120mM) at a 1:1.5:1 (v/v) ratio. Internal standard (A2amide, 110 pmole) was added and the mixture was incubated at 60°C for 30 mins. Iodoacetamide (IAA, 10 μ L of 123 mM) was added and incubated at room temperature for 1 hour in the dark. Trypsin (5 μ L, 40 U/ μ L in 1mM HCl) was added and the mixture was incubated at 37°C for 1 hour. The digested mixture was heat inactivated (90°C, 10 mins) and cooled to room temperature. PNGase F (5U) was added and incubated at 37°C for 2 hours. 20 μ L of digested mixture was applied directly to glycoblotting protocol.

2.8. Releasing *O*-glycans from brain tissue and serum glycoproteins

Sample preparation and trypsin digestion were performed as previously described. Ammonium carbamate ('a' +5mg, where 'a' is the volume original homogenate used) was added to enzyme digested mixture and incubated at 60°C for 40 hrs. The digested mixture was subsequently washed (four times with 500 μ L water) and neutralized (one time with 500 μ L 150 mM acetic acid) with each process being accompanied by drying via SpeedVac. The dried sample was stored at -30°C until use. 400 μ g of protein dissolved in deionized water was used for glycoblotting protocol.

2.9. Releasing glycan moieties from brain tissue GSLs

Perfused and subfragmented mouse brain tissue (n=5 of each sex of HD transgenic and control mice) was homogenized in PBS (20 mg/mL) using the same method described previously. The

lysates were centrifuged at 100 g for 10 mins at 4°C and the supernatant was transferred into another 15 mL Falcon tube. The precipitate was dissolved with 600 µL of PBS and sonicated. The mixture was centrifuged at 800 g for 10 mins at 4°C and the supernatant was mixed with the first homogenates. The whole homogenates were centrifuged at 13,000 g for 10 mins at 4°C and the resulting precipitate was again dissolved in 500 µL PBS. 5 µL of this mixture was used for the BCA assay. The remaining solution was centrifuged (13,000 g, for 10 mins at 4°C). The precipitate was dissolved, successively, with a mixture of 1 mL chloroform/methanol to produce three solutions (1:2, 1:1 and 2:1 v/v). Each solution was subsequently sonicated for approximately 10 mins. Each extraction was followed by centrifugation at 800 g and 4°C and the resulting supernatants were collected in another 15 mL Falcon tube. The resulting supernatants (total lipids) were dried at room temperature via SpeedVac. The total lipids were suspended in 1 mL chloroform/methanol (1:1 v/v) and sonicated. Ozone was generated from oxygen using a benchtop generator (NG81-NO3, Nigoriwakogyo, Japan) at a flow setting of 1 mL/min. The output from the ozone generator was bubbled for 5 mins and the reaction was quenched by passing N₂ gas for a minute. The pipettes were rinsed with CHCl₃/MeOH (1:1 v/v), and the resulting solution was dried by SpeedVac. Next, MeOH:0.5 M NaOMe/MeOH (4:1 v/v) was added to the dried sample and allowed to stand for 30 mins at room temperature. The reaction mixture was transferred to a 1.5 mL eppendorf tube, neutralized with 6 µL concentrated acetic acid and then dried. A sample equivalent to 500 µg of protein adjusted with de-ionized water was used for glyco blotting protocol.

2.10. Releasing glycan moieties from serum GSLs

A 100 µL of freeze-dried serum was used to analyze serum glycan moieties released from GSLs. Briefly, GSLs were extracted in 3 mL of chloroform:MeOH mixtures at 1:2, 1:1, 2:1 (v/v) and

achloroform:MeOH:water mixture at 2:5:1 (v/v). Each step was accompanied by sonication for 10 mins and centrifugation at 800 rpm for 10 mins. The resulting supernatants of each extraction were collected together in another 15 mL Falcon tube. The remaining steps were performed in accordance with the procedure of brain GSL-omics described above.

2.11. Glycoblotting (enrichment, on bead esterification, and labelling total glycomes)

500 μ L of BlotGlyco[®] H beads (10 mg/mL) was added to the wells of MultiScreen Solvinert filterplate and the water was removed by vacuum. Digested mixtures of brain tissue (20 μ L) containing 100-500 μ g protein (specified in each *N*-, *O*-, and GSL-glycan analysis) and serum containing IS (A2amide for *N*-glycans, GN4 for *O*- and GSL-glycans, which accurate concentration was known) were added together to each well along with 180 μ L of 2% AcOH/Acetonitril (ACN). The plate was incubated at 80°C for 45 mins followed by two successive washings using 200 μ L each of 2M guanidine-HCl in 16.6 mM ABC and, water and 1% triethylamine in MeOH. After unreactive hydrazide functional groups were acetyl capped using 10% acetic anhydride in MeOH at room temperature for 30 mins, the remaining acetic anhydride was removed by vacuum. Each well was washed two times with 10 mM HCl, MeOH and dioxane, consecutively. 150 mM MTT was added and incubated at 60°C for 90 mins for on bead methyl esterification. Next, washing was performed using 200 μ L of dioxane, water, methanol and water. For an effective transimination reaction, 20 mM Na (aminoxyl) acetyl tryptophanarginine methyl ester (aoWR) (for brain tissue *N*- and serum *O*-glycans) and 50 mM *O*-benzylhydroxylamine (BOA) (for serum *N*-, brain tissue *O*- and GSL-glycans) were added and incubated at 80°C for 45 mins. Labeled glycans were eluted with 100 μ L water. The aoWR-tagged solution was further purified using a hydrophilic interaction chromatography purification plate (Mass PREP[™] HILIC μ Elution Plate, Waters, MA). The resulting glycan solutions were

concentrated by SpeedVac and subsequently dissolved in 10 μ L of distilled water. In general, BOA-based labeling should be employed due to its chemical stability, easy handling, and lower cost. However, it is known that use of aoWR reagent often gives much improved MS spectra because of its higher ionization potential than that of BOA reagent [47,56]. In the present study, we used aoWR labeling in cases for brain tissue N-glycans and serum O-glycans enrichment processes in order to purify tagged tissue N-glycans from highly complicated mixtures containing heavy contaminations and extremely low concentration of serum O-glycans, respectively.

2.12. Mass spectrometric analysis

The recovered glycans from mice brain tissues and serum, which were dissolved in 10 μ L water, were analyzed after being spotted on MTP 384 target plate (Polished steel TF, Bruker Daltonics) and crystallized together with matrices [10 mg/mL DHB in 30% ACN for aoWR-labeled glycans, and DHB/DHB- Na^+ (10 mg/mL DHB- Na^+ in 30% ACN, 9:1) for BOA-labeled O- and GSL-glycans]. MALDI-TOF/MS data were acquired on an Ultraflex III (Bruker Daltonics, Bremen, Germany) equipped with a reflector and controlled by the FlexControl 3.0 software package according to the general protocol. All spectra were obtained using reflectron mode with an acceleration voltage of 25 KV, a reflector voltage of 26.3 KV, pulsed ion extraction of 160 ns in the positive mode and sum up to 2,000 shots for each spot. Further fragment analysis was performed by MALDI-TOF/TOF analysis (Bruker Daltonics, Bremen, Germany). Annotation of the MS and TOF/TOF data was done using the FlexAnalysis 3.0 software package (Bruker Daltonics GmbH, Bremen, Germany, S/N = 6 and quality factor threshold of 30). The experimental masses were used to predict possible glycan compositions reported on <http://web.expasy.org/glycomod/> and CFG (<http://www.functionalglycomics.org>). The Lipid Bank (<http://lipidbank.jp/>) and in-house databases were used to estimate GSL-glycan structures.

