Deficiency in ST6GAL1, one of the two α2,6-sialyltransferases, has only a minor effect on the pathogenesis of prion disease

Natallia Makarava1,2, Elizaveta Katorcha1,2, Jennifer Chen-Yu Chang1,2, Joseph T. Y. Lau3 and Ilia V. Baskakov1,2*

1Center for Biomedical Engineering and Technology, University of Maryland School of Medicine, Baltimore, MD, United States, 2Department of Anatomy and Neurobiology, University of Maryland School of Medicine, Baltimore, MD, United States, 3Department of Molecular and Cellular Biology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, United States

Prion diseases are a group of fatal neurodegenerative diseases caused by misfolding of the normal cellular form of the prion protein or PrP\textsuperscript{C}, into a disease-associated self-replicating state or PrP\textsuperscript{Sc}. PrP\textsuperscript{C} and PrP\textsuperscript{Sc} are posttranslationally modified with N-linked glycans, in which the terminal positions occupied by sialic acids residues are attached to galactose predominantly via α2-6 linkages. The sialylation status of PrP\textsuperscript{Sc} is an important determinant of prion disease pathogenesis, as it dictates the rate of prion replication and controls the fate of prions in an organism. The current study tests whether a knockout of ST6Gal1, one of the two mammalian sialyltransferases that catalyze the sialylation of glycans via α2-6 linkages, reduces the sialylation status of PrP\textsuperscript{Sc} and alters prion disease pathogenesis.

We found that a global knockout of ST6Gal1 in mice significantly reduces the α2-6 sialylation of the brain parenchyma, as determined by staining with Sambucus Nigra agglutinin. However, the sialylation of PrP\textsuperscript{Sc} remained stable and the incubation time to disease increased only modestly in ST6Gal1 knockout mice (ST6Gal1-KO). A lack of significant changes in the PrP\textsuperscript{Sc} sialylation status and prion pathogenesis is attributed to the redundancy in sialylation and, in particular, the plausible involvement of a second member of the sialyltransferase family that sialylate via α2-6 linkages, ST6Gal2.

KEYWORDS
prion, prion diseases, N-glycosylation, sialic acid, sialyltransferases, ST6GAL1, ST6GAL2, mouse

Introduction

Prion diseases are a group of fatal neurodegenerative diseases in humans and animals that can arise spontaneously or via transmission (Prusiner, 2001). Prions or PrP\textsuperscript{Sc} are transmissible and spread from host-to-host or cell-to-cell by recruiting and converting the cellular form of the prion protein or PrP\textsuperscript{C} into a disease-associated PrP\textsuperscript{Sc} state (Prusiner,
Prions elicit multiple disease phenotypes characterized by different clinical symptoms, cell tropism, affected brain regions, deposition patterns and incubation times to disease (Collinge and Clarke, 2007). The diversity of disease phenotypes within the same host has been attributed to the ability of PrPSc to acquire multiple, structurally distinct, self-replicating PrPSc states referred to as prion strains (Bessen and Marsh, 1992; Safar et al., 1998; Peretz et al., 2001; Ayers et al., 2011; Gonzalez-Montalban et al., 2011; Klimova et al., 2015; Morales et al., 2016). The fact that prion strains are structurally different has been well documented (Caughey et al., 2015; Morales et al., 2016). The fact that prion strains are prion diseases. Moreover, learning how carbohydrates dictate strain-specific pathology will help to establish the relationship between strain-specific structure and disease phenotype in a predictable manner. In mammals, sialylation of glycans takes place in the trans-Golgi and is executed by twenty sialytransferases (STs) (Audry et al., 2011). STs are classified into four families according to the type of linkages synthesized (α-2-3, α-2-6, α-2-8 or α-2-9) and the selectivity towards N- or O-linked glycans (Takashima, 2008; Audry et al., 2011). Among the twenty STs, three enzymes (ST3Gal3, ST3Gal4 and ST3Gal6) sialylate N-linked glycans via an α-2-3 linkage, whereas only two, ST6Gal1 and ST6Gal2, sialylate via an α-2-6 linkage (Takashima, 2008; Audry et al., 2011). In PrPSc, an α-2-6 linkage is predominant (Endo et al., 1989; Katorcha and Baskakov, 2017), so we decided to target the ST6 family for manipulating the sialylation status of PrPSc and PrPSc. Between the two members of the ST6 family, ST6Gal1 is believed to be the main enzyme responsible for α-2-6 sialylation in mammals. In adults, it is expressed ubiquitously in almost all tissues, including the brain (Manzi et al., 2000; Martin et al., 2002). Numerous reports document the involvement of ST6GAL1-mediated sialylation in pathologies relating to inflammation, immune responses, hematopoietic regulation, and cancer (e.g. recently reviewed in (Gc et al., 2022). Very little is known of ST6GAL2 and its catalytic properties. In fact, nothing is known about the physiologic roles of ST6GAL2, and its expression appears to be restricted to a brain, lung and spleen (Krezwinski-Recchi et al., 2003; Laporte et al., 2009; Lehoux et al., 2010; Ohmi et al., 2021).