Glycan profiling was performed basically by focusing on exclusively known glycan structures sourced from the brain/nervous system and serum of *Mus musculus* and *Ratus norvegicus* and other mammals (especially known glycoforms sourced from human brain). After the peaks for the detected glycans were selected, absolute quantification was performed by comparative analysis, i.e., the area under each isotopic peak was normalized to the internal standard. Glycan expression levels were determined analytically as HD transgenic (R6/2) versus the control group (BCF1) (n=3 and 5 for brain tissue and serum, respectively, and N=2, where n and N are the number of samples and experiments, respectively) and the corresponding statistical significance was calculated using the student t-test.

3. Results

3.1. Strategy of total glycomics of HD transgenic (R6/2) and WT (BCF1) mice

The comprehensive glycomics of 12-week-old HD transgenic mice (R6/2) and the WT littermate (BCF1) mice were performed by a general protocol outlined in Figure 1. Firstly, the brain and serum proteins (for *N*- and *O*-glycomics) were directly solubilized, reduced and alkylated. Preparation of tryptic digested glycopeptides facilitated the release of *N*- and *O*-glycans by means of peptide *N*-glycosidase F (PNGase F) digestion and non-reductive β -elimination, respectively. Likewise, GSLs were extracted from the brain tissue and serum using a chloroform/methanol mixture. Glycans were released from GSLs using ozonolysis and alkaline degradation. All glycan analysis followed the same glycoblotting process, viz., released glycans were selectively captured onto high density hydrazide beads (BlotGlyco[®] H) for highly efficient purification of oligosaccharides followed by on-bead methyl esterification to stabilize sialic acid(s) for the simultaneous quantitation of neutral and sialylated oligosaccharides by MALDI-TOF/MS. Finally the labeled oligosaccharides were recovered as aoWR or BOA derivatives *via* imine exchange that enabled highly sensitive and quantitative analysis via MALDI-TOF/MS. It is noteworthy that the efficient ionization of glycans after aoWR derivatization and following selective capturing and HILIC purification of glycans enhanced the removal of impurities in case for brain tissue *N*-glycomics and drastically improved the signal-to-noise ratio. This was found to be especially effective for higher molecular weight oligosaccharides such as multi-antennary *N*-glycans [47] and extremely low concentration of serum *O*-glycan analysis [50]. Glycans were measured by MALDI-TOF/MS as a single proton and sodium adduct molecular ions ($[M + H]^+$ and $[M + Na]^+$ for aoWR and BOA labeled, respectively). The results of these analyses were used to select target ions for further analysis by tandem TOF/TOF to investigate possible

structures, sequence positions and branching patterns. However, it should also be noted that, since MS-based analyses can provide only possible *N*-glycans by estimating sugar compositions predicted using known *N*-glycome database, further NMR-based structural characterization using synthetic standards may be necessary to determine precise structures with respect to stereochemistry and branching positions in the glycoside linkages.

3.2. Brain *N*-glycomics analysis of HD transgenic and WT mice

Using glycoblotting-based sample preparation combined with MALDI-TOF/MS analysis of brain total glycomics of HD transgenic (R6/2) and WT mice (BCF1), we could efficiently estimate the type of *N*-, *O*-, GSL-glycan moieties. As a preliminar trial, we first analyzed the whole brain (20 mg randomly taken) of perfused and unperfused BCF1 mice to assess the dominant glycans present in the brain, enabling us to establish an in-house database, and to exclude the possible impingement of *N*-glycans from serum. While perfused males BCF1 (BCF1 ♂) and unperfused males BCF1 (BCF1 ♂) were found to have 240.71 ± 53.22 and 20.45 ± 7.26 pmoles, respectively, perfused females BCF1 (BCF1 ♀) and unperfused females BCF1 (BCF1 ♀) were found to have 591.07 ± 141.67 and 173.54 ± 39.45 pmoles, respectively ($p < 0.01$ for male and $p < 0.05$ for female mice). We have found significant differences in the amount of *N*-glycans found in the perfused compared to the unperfused mouse brain tissues. Only trace amounts of terminal sialic acid containing *N*-glycans were found in the perfused mice as compared to the unperfused mice. Surprisingly, total *N*-glycan expression levels in the brain tissue were found to be higher in female BCF1 mice as compared to males in both the perfused and unperfused groups when the glycoblotting protocol was used directly for whole brain tissues in which the striatum and cortex had not been separated. We also found that the *N*-glycans intra-mice deviation was reduced in the perfused mice relative to those of the unperfused mice. As a result,

we decided to employ isolated striatum and cortex section from the perfused mouse brain tissues for total glycomics of HD transgenic and the control group mice.

We have estimated 75 and 78 *N*-glycans in the striatum and the cortex, respectively, in which 67 of those were common between the two. There were 9 striatum-specific [Peak #s 48, 52, 56, 63, 69, 74, 78, 83 & 84] and 11 cortex specific [Peak #s 45, 47, 58, 72, 77, 80, 81, 82, 85, 86, 87] *N*-glycans. All 61 peaks in the striatum (80.3%) and 64 peaks in the cortex (82.1%) were reported in the GlycosuitDB (<http://www.glycosuitdb.expasy.org/results>), as summarized in Table 1. We determined, using database sample sources, that more than 85% of those *N*-glycans were sourced from the brain of the *M. Musculus* and *R. norvegicus* species.

From MALDI-TOF/MS analysis, we are able to estimate high mannose and complex types of *N*-glycans with the latter existing in bi-, tri-, and tetra-antennary forms, most of which are highly core-fucosylated and have bisecting GlcNAc (Figure 2). Even though quantitatively small in the whole brain, sialylated *N*-glycans were also present with high amount in the cortex than in the striatum (details are shown in section 3.3). Although Lewis^x (Le^x) and sialyl Lewis^x (sLe^x) structures could be considered from the compositional analysis, further structural characterization is needed to confirm the existence of these specific antigenic oligosaccharides. While terminal GalNAc residues (peak #s 34, 45, 53, 56, 61, 68) also seem to exist, there was no direct evidence for the presence of NeuGc in HD transgenic and WT mice in the present study. We performed all glycomics experiments independently and at least two times. The molecular weights and estimated compositions of *N*-glycans of the striatum and cortex of the HD model and its control mice were tabulated in Table 1, peak numbers of striatum and serum *N*-glycans were listed in supporting information (Figure S1) and differences in the *N*-glycans expression

levels in the striatum, cortex and serum of each mouse are given in supporting information (Table S1).