The current study tested whether a deficiency of ST6Gal1 reduces the sialylation status of PrPSc and alters prion disease pathogenesis. We found that a global knockout of ST6Gal1 significantly reduces α-2-6 sialylation of the brain parenchyma, as judged from staining with Sambucus Nigra agglutinin (SNA). However, the sialylation of PrPSc remained stable and the incubation time to disease showed only a modest increase in ST6Gal1 knockout mice (ST6Gal1-KO). Lack of significant changes is attributed to the redundancy in sialylation of PrPSc and, in particular, the involvement of ST6GAL2. Our data indirectly points to the involvement of ST6GAL2, the only other known sialyltransferase to be able to construct the α2,6-Sia linkage in PrP, in the pathogenic progression of prion disease.
Materials and methods

Ethics statement

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Maryland, Baltimore (Assurance Number A32000-01; Permit Number: 0215002).

Animals

The ST6Gal1-KO mouse has a globally inactivated ST6Gal1 gene and was originally produced by Marth and co-workers (Henret et al., 1998). The strain was backcrossed >10 generations into the C57Bl/6 background. Age-matched C57Bl/6 animals were used as controls.

ST6Gal1-KO and C57BL/6 mice (females and males) were inoculated intracerebrally into the left hemisphere ~2 mm to the left of the midline and ~2 mm anterior to a line drawn between the ears with 20 μl of 1% 22L brain homogenate under isoflurane anesthesia. Inoculum is delivered slowly by a 26 G needle inserted to a depth of approximately 3 mm. Signs of neurological disease were detected between 127–138 days post inoculation and consisted of hind-limb clasp, ataxia, and weight loss. Within 13–26 days after the first clinical signs, mice became unable to walk on a beam, developed kyphosis and became lethargic. Mice were considered terminally ill when they were unable to reach or lost 20% of their weight. At this point they were euthanized by CO2 asphyxiation and decapitation.

Western blot

10% (wt/vol) homogenates in PBS from brains and spleens were prepared using glass/Teflon homogenizers as described previously (Makarava et al., 2012). For analysis of brain-derived PrPSc, an aliquot of 10% (wt/vol) homogenate was diluted with nine volumes of 1% Triton X-100 in PBS, sonicated for 30 s inside a Misonix S-4000 microplate horn (Qsonica), and treated with 20 μg/ml of proteinase K for 1 h at 37°C. For analysis of spleen-derived PrPSc, an aliquot of 10% homogenate was diluted with nine volumes of 1% Triton X-100 in PBS, sonicated for 30 s inside a Misonix S-4000 microplate horn (Qsonica), and treated with 20 μg/ml of proteinase K for 1 h at 37°C. Resulting brain and spleen samples were supplemented with 4xSDS loading buffer and heated for 10 min in a boiling water bath. Samples were loaded onto NuPAGE 12% Bis-Tris gels, transferred to PVDF membranes, and probed with anti-prion antibodies ab3531 (Abcam, Waltham, MA). Western blot signals were visualized using the FlourChem M imaging system (Protein Simple, San Jose, CA). For generating glycoform profiles and calculating total PrPSc amount, densitometry analysis of 1D blots was performed using the “Lane profile” and “Multiplex Band Analysis” functions in AlphaView software (Protein Simple), respectively.