For quantitative analysis, the area of *N*-glycans detected in MS were normalized with a known concentration of IS (20 pmoles of A2 amide, namely non-natural amidated A2 prepared originally in our laboratory). There were no significant differences in *N*-glycan expression levels in the striatum and cortex of HD transgenic and control group mice (Table 2). However, the groups differ with respect to a few *N*-glycans that were specific to brain regions and gender as summarized in supporting information (Table S1).

3.3. Glycotyping analysis of brain *N*-glycans

To facilitate the glycome profiling analysis and their changes between HD transgenic and control group mice, we applied the glycotyping analysis approach [49,53,57]. Here, we focused on the high mannose, fucosylated, bisecting-GlcNAc and antennary type of complex *N*-glycan moieties. Typically, we found high mannose (>50%), core fucosylated (>28%), bisecting GlcNAc (>35%) and biantennary (>32%) as major types of *N*-glycans in the striatum and cortex of HD transgenic and WT mice of both sexes. While the high mannose types were decreased, those of core fucosylated, bisecting-GlcNAc and biantennary types of *N*-glycans were increased in the HD transgenic mice compared to that of the WT mice, albeit these numbers statistically insignificant (Table 3). Using TOF/TOF analysis, possible major glycoforms were high mannose, core-fucosylated, biantennary and bisecting-GlcNAc types of *N*-glycans (supporting information, Figure S2 A-C). Moreover, hybrid and complex types of *N*-glycans with bisecting-GlcNAc show unfragmented HexHexNAc and Hex₂HexNAc₂ (peak #s 7, 12, 14, 21, 23, 30) under the laser irradiation during the TOF/TOF analysis. This result might indicate that the bisecting GlcNAc

residue both found in the hybrid and complex type *N*-glycans contribute significantly to the stability of these *N*-glycans under high energy laser irradiation in the TOF/TOF fragmentation processes. Interestingly, it was also demonstrated that Golgi α -mannosidase, one of the key exoglycosidases in a general biosynthetic pathway of the hybrid and complex type *N*-glycans, can not remove non-reducing Man residues of the key intermediates in the presence of the neighboring bisecting GlcNAc residue [19]. It seems likely that interaction of this GlcNAc with outer-chain sugar residues as well as steric effect might be critical for such unique resistance observed both in the TOF/TOF and enzymatic fragmentation experiments.

3.4. Serum *N*-glycomics of HD transgenic and WT mice

Towards the discovery of a potential glyco-biomarker for easy diagnosis and therapy, we extended efforts to profile and quantify *N*-glycans from the sera of HD transgenic and WT mice (n=5, N=2, where n and N are the number of samples and experiments, respectively). As estimated, 58 (plus 8 with same m/z with different composition) *N*-glycans were found in serum, 51 peaks (85%) of which were reported in the GlycosuitDB (<http://www.glycosuitdb.expasy.org/results>). All of the peaks (m/z) specific to serum and common with brain *N*-glycans composition are shown in Table 1 and the spectra containing peak numbers are shown in supporting information (Figure S1).

Quantitatively, the amount of *N*-glycans released from the serum of BCF1 ♂ and ♀ is shown in Table 2. *N*-glycans decreased in male and female HD transgenic (with the female decrease being slightly less pronounced) as compared to the WT mice. We have selected a few peaks that show a significant difference in *N*-glycan expression levels between HD transgenic and WT mice (Table 4). The largest peak in the serum *N*-glycan level (peak#42) is higher in the control group than in the HD transgenic mice, and the peak of the fucosylated form of this glycan (peak #48) is

higher for the HD transgenic mice. We specified peak 42 using TOF/TOF analysis (supporting information, Figure S3). From glycotyping analysis, we have estimated that core-fucoylated *N*-glycans are higher in HD transgenic than the control group mice (increased from 12.1 to 38.6% in male and 29.9 to 43.8% in female mice, Figure 3). Moreover, high levels of disialic acid (majority of *N*-glycolylneuraminic acid) containing biantennary type *N*-glycans were found in the HD transgenic and WT mice. However, the levels of high mannose and bisecting type *N*-glycans were small compared to those in the striatum and cortex of the same experimental mice (data not shown).

3.5. Avidin-biotin complex lectin staining of brain tissue sections

We applied avidin conjugated lectins specific to high mannose (ConA), core-fucosylated (LCA), bisecting-GlcNAc (PHA-E₄), LacNAc (DSA), terminal sialic acid (SSA) and non-sialylated T-antigen (PNA) to study the localization of glycoconjugates in brain tissue (supporting information, Figure S6). Around the nucleus, we were able to observe less intensely stained regions of the striatum and cortex of R6/2 by ConA (highly pronounced in male R6/2), the striatum of R6/2 ♂ and cortex of R6/2 ♀ by LCA, and both the striatum and cortex of R6/2 ♀ by PHA-E₄ as compared to those of the WT mice. In addition, SSA and PNA staining both in the striatum and cortex confirmed the presence of the terminal sialic acid and *O*-glycans, respectively. In general, there were significant differences in the staining intensity with the lectins in the brain sections of HD transgenic and WT mice dependent on the total amount of *N*-glycans expression levels. However, the results stained by means of LCA and PHA-E₄ did not support the specific glycoform alterations suggested by the comprehensive glycomics described above, while the ConA staining clearly indicated significant changes found in *N*-glycan analysis. Conventional PAS approach appeared to give non-specific staining in all brain regions.

3.6. *O*-glycomics of HD transgenic and WT mice

We profiled the possible mucin type *O*-glycans from the brains and sera of the HD transgenic and WT mice. The total amount of mucin type *O*-glycans in the striatum and cortex was found in increased levels in male and decrease in female HD transgenic mice (Table 2 and supporting information, Table S2). The estimated composition of mucin type *O*-glycans in the striatum, cortex and serum were summarized in supporting information Table S2A. Regarding the brain tissue, Tn antigen (HexNAc, m/z 349.11) was only found to have decreased in the cortex of R6/2 ♀ compared to the same section of BCF1 ♀. Core 1 or T antigen (HexNAcHex, m/z 511.22) was exclusively detected in the HD transgenic mice (supporting information Table S2 A). Core 3 (HexNAc₂, m/z 552.2) increased in R6/2 ♂ in both the striatum and cortex whereas it decreased in R6/2 ♀. Levels of HexNAcNeuAc (m/z 640.3), which is supposed to be the final product that can no longer be an acceptor for other glycosyltransferases, increased in the HD transgenic mice, except in the cortex of female R6/2 mice (Supporting information Table S2 B).

From 100 μ L of serum, mucin type *O*-glycans levels were found in decreased amounts in the HD transgenic mice, albeit the decrease is statistically insignificant. However, core 1 (HexHexNAc) decreased in R6/2 as compared to BCF1 and, core 2 (Hex₁HexNAc₂) was not detected in the HD transgenic mice. In addition to mucin type, we also found other modification such as *O*-mannosyl, *O*-fucosyl, and *O*-GlcNAcylation, all of which are potential markers (data not shown).