2D electrophoresis

For 2D electrophoresis of brain-derived materials, an aliquot of 10% (wt/vol) homogenate was diluted with nine volumes of 1% (vol/vol) Triton X-100 in PBS, sonicated as above, and treated with 25 μg/ml of proteinase K for 30 min at 37°C. For 2D electrophoresis of spleen-derived material, 250 μl of 10% (wt/vol) homogenate was diluted 1:1 with PBS, aliquotted into 0.2-ml thin-wall PCR tubes, sonicated as above, and combined into one tube, which was subjected to a 30-min 16,000 g centrifugation at 4°C. The pellet was resuspended in 25 μl of 1% (wt/vol) Triton X-100 in PBS, treated with 20 μg/ml of proteinase K for 30 min at 37°C, then supplemented with 4xSDS loading buffer, heated for 10 min in a boiling water bath, and processed for 2D electrophoresis. 25 μl of the resulting samples were solubilized for 1 h at room temperature in 200 μl of solubilization buffer (8 M Urea, 2% (wt/vol) CHAPS, 5 mM TBP, 20 mM TrisHCl pH 8.0), then alkylated by adding 7 μL of 0.5 M iodoacetamide and incubated for 1 h at room temperature in the dark. Then, 1150 μL of ice-cold methanol was added and samples were incubated for 2 h at −20°C. After 16,000 g centrifugation at 4°C, the supernatant was discarded, and the pellet was resolubilized in 160 μl rehydration buffer (7 M urea, 2 M thiourea, 1% (wt/vol) DTT, 1% (wt/vol) CHAPS, 1% (wt/ vol) Triton X-100, 1% (vol/vol) ampholyte, and a trace amount of Bromophenol Blue). Fixed immobilized pre-cast IPG strips (cat. # ZM0018, Life Technologies, Carlsbad, CA) with a linear pH gradient 3–10 were rehydrated in 155 μl of the resulting mixture overnight at room temperature inside IPG Runner cassettes (cat. # ZM0008, Life Technologies). Isoelectrofocusing (first dimension separation) was performed at room temperature with increasing voltage (175 V for 15 min, then 175–2,000 V linear gradient for 45 min, then 2,000 V for 30 min) on a Life Technologies Zoom Dual Power Supply using an XCell SureLock Mini-Cell Electrophoresis System (cat. # EI0001, Life Technologies). The IPG strips were then equilibrated for 15 min consecutively in 1) 6 M Urea, 20% (vol/vol) glycerol, 2% SDS, 375 mM Tris-HCl, pH 8.8, 130 mM DTT, then 2) 6 M Urea, 20% (vol/vol) glycerol, 2% SDS, 375 mM Tris-HCl, pH 8.8, 135 mM iodoacetamide, and loaded on 4–12% Bis-Tris ZOOM SDS-PAGE pre-cast gels (cat. # NP0330BOX, Life Technologies). For the second dimension, SDS-PAGE was performed for 1 h at 170 V. Immunoblotting was performed with ab3531 (Abcam).
Histopathological study

Formalin-fixed brain halves divided at the midline (left hemisphere) were treated in formic acid (95%) to deactivate prion infectivity before being embedded in paraffin. 4 μm sections mounted on slides were processed for hematoxylin-eosin (H&E) staining and immunohistochemistry. To expose epitopes, slides were subjected to 20 min hydrated autoclaving at 121°C in trisodium citrate buffer, pH 6.0 with 0.05% Tween 20. For detection of disease-associated PrP, a 5 min treatment with 88% formic acid was used following autoclaving. PrP was stained with anti-prion antibody SAF-84 (Cayman Chemical, Ann Arbor, MI). Rabbit anti-Iba1 (Wako, Richmond, VA) was used to stain microglia. Detection was performed using 3,3’ Diaminobenzidine (DAB) Quanto chromogen and substrate (VWR, Radnor, PA).

For SNA staining, 4 μm brain sections mounted on slides were subjected to the standard rehydration procedure, then submerged in 10 mM tri-sodium citrate buffer, pH 6.0, boiled for 5 min by microwaving at 20% power, and cooled for 1 h before proceeding with lectin staining. Incubation in 3% hydrogen peroxide in methanol for 20 min was used to remove endogenous peroxidase activity. After a 5 min wash in running water, slides were incubated for 1 h with 5 μg/ml biotin-labeled elderberry bark lectin (SNA, Vector laboratories, Burlingame, CA) diluted in lectin buffer, pH 7.6 (50 mM Tris, 150 mM NaCl, 1 mM MgCl2, 0.75 mM CaCl2). Following three 5 min washes in lectin buffer, the slides were incubated for 30 min in 5 μg/ml horse radish peroxidase-labeled streptavidin (Thermo Fisher scientific, Waltham, MA), then again washed three times with lectin buffer, and developed with DAB Quanto chromogen and substrate (VWR, Radnor, PA).

RT-qPCR

Brains were divided at the midline, and the right hemispheres were used to dissect cortex, hippocampus, midbrain and thalamus regions. 10% (wt/vol) homogenates were prepared within 1.5 ml tubes in Trizol (Thermo Fisher Scientific, Waltham, MA, United States), using RNase-free disposable pestles (Fisher scientific, Hampton, NH). Total RNA was isolated using the Aurum Total RNA Mini Kit (Bio-Rad, Hercules, CA, United States) and subjected to DNase I digestion to remove contaminating genomic DNA. Total RNA was dissolved in elution buffer and stored at −80°C. An absorbance 260/280 value of ~2.0, determined using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, United States) proved RNA purity. Reverse transcription was performed using 1 μg of extracted RNA and an iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA, United States). RT-qPCR was performed in triplicate from one ST6Gal1-KO and one C57Bl/6 (WT) brain using the SsoAdvanced universal SYBR Green Supermix (Bio-Rad, Hercules, CA, United States) with Bio-Rad designed and validated primers for ST6Gal1 (qMmuCID0009827). Alternative ST6Gal1 primers designed for exon 2 were as follows: forward 5’- ACACCACCTGAATGGGAGGGT - 3’, and reverse 5’- CAGAGATCTTCAGAGAGACAGC - 3’. The housekeeping gene TBP (Bio-Rad, qMmuCID0040542) was used for normalization. The PCR protocol consisted of a treatment at 95°C for 2 min followed by 40 amplification cycles with the following steps: 95°C for 5 s, and 60°C for 30 s. The RT-qPCR was performed with a CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA, United States), and analyzed with Bio-Rad CFX Manager software (Bio-Rad, Hercules, CA, United States).

Results

The knockout of ST6Gal1 significantly reduces α2-6 sialylation of the brain parenchyma

In mice, the highest level of ST6Gal1 expression was found in the spleen (Takashima et al., 2002). In the current study, spleens were used to confirm the global knockout of ST6Gal1. As expected, ST6Gal1 was found in control C56Bl/6 mice (WT) but was lacking in ST6Gal1-KO mice (Figure 1A). The level of ST6Gal1 expression in the brains of WT mice was below the detection limits of the Western blot (data not shown). To confirm the knockout of ST6Gal1 in animal brains, we used RT-qPCR with two sets of ST6Gal1 primers, including primers to the exon 2. ST6Gal1-KO mice were generated by deleting the exon 2 that resulted in a loss of over 50% of the ST6Gal1 coding sequence including cytoplasmic, transmembrane, and considerable catalytic domain sequences (Hennet et al., 1998). As expected, RT-qPCR with primers to exon 2 detected expression of ST6Gal1 in multiple brain areas of WT mice, but not in ST6Gal1-KO mice (Figure 1B).

To test whether the knockout of ST6Gal1 reduces sialylation of the brain parenchyma, brain slices were stained with SNA lectin that detects terminal α2-6 sialylation of both N- and O-linked glycans. Consistent with previous studies (Martin et al., 2002), ST6Gal1-KO mice showed significant loss in α2-6 sialylation of the brain parenchyma across multiple brain regions including thalamus, hippocampus and cortex in comparison to control WT mice (Figure 1C).