3.7. Glycosphingolipidomics of brain tissue of HD transgenic and WT mice

The carbohydrate moieties of unprotected (native) GSLs were released by extraction of total lipids from the brain tissue and serum, with subsequent liberation by ozonolysis giving rise to GSL-aldehyde and glycan release by alkaline degradation [58]. Using the glycoblotting technique, GSL-glycans were efficiently captured and further structural estimation and

quantitative analysis by MALDI-TOF/MS and TOF/TOF analysis was performed. From the brain tissue, we have profiled GM3, GM2, GM1, GD1a, GD2 (except in striatum of male mouse), GA1 (only in the cortex of both sexes) and Type IIA glycosyl ceramide (only in the striatum of male mice). With the exception of the cortex of R6/2♂, the total amount of oligosaccharides released from GSLs increased in the striatum and cortex of the R6/2 compared to that of BCF1 (Table 2). Focusing on a-series gangliosides expression levels, the relative abundance was found to be GD1a >> GM1 > GM2 > GM3. GD1a is exclusively increased in the striatum (brain region that is most severely affected by HD) (Table 5). The possible sequence of GD1a is shown in supporting information Figure S4. Ganglioside expression levels were found to differ between male and female mice as well as between intra-brain regions.

3.8. Serum glycosphingolipidomics

According to the same approach used in the brain GSL-omics, the amounts of serum gangliosides (containing NeuAc and NeuGc) and globo-series were estimated (Figure 4, supporting information Figure S5). The compositions and amounts of GSL-glycans (pmoles $\pm$ SD) are summarized in Table 6. Total GSL-glycans were decreased by 39% and 36% in male and female HD transgenic mice, respectively, compared to that of the control group mice. This reduction was highly pronounced for NeuGc-containing a-series gangliosides, such as GM2-NeuGc, GM1-NeuGc and GD1a-NeuGc. GM2-NeuGc was found to be the most abundant ganglioside (>76%), decreasing 32% and 40% in male and female HD transgenic mice, respectively, compared to that of the control group mice. From these results, we determined GM2-NeuGc to be a promising candidate as a potential glyco-biomarker in HD and should be studied further.

4. Discussion

Neurodegenerative diseases are, collectively, the leading cause of disability in the elderly, and Huntington's disease is a hallmark of genetic disorders. In most cases, scientists have chosen diagnostic techniques based on physical examinations such as CT, PET, MRI, and fMRI scans, immunohistochemistry and neurochemistry, all of which are used to determine the levels of amino acids, lipids, small peptides and metabolic pathways in brain [59-62]. When A. B. Young predicted [59] that, within the next 40 years, "we will have silver bullets for neurodegenerative diseases," she targeted biomarkers by blood proteomics and/or metabolomics to be used as screening tools and predictors of subsequent diseases. We targeted the glyco-biomarkers that are enzymatically or chemically released from glycoproteins and glycolipids. Thematically, we proposed and established the *total glycomics* of R6/2 and BCF1 for comprehensive structural estimation and quantitative analysis of total glycomes, viz., *N*-, *O*-, and GSL-glycans. To the best of our knowledge, this is the first study on the total glycomics of Huntington's disease.

From the whole brain *N*-glycomics, we found no significant difference in the amount of *N*-glycomes expression levels between R6/2 and BCF1. However, in serum, *N*-glycans were found in decreased levels in HD transgenic mice. Core-fucosylated, bisecting-GlcNAc and biantennary complex type *N*-glycans were slightly increased in the brain tissue of HD transgenic mice. We performed a glycan-type focus lectin staining to crosscheck *N*-glycan analysis and confirmed that the localization of glycoconjugates in brain tissue were not significantly different between HD transgenic and WT mice. Conventional PAS showed no specific stained regions in brain tissue of HD transgenic and WT mice.

The total amount of *O*-glycans found in the striatum and cortex was elevated in males and lower in females from the R6/2 group. We found that the amount of *O*-glycans was reduced in the serum of R6/2. The most dominant mucin type of *O*-glycan in the brain tissue was found to be core 3 (GlcNAc β 1 $\rightarrow$ 3GalNAc) which was elevated in R6/2 ♂ and lower in R6/2 ♀ compared to the corresponding BCF1 group. Consequently, we believe that this particular *O*-glycan could serve as a promising mucin type *O*-glycan biomarker for HD pathophysiology. With respect to serum *O*-glycomics, Core 1 levels were decreased and, Core 2 was not detected in R6/2 at all.

The total amount of GSL-glycans detected in the striatum of male HD transgenic mice was three times higher than that for the control group mice. However, the glycans released from the cortex of the same HD transgenic mice were lower than those in the control group mice. The whole brain regions of female HD transgenic mice displayed higher levels of GSL-glycans relative to those of the WT mice. GD1a was drastically increased in the striatum of HD transgenic mice. Most gangliosides are synthesised by liver and secreted into the serum. Ganglioside composition can be altered as a result of liver disease of various etiologies. Since the serum ganglioside concentration, especially that of GM2-NeuGc, was markedly reduced in mice with metastatic VM tumors [63], our study can provide further evidence demonstrating the alteration of liver functions due to the effect of HD. Consequently, GM2-NeuGc in serum was determined as a potential glyco-biomarker in the ganglioside-family. In serum, GSL-glycans are more strongly expressed in male (both R6/2 and BCF1) as opposed to female mice. This difference in the glycan expression levels may be linked to sex hormones or gender sensitivity to the overexpression of circulatory HTT. The higher levels of gangliosides in HD model mice might be due to inadequate amounts of hexosaminidase in lysosomal compartments, suggesting that HD transgenic mice experienced the same symptoms of GSL lysosomal storage disease[19,64].

As stated early, it is likely that HD might affect significantly cortex as well as striatum, and also even other parts of the body. Comparison of the intra-brain region glycome may uncover the significance of the region-specific sensitivity of the neuronal and glial cells to the cytotoxic effect of the huntingtin aggregates. The total amount of *N*- and GSL-glycans were found in increased levels in the striatum and the cortex, respectively. The samples that were taken from the same mouse brain ($p < 0.001$). However, there was no significant difference in total amounts of *O*-glycans between the striatum and cortex (Figure 5A-C). The increase of *N*-glycans quantities in the striatum is related to the higher neuronal numbers [65]. Lower *N*-glycans and higher GSL-glycans expression levels in the cortex indicate selective vulnerability of the HD cortices (especially in the later weeks) and accumulation of gangliosides due to the low activity of specific hydrolase enzymes. The difference in total glycomes expression levels between male and female mice is a useful finding as it would facilitate sex focused diagnosis and therapeutic targets.

The decrease mechanism and biochemical effects of HD to other sequelae of diseases, including diabetes mellitus, changes in liver function and the aggregation of proteins in different cellular compartments (as characteristic neurodegenerative disease neuropathological lesions involve deposition of abnormal proteins) is area of great interest. In addition to glycome profiling of the brain, serum *total-glycomics* provides an alternative method for quantifying the glycome expression levels of HD to help assess the liver function. Furthermore, the alteration of specific glycomes can predict whether HD transgenic mice are predisposed for other sequela.

It has been known that an omnipresent huntingtin (HTT) protein is responsible for the aggregation of amyloid like proteins, termed as “protein-cancers”, in the bodies of mice. The gradual increase in the amount of aggregates significantly affects liver (the source of most serum

glycoproteins) and pancreas (where the whole sugar metabolism is governed) function. Some researchers believe that age-dependent HD neurological defects are accompanied by progressive decline in glucose tolerance that ultimately leads to diabetes mellitus and insulin deficiency [66]. This hypothesis is further supported by the observation that diabetes frequently develops in HD patients and transgenic mice [67]. In contrast, other researchers also claim that HD does not increase the possible risk of diabetes mellitus, albeit their results do not exclude changes in glucose tolerance in end-stage HD patients or in patients with juvenile onset HD [68]. The present result reveals that the expression levels of FUT8 mRNA were significantly increased in the liver and *N*-glycans possessing alpha1,6-fucose was increased in diabetic mice (db/db) relative to the db/+ of the control group [69]. Core fucosylation by FUT8 might increase the survival rate of HD transgenic mice. Miyoshi *et al.* reported that in HCC, chronic hepatitis and liver cirrhosis, the expression of FUT8 is quite low in comparison to that of a normal liver. Further, increased levels of fucosylated proteins in serum can be used as tumour markers because numerous serum proteins are produced in liver [70,71]. Combining with the current serum *N*-glycomics, it is considered that HD transgenic mice might share a similar glycosylation effect as that observed in case of the diabetic mice, in which the liver function might be influenced significantly by the progression of the disease. However, identifying the specific protein to which those glycans attach required further investigation.

5. Conclusion

In this study, we demonstrated the importance of the total glycomics of HD transgenic (R6/2) and WT mice (BCF1) using the glycoblotting method [35-41,47,56]. This technique enabled us to efficiently estimate the structure and quantify of the total glycomes derived from brain and serum glycoproteins and glycosphingolipids. Brain tissue and serum total glycomics were key in determining the amount of *N*-, *O*-, and GSL-glycans associated with post-translational modifications as well as the characterization of glycan repertoires as novel glyco-biomarkers. Accordingly, we determined that the trend and expression levels of total glycomes were significantly different between the HD transgenic and control group mice. Glycans play critical roles in nervous system development, regeneration and synaptic plasticity [13] and their structural richness makes promising candidates as potential disease markers [48]. Therefore, glycoblotting-based glycome profiling of HD transgenic mice is an alternative approach towards the identification of potential glyco-biomarkers specific to CNS disorders viz., Alzheimer's, Parkinson's, Spinocerebellar ataxia, and Amyotrophic lateral sclerosis. We believe the present study will be of great assistance in the field of neuroscience and future drug discovery.

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Table 1. Estimated composition of *N*-glycans from striatum, cortex and serum of R6/2 and BCF1. These peaks are common to both the disease and control groups in this study. The asterisk (*) shows peak number 16 and 23 containing NeuGc that are detected only in serum, not in brain tissues. GlcNAc, *N*-acetylglucosamine; Man, Mannose; Deoxyhexose, fucose; HexNAc, *N*-acetylhexoseamine (*N*-acetylglucosamine or *N*-acetylgalactoseamine depends on the description); Hex, hexose (Mannose, glucose and galactose depend of the description); NeuAc, *N*-acetylneuraminic acid; and NeuGc, *N*-glycolylneuraminic acid.

Brain and serum Common <i>N</i> -glycans		Brain	Serum
Composition	ExPasy MW	Peak #	Peak #
(HexNAc)1 + (Man)3 (GlcNAc)2	1095.40	3	1
(Hex)2 + (Man)3 (GlcNAc)2	1216.42	4	2
(HexNAc)1 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1241.45	5	3
(Hex)1 (HexNAc)1 + (Man)3 (GlcNAc)2	1257.45	6	4
(Hex)3 + (Man)3 (GlcNAc)2	1378.48	8	5
(Hex)2 (HexNAc)1 + (Man)3 (GlcNAc)2	1419.50	11	6
(HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1444.53	12	7
(Hex)1 (HexNAc)2 + (Man)3 (GlcNAc)2	1460.53	13	8
(Hex)4 + (Man)3 (GlcNAc)2	1540.53	15	9
(Hex)1 (HexNAc)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1548.54	17	10
(Hex)1 (HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1606.59	21	12
(Hex)2 (HexNAc)2 + (Man)3 (GlcNAc)2	1622.58	22	13
(HexNAc)3 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1647.61	23	14
(Hex)5 + (Man)3 (GlcNAc)2	1702.58	25	15
(Hex)2 (HexNAc)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1710.60	28	16*
(Hex)1 (HexNAc)1 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNAc)2	1710.60		
(Hex)2 (HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1768.64	31	19
(Hex)1 (HexNAc)3 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1809.67	33	20
(Hex)6 + (Man)3 (GlcNAc)2	1864.63	35	21
(Hex)2 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNAc)2	1913.68	41	23*
(Hex)1 (HexNAc)3 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1955.72	43	25
(Hex)2 (HexNAc)3 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1971.72	44	26
(HexNAc)4 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1996.75	45	27
(Hex)1 (HexNAc)4 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	2012.75	46	28
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2059.73	50	30
(Hex)3 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2075.73	52	31
(Hex)1 (HexNAc)3 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2101.78	53	32
(Hex)1 (HexNAc)4 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	2158.80	57	34

(Hex)1 (HexNAc)4 (NeuAc)1 + (Man)3 (GlcNAc)2	2157.78	58	35
(Hex)2 (HexNAc)4 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	2174.80	59	36
(Hex)2 (HexNAc)2 (Deoxyhexose)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2205.79	62	37
(Hex)3 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2221.79	63	39
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNAc)2	2350.83	70	46
(Hex)3 (HexNAc)4 (Deoxyhexose)3 (NeuAc)1 + (Man)3 (GlcNAc)2	2920.06	84	53