Knockout of ST6Gal1 had a modest impact on the incubation time to disease

To test whether the knockout of ST6Gal1 modifies prion disease pathogenesis, ST6Gal1-KO and control WT mice were
inoculated intracerebrally with 1% scrapie brain homogenate of 22L, a mouse-adapted prion strain. ST6Gal1-KO and control WT groups succumbed to the diseases at 155.3 ± 4.9 and 150.3 ± 4.1 days post-inoculation, respectively (Figure 2A). While ST6Gal1-KO mice showed slightly longer incubation times relative to the control group, the difference in their survival curves was bordering statistical significance (Figure 2A). No statistically significant difference was found between the two groups with respect to the amounts of PK-resistant PrPSc in their brains (Figure 2B). The knockout of ST6Gal1 did not change the ratios of un-, mono-, and diglycosylated glycoforms within PrPSc (Figure 2C). As reported previously (Katorcha et al., 2015b), in the 22L mouse adapted strain, the diglycosylated glycoform is predominant, whereas the unglycosylated is the least abundant PrPSc glycoform. In both groups, PrPSc spread to spleen showing large variations between individual animals with respect to its amounts, which is expected for the 22L strain inoculated intracranially (Figure 2B). In spleen, the amounts of PrPSc was lower in ST6Gal1-KO group relative to WT group (Figure 2B). Histopathological analysis showed the main pathological hallmarks of prion disease including PrPSc deposition, spongiosis and reactive microglia in both ST6Gal1-KO and control WT mice (Figure 3).

A ST6Gal1 knockout did not affect the sialylation status of PrPSc in the brain or spleen

To test whether the knockout of ST6Gal1 reduces the sialylation status of PrPSc, brain- and spleen-derived material was analyzed using two-dimensional (2D) gel electrophoresis. Prior to 2D analysis, the samples were treated with PK and then denatured, so that the sialylation status of individual PrP molecules could be visualized as a distribution of charge isoforms in the horizontal dimension of 2D. Each sialic acid residue adds one negative charge and hypersialylated PrP molecules move toward an acidic pH, whereas hyposialylated PrP molecules move toward a basic pH in the horizontal dimension of 2D gels (Figure 4). Three horizontal rows of charged isoforms from bottom to top correspond to the non-, mono- and diglycosylated PrP molecules.
Consistent with previous studies, non-glycosylated PrP molecules showed multiple charge isoforms (all of which were at basic pH) that were attributed to the structural heterogeneity of GPI anchors (Katorcha et al., 2016b). No notable differences between ST6Gal1-KO and control group brains with respect to the distribution of PrP sialoglycoforms can be found in brain- or spleen-derived PrP Sc (Figures 4A,B). As reported in previous studies (Srivastava et al., 2015), the sialylation pattern of brain-derived PrP Sc was different from that of spleen-derived PrP Sc (Figures 4A,B). As expected, treatment with sialidase *Arthrobacter ureafaciens* (AU), that has broad substrate specificity, shifted the distribution of PrP sialoforms to basic pH (Figures 4A,B). These results illustrate that the global knockout of ST6Gal1 did not change the sialylation pattern of PrP Sc in the brain or spleen.

**Discussion**

Considering the important role of sialylation in dictating the rate of prion replication and their fate in an organism, manipulation of the sialylation status of PrP Sc via altering the steady-state sialylation of its precursor PrP C might offer a novel strategy for developing therapeutic intervention against prion diseases. Sialylation of glycoproteins is controlled by two groups of enzymes: sialyltransferases (STs) and neuraminidases or sialidases (Audry et al., 2011; Miyagi and Yamaguchi, 2012). Previously, we found that a knockout of Neu1, Neu3, Neu4, or both Neu3 and Neu4, which are all expressed in the brain, does not affect the steady-state levels of PrP Sc sialylation in mice (Katorcha et al., 2015a). This fact suggests that desialylated PrP C molecules do not contribute to the steady-state level of PrP C sialoforms, presumably, due to degradation. In the
absence of detectable changes in PrPSc sialylation in Neu’s knockout models, targeting STs seemed to offer a more effective strategy for manipulating the sialylation levels of PrPSc and PrPSc3. In support of this notion, the sialylation levels of PrPSc in cultured N2a cells were suppressed to a large degree by the administration of a general inhibitor of STs, 3Fax-Neu5Ac (Katorcha et al., 2015a). However, because 3Fax-Neu5Ac targets a broad spectrum of STs and blocks global sialylation (Burkart et al., 2000; Lairson et al., 2008), it causes irreversible kidney dysfunction in animals which leads to rapid death (Macauley et al., 2014). In the absence of inhibitors that selectively target individual STs (Wang et al., 2016; Szabo and Skropeta, 2017), we decided to test the effect of a knockout of ST6Gal1.