Brain specific *N*- glycans

Composition	ExPasy MW	m/z	Peak #
(Deoxyhexose)1 + (Man)3 (GlcNAc)2	1038.38	1486.6	1
(Hex)1 + (Man)3 (GlcNAc)2	1054.37	1502.6	2
(HexNAc)2 + (Man)3 (GlcNAc)2	1298.48	1746.7	7
(HexNAc)1 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1387.51	1835.8	9
(Hex)1 (HexNAc)1 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1403.51	1851.7	10
(HexNAc)3 + (Man)3 (GlcNAc)2	1501.56	1949.8	14
(Hex)1 (HexNAc)1 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1549.57	1997.8	16
(Hex)2 (HexNAc)1 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1565.56	2013.8	18
(Hex)3 (HexNAc)1 + (Man)3 (GlcNAc)2	1581.56	2029.8	19
(HexNAc)2 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1590.59	2038.8	20
(Hex)1 (HexNAc)3 + (Man)3 (GlcNAc)2	1663.61	2111.8	24
(Hex)1 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1694.60	2156.9	26
(Hex)3 (HexNAc)1 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1727.61	2175.9	29
(Hex)1 (HexNAc)2 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1752.64	2200.9	30
(Hex)3 (HexNAc)2 + (Man)3 (GlcNAc)2	1784.63	2232.9	32
(HexNAc)4 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1850.69	2298.9	34
(Hex)2 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1856.66	2318.9	36
(Hex)3 (HexNAc)1 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1873.67	2321.9	37
(Hex)3 (HexNAc)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1872.65	2334.9	38
(Hex)1 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	1897.68	2359.9	39
(Hex)2 (HexNAc)2 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	1914.70	2362.9	40
(Hex)3 (HexNAc)2 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	1930.69	2378.9	42
(Hex)7 + (Man)3 (GlcNAc)2	2026.69	2474.9	47
(Hex)3 (HexNAc)1 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2018.71	2481.0	48
(Hex)2 (HexNAc)2 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2060.76	2509.0	49
(Hex)3 (HexNAc)2 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	2076.75	2525.0	51
(Hex)1 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2100.76	2563.0	54
(Hex)2 (HexNAc)3 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	2117.78	2566.0	55
(HexNAc)4 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2142.81	2591.0	56
(Hex)8 + (Man)3 (GlcNAc)2	2188.74	2637.0	60

(Hex)1 (HexNAc)5 (Deoxyhexose)1 + (Man)3 (GlcNAc)2	2215.82	2664.1	61
(Hex)2 (HexNAc)3 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2263.83	2712.1	64
(Hex)2 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2262.81	2725.1	65
(Hex)3 (HexNAc)3 (Deoxyhexose)2 + (Man)3 (GlcNAc)2	2279.83	2728.1	66
(Hex)1 (HexNAc)4 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2303.84	2766.1	67
(Hex)2 (HexNAc)4 (Deoxyhexose)2 + (Man)3 GlcNAc)2	2320.86	2769.1	68
(Hex)2 (HexNAc)2 (Deoxyhexose)3 (NeuAc)1 + (Man)3 (GlcNAc)2	2351.85	2814.1	69
(Hex)2 (HexNAc)3 (Deoxyhexose)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2408.87	2871.1	71
(Hex)3 (HexNAc)3 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2425.89	2874.1	72
(Hex)2 (HexNAc)4 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2466.91	2915.2	73
(Hex)2 (HexNAc)2 (Deoxyhexose)2 (NeuAc)2 + (Man)3 (GlcNAc)2	2496.89	2973.2	74
(Hex)2 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNAc)2	2553.91	3030.2	75
(Hex)2 (HexNAc)4 (Deoxyhexose)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2611.95	3074.2	76
(Hex)3 (HexNAc)4 (Deoxyhexose)3 + (Man)3 (GlcNAc)2	2628.97	3077.2	77
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)3 + (Man)3 (GlcNAc)2	2641.93	3132.2	78
(Hex)3 (HexNAc)3 (Deoxyhexose)3 (NeuAc)1 + (Man)3 (GlcNAc)2	2716.98	3179.2	79
(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNAc)2	2715.96	3192.2	80
(Hex)2 (HexNAc)4 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNAc)2	2756.99	3233.3	81
(Hex)2 (HexNAc)5 (Deoxyhexose)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2815.03	3277.3	82
(Hex)3 (HexNAc)3 (Deoxyhexose)2 (NeuAc)2 + (Man)3 (GlcNAc)2	2862.02	3338.3	83
(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)3 + (Man)3 (GlcNAc)2	3007.06	3497.3	85
(Hex)4 (HexNAc)4 (Deoxyhexose)3 (NeuAc)2 + (Man)3 (GlcNAc)2	3373.21	3849.5	86
(Hex)4 (HexNAc)4 (Deoxyhexose)2 (NeuAc)3 + (Man)3 (GlcNAc)2	3518.25	4008.5	87

Serum specific <i>N</i> - glycans		Serum		
Composition	ExPasy MW	m/z	Peak #	
(Hex)1 (HexNAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	1564.54	1724.6	11	
(Hex)2 (HexNAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	1726.59	1886.7	17	
(Hex)1 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNAc)2	1751.62	1911.7	18	
(HexNAc)5 + (Man)3 (GlcNAc)2	1907.71	2053.6	22	
(Hex)2 (HexNAc)2 (NeuGc)1 + (Man)3 (GlcNAc)2	1929.67	2089.8	24	
(Hex)2 (HexNAc)4 + (Man)3 (GlcNAc)2	2028.74	2174.7	29	
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2075.73	2235.8	31	
(Hex)2 (HexNAc)3 (NeuAc)1 + (Man)3 (GlcNAc)2	2116.76	2276.8	33	
(Hex)2 (HexNAc)2 (NeuAc)2 + (Man)3 (GlcNAc)2	2204.77	2378.8	38	
(Hex)2 (HexNAc)2 (Deoxyhexose)2 (NeuGc)1 + (Man)3 GlcNAc)2	2221.79	2381.9	39	

(Hex)2 (HexNAc)2 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2220.77	2394.8	40
(Hex)4 (HexNAc)2 (NeuAc)1 + (Man)3 (GlcNAc)2	2237.78	2397.9	41
(Hex)3 (HexNAc)2 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2236.76	2410.9	42
(Hex)3 (HexNAc)3 (NeuAc)1 + (Man)3 (GlcNAc)2	2278.81	2438.9	43
(Hex)2 (HexNAc)3 (Deoxyhexose)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2319.84	2479.9	44
(Hex)4 (HexNAc)4 + (Man)3 (GlcNAc)2	2352.85	2498.9	45
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2366.83	2540.9	47
(Hex)2 (HexNAc)2 (Deoxyhexose)1 (NeuGc)2 + (Man)3 (GlcNAc)2	2382.82	2556.9	48
(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2424.87	2585.0	49
(Hex)3 (HexNAc)3 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	2585.90	2760.0	50
(Hex)3 (HexNAc)4 (Deoxyhexose)1 (NeuAc)1 + (Man)3 (GlcNAc)2	2627.95	2788.1	51
(Hex)2 (HexNAc)2 (Deoxyhexose)3 (NeuAc)2 + (Man)3 (GlcNAc)2	2642.95	2817.0	52
(Hex)3 (HexNAc)3 (NeuGc)3 + (Man)3 (GlcNAc)2	2908.98	3097.1	54
(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuAc)2 (NeuGc)1 + (Man)3 (GlcNAc)2	3023.05	3211.1	55
(Hex)3 (HexNAc)3 (Deoxyhexose)1 (NeuGc)3 + (Man)3 (GlcNAc)2	3055.04	3243.2	56
(Hex)5 (HexNAc)4 (NeuAc)2 + (Man)3 (GlcNAc)2	3097.09	3271.3	57
(Hex)6 (HexNAc)5 (Deoxyhexose)1 (NeuAc)2 + (Man)3 (GlcNAc)2			
(Hex)5 (HexNAc)5 (Deoxyhexose)2 (NeuAc)1 (NeuGc)1 + (Man)3 (GlcNAc)2	3608.28	3782.4	58
(Hex)4 (HexNAc)5 (Deoxyhexose)3 (NeuGc)2 + (Man)3 (GlcNAc)2			

Table 2. Total glycome (pmole $\pm$ SD) of HD transgenic and control group mice based on protein concentration (100 μ g for *N*- and *O*-glycans, and 100 μ g protein equivalent of GSL-glycans) and 20 μ L of serum of each sample.

Total glycomes	Sample	Control		HD	
		BCF1_♂	BCF1_♀	R6/2_♂	R6/2_♀
<i>N</i> - glycomes	Striatum	426.44 $\pm$ 4.98	404.71 $\pm$ 18.5	401.64 $\pm$ 15.48	379.99 $\pm$ 36.28
	Cortex	253.77 $\pm$ 58.73	241.32 $\pm$ 51.05	255.86 $\pm$ 29.62	264.15 $\pm$ 51.65
	Serum	306.65 $\pm$ 11.35	250.83 $\pm$ 10.98	246.90 $\pm$ 26.13	233.15 $\pm$ 34.62
<i>O</i> -glycomes	Striatum	247.83 $\pm$ 88.75	208.69 $\pm$ 82.94	354.33 $\pm$ 194.47	170.42 $\pm$ 81.36
	Cortex	148.10 $\pm$ 99.42	158.91 $\pm$ 66.72	170.83 $\pm$ 104.46	114.68 $\pm$ 36.35
	Serum	6.48 $\pm$ 3.63	3.60 $\pm$ 2.23	1.84 $\pm$ 0.53	2.21 $\pm$ 0.75
<i>GSL</i> -glycomes	Striatum	267.55 $\pm$ 35.53	436.93 $\pm$ 130.22	942.35 $\pm$ 124.96	472.72 $\pm$ 127.61
	Cortex	1433.72 $\pm$ 192.02	1110.77 $\pm$ 377.88	1185.54 $\pm$ 261.57	1839.34 $\pm$ 383.31
	Serum	325.84 $\pm$ 35.44	242.26 $\pm$ 58.10	198.78 $\pm$ 36.98	154.10 $\pm$ 49.87

Table 3. Glycotyping analysis (in %) of *N*-glycomes of HD transgenic (R6/2) and WT (BCF1) mice

Glycotype	No. peaks	Striatum				No. peaks	Cortex			
		BCF1_♂	BCF1_♀	R6/2_♂	R6/2_♀		BCF1_♂	BCF1_♀	R6/2_♂	R6/2_♀
High Mannose	7	53.9	56.0	49.6	49.8	7	51.4	50.3	48.3	49.3
Mono-Fucose	28	28.1	27.4	31.6	30.4	28	28.4	28.5	30.1	30.1
Di-Fucose	16	13.2	12.2	13.5	14.0	19	15.0	15.2	15.7	14.8
Tri-Fucose	9	1.6	1.2	1.4	1.7	8	1.9	2.0	2.1	1.8
Mono-Sialic acid	20	2.2	1.2	2.7	1.9	19	2.4	2.6	2.5	1.9
Di-Sialic acid	4	0.2	0.0	0.1	0.1	5	0.2	0.2	0.2	0.1
Bisect-GlcNAc	24	35.5	34.4	38.0	37.9	23	34.8	35.3	37.0	36.3
Bi-antennary	35	27.9	26.1	31.3	31.6	29	31.6	32.1	34.8	33.5
Tri-antennary	8	1.0	0.5	1.1	1.1	12	1.4	1.5	1.9	1.4
Tetra-antennary	2	0.2	0.2	0.3	0.4	6	0.4	0.4	0.5	0.5

Table 4. Selected peaks of *N*-glycans (pmole $\pm$ SD) from 10 μ L serum of HD transgenic and control group mice. Glycan cartoons were constructed using the standard Consortium for Functional nomenclature, i.e., blue square, *N*-acetylglucosamine; yellow square, *N*-acetylgalactosamine; red triangle, fucose; green circle, mannose; yellow circle, galactose; purple diamond, *N*-acetylneuraminic acid; and white diamond, *N*-glycolylneuraminic acid.

Peak No.	m/z	Control		HD		Structure
		BCF1_♂	BCF1_♀	R6/2_♂	R6/2_♀	
24	2089.75	16.62 $\pm$ 0.84	10.04 $\pm$ 1.09	8.85 $\pm$ 1.11	8.27 $\pm$ 0.96
42	2410.06	205.83 $\pm$ 17.73	120.28 $\pm$ 6.50	98.80 $\pm$ 8.15	82.06 $\pm$ 9.14
48	2556.93	16.85 $\pm$ 2.96	43.90 $\pm$ 3.23	63.21 $\pm$ 12.63	69.00 $\pm$ 13.48
49	2584.98	0.68 $\pm$ 0.26	2.02 $\pm$ 0.86	3.29 $\pm$ 1.37	4.24 $\pm$ 1.66
54	3097.12	13.89 $\pm$ 2.44	7.40 $\pm$ 0.93	5.29 $\pm$ 1.52	3.99 $\pm$ 0.98

Table 5. Quantitative comparison of a-series gangliosides (pmole $\pm$ SD) in brain tissue of R6/2 and BCF1 (from 500 μ g protein equivalent applied to glycoblotting).

Striatum				
Gangliosides	BCF1_♂	R6/2_♂	BCF1_♀	R6/2_♀
GM3	2.61 $\pm$ 4.52	3.03 $\pm$ 5.25	8.17 $\pm$ 0.90	3.24 $\pm$ 2.89
GM2	34.85 $\pm$ 7.77	171.70 $\pm$ 50.38	78.01 $\pm$ 14.96	54.15 $\pm$ 3.63
GM1	341.12 $\pm$ 47.99	1349.14 $\pm$ 321.12	600.00 $\pm$ 130.70	600.27 $\pm$ 127.34
GD1a	887.12 $\pm$ 119.58	2658.44 $\pm$ 378.81	1349.81 $\pm$ 493.27	1652.84 $\pm$ 535.48

Cortex				
Gangliosides	BCF1_♂	R6/2_♂	BCF1_♀	R6/2_♀
GM3	17.40 $\pm$ 3.34	11.04 $\pm$ 7.68	8.14 $\pm$ 6.31	22.00 $\pm$ 2.66
GM2	138.52 $\pm$ 44.13	86.45 $\pm$ 28.31	82.70 $\pm$ 25.08	163.20 $\pm$ 36.18
GM1	172.37 $\pm$ 228.75	1459.61 $\pm$ 321.83	1331.16 $\pm$ 388.00	2256.05 $\pm$ 508.23
GD1a	4819.79 $\pm$ 736.96	3959.94 $\pm$ 1046.83	3798.44 $\pm$ 1366.83	6112.56 $\pm$ 1365.02

Table 6. Serum glycosphingolipidomics. All serum GSL-glycans compositions, common names and amounts (pmole $\pm$ SD) released from 100 μ L of sera from HD transgenic and WT mice are summarized (Hex, glucose or galactose; HexNAc, *N*-acetylgalactosamine; NeuAc, *N*-acetylneuraminic acid; NeuGc, *N*-glycolylneuraminic acid; and ND, not detected)