Western blotting of spleen tissues from the ST6Gal1-KO cohort confirmed a global knockout of ST6Gal1, while RT-qPCR documented the lack of ST6Gal1 expression in brain tissue. Consistent with previous studies (Martin et al., 2002), SNA staining revealed significantly lower levels of α2-6 sialylation of the brain parenchyma in ST6Gal1-KO mice relative to that of control WT animals. However, despite a global knockout of the ST6Gal1 gene, the sialylation status of brain- and spleen-derived PrPSc did not show notable changes. In spleen-derived PrPSc, diglycosylated PrP molecules shifted toward acidic pH relative to those of monoglycosylated PrP molecules in both WT and ST6Gal1 mice (Figure 4B), suggesting that spleen-derived PrPSc is fully sialylated. Such sialylation pattern is consistent with very high levels of sialyltransferase activity in secondary lymphoid organs. In contrast, in brain-derived PrPSc of WT and ST6Gal1, the pattern of charged isoforms of diglycosylated PrP molecules was very similar to that of monoglycosylated PrPs (Figure 4A). Such pattern suggests that in a brain, diglycosylated PrP molecules are only partially sialylated and on average have a similar number of sialic acid residues per PrP molecule as monoglycosylated PrPs, despite the fact that diglycosylated PrPs have two glycans, while monoglycosylated PrPs have...
only one glycan. Nevertheless, similar sialylation levels of PrPSc in ST6Gal1-KO and WT mice could be due to 1) the possibility that sialylation is conducted primarily by the ST6Gal2 enzyme, 2) redundancy in activities of ST6Gal1 and ST6Gal2 enzymes, and/or 3) redundancy between the ST6 and ST3 families of STs.

As the last member of STs discovered, ST6Gal2 has not been characterized as thoroughly as ST6Gal1 (Krzewinski-Recchi et al., 2003). ST6Gal2 seems to exhibit a more narrow enzymatic substrate specificity in comparison to ST6Gal1 (Takashima et al., 2002; Takashima et al., 2003). While the ST6Gal1 gene is expressed in almost all tissues, the expression of ST6Gal2 shows a restricted tissue-specific pattern and is primarily limited to the brain and spleen (Krzewinski-Recchi et al., 2003; Laporte et al., 2009; Lehoux et al., 2010; Ohmi et al., 2021). A direct comparison of expression using RT-qPCR revealed that in an adult mouse brain, ST6Gal1 and ST6Gal2 genes are expressed at similar levels (Ohmi et al., 2021). However, ST6Gal1 mRNAs were found across different cell types in mice including neurons, astrocytes and microglia, whereas the expression of ST6Gal2 gene was detected primarily in astrocytes and neurons. Notably, the expression of ST6Gal2 in primary epithelial cultures was found to be upregulated by treatment with IL-6, suggesting that ST6Gal2 responds to pro-inflammatory conditions (Laporte et al., 2009; Lehoux et al., 2010). In a mouse brain, region-specific expression of the ST6Gal2 gene and the region-specific sialylation status of PrPSc showed the same ranking order (Makarava et al., 2020a). Lower levels of sialylation promotes faster prion replication and triggers profound neuroinflammation (Katorcha et al., 2015b; Srivastava et al., 2018). As such, testing a knockout of ST6Gal2 in future studies might provide new insight into etiology of sporadic CJD.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was reviewed and approved by Institutional Animal Care and Use Committee of the University of Maryland, Baltimore (Assurance Number A32000-01).
Author contributions

IB and NM conceived the idea and designed the experiments; JL provided ST6Gal1 KO mice; NM, EK, and JC performed experiments; NM analyzed the data; IB wrote the manuscript. All authors read and approved the final manuscript.

Funding

Financial support for this study was provided by National Institute of Health (Grants R01 NS045585 and AI128925) to IB.

Acknowledgments

We thank Daryl Butler for his assistance in handling animals, Regina Savtchenko for her assistance in running Western blots, and Kara Molesworth for editing the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.
a host-tissue and cell-specific manner. *J. Biol. Chem.* 291 (33), 17009–17019. doi:10.1074/jbc.M116.732040

Klimova, N., Makarava, N., and Baskakov, I. V. (2015). The diversity and relationship of prion protein self-replicating states. *Virus Res.* 207 (1), 113–119. doi:10.1016/j.virusres.2014.10.002

Krzewinski-Recchi, M. A., Julien, S., Iuliani, S., Teintenier-Lehvé, M., Samyn-Petit, B., Montiel, M. D., et al. (2003). Identification and functional expression of a second human beta-galactoside alpha2,6-sialyltransferase, ST6Gal II. *Europ. J. Biochem.* 270 (5), 950–963. doi:10.1046/j.1432-1323.2003.03458.x

Kushwaha, R., Sinha, A., Makarava, N., Molesworth, K., and Baskakov, I. V. (2021). Non-cell autonomous astrocyte-mediated neuronal toxicity in prion diseases. *Acta Neuropathol. Commun.* 9 (1), 22. doi:10.1186/s40478-021-01123-8

Lairson, L. L., Henrissat, B., Davies, G. J., and Withers, S. G. (2008). Glycosyltransferases: structures, functions, and mechanisms. *Annu. Rev. Biochem.* 77, 521–555. doi:10.1146/annurev.biochem.76.061005.092322

Laporte, B., Gonzalez-Hilarion, S., Maftah, A., and Petit, J.-M. (2009). The second bovine beta-galactoside-alpha2,6-sialyltransferase (ST6Gal II): genomic organization and stimulation of its expression by IL-6 in bovine mammary epithelial cells. *Glycobiol.* 19 (10), 1082–1093. doi:10.1093/glycob/cwp094

Legname, G., Baskakov, I. V., Nguyen, H. O. B., Riesner, D., Cohen, F. E., DeArmon, S. J., et al. (2004). Synthetic mammalian prions. *Science* 305 (5684), 673–676. doi:10.1126/science.1100195

Lehoux, S., Groux-Degroote, S., Cazet, A., Dhaenens, C.-M., Maurage, C.-A., Cailler-Boudin, M.-L., et al. (2010). Transcriptional regulation of the human ST6Gal2 gene in cerebral cortex and neuronal cells. *Glycogen.* 27, 99–114. doi:10.1017/S009799040900964-y

Linnartz, B., Bodea, L.-G., and Neumann, H. (2012). Microligand carbohydrate-binding receptors for neural repair. *Cell Tissue Res.* 349, 215–227. doi:10.1007/s00441-012-1342-7

Liu, Z., Swinndall, A. F., Kesterson, R. A., Schoe, T. R., Bullard, D. C., and Bells, S. L. (2011). ST6Gal-I regulates macrophage apoptosis via a2-6 sialylation of the TNFRI1 death receptor. *J. Biol. Chem.* 286 (45), 39654–39662. doi:10.1074/jbc.M111.276862

Macaulay, M. S., Arlian, B. M., Billahan, C. D., Pang, P. C., Bortell, N., Marcondes, C. M., et al. (2014). Systematic blockade of sialylation in mice with a global inhibitor of sialyltransferases. *J. Biol. Chem.* 289 (35149–35158), 35149–35158. doi:10.1074/jbc.M114.606517

Makarava, N., Chang, J.-C. Y., and Baskakov, I. V. (2020a). Region-specific sialylation pattern of prion strains provides novel insight into prion neurotropism. *Int. J. Mol. Sci.* 21 (3), 828. doi:10.3390/ijms21030828