GSL-glycan composition	Common name	MS_MW	Control		HD	
			BCF1_♂	BCF1_♀	R6/2_♂	R6/2_♀
(Hex)2 (HexNAc)1	GA2	673.2	3.88 $\pm$ 0.85	4.88 $\pm$ 1.06	2.73 $\pm$ 0.59	2.43 $\pm$ 0.46
(Hex)2 (NeuAc)1	GM3 (NeuAc)	775.3	0.60 $\pm$ 0.22	0.64 $\pm$ 0.07	0.54 $\pm$ 0.18	0.63 $\pm$ 0.14
(Hex)2 (NeuGc)1	GM3 (NeuGc)	791.3	2.22 $\pm$ 0.27	1.99 $\pm$ 0.34	1.28 $\pm$ 0.48	1.21 $\pm$ 0.26
(Hex)3 (HexNAc)1	Asialo GM1	835.3	2.47 $\pm$ 0.33	2.78 $\pm$ 0.26	2.32 $\pm$ 0.30	3.08 $\pm$ 0.61
(Hex)2 (HexNAc)1 (NeuAc)1	GM2 (NeuAc)	978.4	8.54 $\pm$ 1.17	9.05 $\pm$ 3.24	7.94 $\pm$ 1.54	4.67 $\pm$ 1.27
(Hex)2 (HexNAc)1 (NeuGc)1	GM2 (NeuGc)	994.3	247.05 $\pm$ 21.27	196.52 $\pm$ 46.14	167.49 $\pm$ 29.07	118.04 $\pm$ 29.92
(Hex)3 (HexNAc)2	Forssman Ag/Globo-5	1038.4	3.13 $\pm$ 0.37	2.76 $\pm$ 0.57	2.01 $\pm$ 0.47	1.93 $\pm$ 0.24
(Hex)3 (HexNAc)1 (NeuAc)1	GM1 (NeuAc)	1140.4	1.56 $\pm$ 0.19	1.36 $\pm$ 0.09	1.18 $\pm$ 0.20	1.20 $\pm$ 0.27
(Hex)3 (HexNAc)1 (NeuGc)1	GM1 (NeuGc)	1156.4	21.43 $\pm$ 4.21	6.26 $\pm$ 1.78	1.76 $\pm$ 2.06	4.13 $\pm$ 3.38
(Hex)3 (HexNAc)2 (Deoxyhexose)1	Type IA/IIA	1184.4	0.76 $\pm$ 0.25	ND	ND	ND
(Hex)4 (HexNAc)2	Globo-13	1200.4	0.43 $\pm$ 0.08	0.55	ND	0.24
(Hex)2 (HexNAc)1 (NeuGc)2	GD2	1315.5	0.56 $\pm$ 0.06	0.38 $\pm$ 0.09	ND	0.39 $\pm$ 0.08
(Hex)3 (HexNAc)2 (NeuAc)1	GalNAc-GM1 (NeuAc)	1343.5	0.71 $\pm$ 0.06	0.60 $\pm$ 0.10	0.97 $\pm$ 0.35	0.57 $\pm$ 0.17
(Hex)3 (HexNAc)2 (NeuGc)1	GalNAc-GM1 (NeuGc)	1359.5	ND	ND	ND	0.23 $\pm$ 0.10
(Hex)3 (HexNAc)1 (NeuAc)2	GD1a (NeuAc)	1445.5	3.97 $\pm$ 0.81	1.82 $\pm$ 0.39	1.38 $\pm$ 1.12	2.13 $\pm$ 1.98
(Hex)3 (HexNAc)1 (NeuAc)1 (NeuGc)1	GD1a (Neu-Ac/Gc)	1461.5	0.92 $\pm$ 0.22	0.63 $\pm$ 0.16	0.56 $\pm$ 0.06	1.08 $\pm$ 0.30

(Hex)3 (HexNAc)1 (NeuGc)2	GD1a (NeuGc)	1477.5	26.69 ± 4.93	11.35 ± 3.66	8.38 ± 0.52	11.60 ± 10.54
(Hex)5 (HexNAc)2 (Deoxyhexose)1	Globo-15	1508.5	0.32 ± 0.04	ND	ND	ND
(Hex)4 (HexNAc)2 (NeuGc)1	Ganglio-a-10/-as-9	1521.5	0.26 ± 0.05	0.34 ± 0.09	ND	0.27 ± 0.07
(Hex)3 (HexNAc)2 (NeuGc)2	GalNac-GD1a	1680.6	0.33 ± 0.04	0.34 ± 0.07	0.24 ± 0.04	0.28 ± 0.08
		Total	325.84 ± 35.44	242.26 ± 58.10	198.78 ± 36.98	154.10 ± 49.87

Figure Captions

Figure 1: Schematic diagram of glycoblotting – combined with MALDI-TOF/MS analysis for the enrichment of total glycomes from brain tissue of HD transgenic mice (R6/2) and WT mice (BCF1). (a) Perfusion of 12-week-old mice. (b) Homogenization of mouse brain tissue. (c) Trypsin digestion followed by PNGase F and ammonium carbamate cleavage of *N*- and *O*-glycans, respectively; chloroform/methanol extraction and native GSL-glycans cleavage by ozonolysis and subsequent fragmentation by alkaline treatment in the case of oligosaccharides from GSLs. (d) Chemoselective capturing of reducing oligosaccharides on BlotGlyco® H beads. (e) On-bead methyl esterification. (f) Derivatization, glycan release, and finally, MALDI-TOF/MS analysis of either aoWR or BOA labeled *N*-, *O*-, or GSL-glycans.

Figure 2: Representative MALDI-TOF/MS spectra of brain tissues *N*-glycans of HD transgenic and WT mice (IS indicates A2 amide used as an internal standard, and $n = 3$ and $N = 2$ where n and N are the number of the mouse and the experiment, respectively. M, male; F, female).

Figure 3: Glycotyping analysis of fucosylated *N*-glycans from serum R6/2 and BCF1 (Mono-Fu, core-fucose; Di-Fu, di-fucose; others, *N*-glycan types except mentioned; M, male; and F, female).

Figure 4: MALDI-TOF/MS spectrum of serum GSL-glycans in R6/2 and BCF1 (IS, internal standard; M, male; and F, female). Glycan cartoons were constructed using the standard Consortium for Functional nomenclature, i.e., blue circle, glucose; yellow circle, galactose; yellow square, *N*-acetylgalactosamine; purple diamond, *N*-acetylneuraminic acid; and white diamond, *N*-glycolylneuraminic acid.

Figure 5: Comparison of total glycome expression levels in the striatum and cortex. (A) *N*-glycans, (B) *O*-glycans, and (C) GSL-glycans.

Figure 1

Figure 2

Figure 3

BCF1_F

R6/2_F

Figure

BCF1_M

R6/2_M

Figure 4

Figure 5A

Figure 5B

O_glycans Striatum vs Cortex

Figure 5C

Graphical abstract

A comprehensive glycome profiling of **Huntington's** disease transgenic mice

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