Makarava, N., Chang, J.-C. Y., Kushwaha, R., and Baskakov, I. V. (2019). Region-specific response of astrocytes to prion infection. *Front. Neurosci.* 13 (1), e01484. doi:10.3389/fnins.2019.01484

Makarava, N., Chang, J.-C. Y., Molesworth, K., and Baskakov, I. V. (2020b). Region-specific glial homeostatic signature in prion disease is replaced by a uniform neuroinflammation signature, common for brain regions and prion strains with different cell tropism. *Neurobiol. Dis.* 137 (1), e014783. doi:10.1016/j.nbd.2020.05.078

Makarava, N., Kovacs, G. G., Satvchenko, R., Alexeeva, I., Budka, H., Rohwer, R. G., et al. (2012). Stabilization of a prion strain of synthetic origin requires multiple serial passages. *J. Biol. Chem.* 287 (36), 30205–30214. doi:10.1074/jbc.M112.392985

Makarava, N., Mychko, O., Chang, J.-C. Y., Molesworth, K., and Baskakov, I. V. (2021). The degree of astrocyte activation is predictive of the incubation time to prion disease. *Acta Neuropathol. Commun.* 9 (1), 87. doi:10.1186/s40478-021-01192-9

Manka, S. W., Wenborn, A., Collinge, J., and Wadsworth, J. D. F. (2022). Prion strains viewed through the lens of cryo-EM. *Cell Tissue Res.* 380 (1), e0223567-2.

Manzi, A. E., Norgard-Sumnicht, K., Argade, S., Marth, J. D., van Halbeek, H., and Varki, A. (2008). Exploring the glycan repertoire of genetically modified mice by isolation and profiling of the major glycan classes and nano-NMR analysis of glycian mixtures. *Glycobiology* 10 (7), 669–689. doi:10.1093/glycob/wjy769

Martin, L. T., Marth, J. D., Variki, A., and Variki, N. M. (2002). Genetically altered mice with sialidase-deficient brain glycosylation show tissue-specific alterations in sialylation and salic acid 9-O-acetylation. *J. Biol. Chem.* 277 (36), 32930–32938. doi:10.1074/jbc.M203362200
adult human life of Swedes. *Acta Neuropathol.* 94, 345–352. doi:10.1007/s004010050717

Szabo, R., and Skropeta, D. (2017). Advancement of sialyltransferase inhibitors: Therapeutic challenges and opportunities. *Med. Res. Rev.* 37 (2), 219–270. doi:10.1002/mrrev.21407

Takashima, S. (2008). Characterization of mouse sialyltransferase genes: their evolution and diversity. *Biosci. Biotechnol. Biochem.* 72, 1155–1167. doi:10.1271/bbb.80025

Takashima, S., Truji, S., and Tsujimoto, M. (2002). Characterization of the second type of human beta-galactoside alpha 2, 6-sialyltransferase (ST6Gal II), which sialylates Galbeta 1, 4GlcNAc structures on oligosaccharides preferentially. Genomic analysis of human sialyltransferase genes. *J. Biol. Chem.* 277 (48), 45719–45728. doi:10.1074/jbc.M206808200

Takashima, S., Tsuji, S., and Tsujimoto, M. (2003). Comparison of the enzymatic properties of mouse beta-galactoside alpha2, 6-sialyltransferases, ST6Gal I and II. *J. Biol. Chem.* 134 (2), 287–296. doi:10.1093/jb/mvg142

Turk, E., Teplow, D. B., Hood, L. E., and Prusiner, S. B. (1988). Purification and properties of the cellular and scrapie hamster prion proteins. *Eur. J. Biochem.* 176 (1), 21–30. doi:10.1111/j.1432-1033.1988.tb14246.x

Wang, L., Liu, Y., Wu, L., and Sun, X. L. (2016). Sialyltransferase inhibition and recent advances. *Biochim. Biophys. Acta* 1864 (1), 143–153. doi:10.1016/j.bbabios.2015.07.007

Zhuo, Y., and Bellis, S. L. (2011). Emerging role of alpha2, 6-sialic acid as a negative regulator of galectin binding and function. *J. Biol. Chem.* 286 (8), 5935–5941. doi:10.1074/jbc.R110.191429