α2, 3-linkage of sialic acid to a GPI-anchor and an unpredicted GPI attachment site in human prion protein

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Table S1. Identified peptides of human PrP^C from KI mouse brains after C18 column.

Fragment	Obsd. m/z	Calcd. m/z	Charge	Score	*P*
QHTVTTTTK + Gln->pyro-Glu (N-term Q)	500.2590	998.5033	2	62	4.7e-006
QHTVTTTTK	508.7723	1015.5298	2	62	6.2e-006
ESQAYYQR	522.7407	1043.4672	2	60	4.1e-006
YPGQGSPGGNR	545.2571	1088.4999	2	55	1.8e-005
GENFTETDVK	570.2645	1138.5142	2	60	4.5e-006
RPKPGGWNTGGSR	457.2410	1368.7011	3	48	0.00011
VVEQMCITQYER	778.3681	1554.7170	2	28	0.011
VVEQMCITQYER + Oxidation (M)	786.3632	1570.7120	2	70	5.5e-007
GENFTETDVKMMER + 2 Oxidation (M)	573.5829	1717.7287	3	21	0.022
HMAGAAAAGAVVGGLGGYMLGSAMSRPIIHFGSDYEDR + 3 Oxidation (M)	768.9607	3839.7818	5	38	0.00046

Table S2. Identified peptides of human PrP^C from KI mouse brains after HILIC column.

Fragment	Obsd. m/z	Calcd. m/z	Charge	Score	*P*
QHTVTTTTK + Gln->pyro-Glu (N-term Q)	500.2587	998.5033	2	57	1.5e-005
QHTVTTTTK	508.7716	1015.5298	2	27	0.024
ESQAYYQR	522.7411	1043.4672	2	60	4.3e-006
YPGQGSPGGNR	545.2562	1088.4999	2	50	4.3e-005
GENFTETDVK	570.2640	1138.5142	2	54	1.6e-005
VVEQMCITQYER	778.3660	1554.7170	2	70	6.6e-007
VVEQMCITQYER + Oxidation (M)	786.3628	1570.7120	2	79	6.5e-008
YPNQVYYRPMDEYSNQNNFVHDCVNITIK	906.1686	3620.6453	4	19	0.028

Table S3. LC-ESI-MS/MS analysis of GPI-anchor in human PrP^C from KI mouse brains.

Fragment	Calcd. m/z	Charge	Calcd. m/z	Peak intensity		%
				2^+ or 3^+	Total	Total
G-GPI core	1211.3^+	2^+	606.2	ND*	0	0
		3^+	404.4	ND		
G-GPI core + 4th Man	1373.4^+	2^+	687.2	ND	0	0
		3^+	458.5	ND		
G-GPI core + GalNAc	1414.4^+	2^+	707.7	ND	0	0
		3^+	472.1	ND		
G-GPI core + GalNAc + 4th Man	1576.4^+	2^+	788.7	$2.54\ E^3$	$2.54\ E^3$	34.5
G-GPI core + GalNAc + Gal		3^+	526.1	ND		
G-GPI core + GalNAc + Gal + 4th Man	1738.5^+	2^+	869.8	$2.78\ E^3$	$2.78\ E^3$	37.7
		3^+	580.2	ND		
G-GPI core + GalNAc + Gal + Neu5Ac	1867.5^+	2^+	934.3	$1.24\ E^3$	$1.28\ E^3$	17.4
		3^+	623.2	$4.42\ E^1$		
G-GPI core + GalNAc + Gal + Neu5Ac + 4th Man	2029.6^+	2^+	1015.3	$5.87\ E^2$	$7.63\ E^2$	10.4
		3^+	677.2	$1.76\ E^2$		
GS-GPI core	1298.3^+	2^+	649.7	ND	0	0
		3^+	433.4	ND		
GS-GPI core + 4th Man	1460.4^+	2^+	730.7	ND	0	0
		3^+	487.5	ND		
GS-GPI core + GalNAc	1501.4^+	2^+	751.2	ND	0	0
		3^+	501.1	ND		
GS-GPI core + GalNAc + 4th Man	1663.5^+	2^+	832.2	ND	0	0
GS-GPI core + GalNAc + Gal		3^+	555.2	ND		
GS-GPI core + GalNAc + Gal + 4th Man	1825.5^+	2^+	913.3	ND	0	0
		3^+	609.2	ND		
GS-GPI core + GalNAc + Gal + Neu5Ac	1954.6^+	2^+	977.8	ND	0	0
		3^+	652.2	ND		
GS-GPI core + GalNAc + Gal + Neu5Ac + 4th Man	2116.6^+	2^+	1058.8	ND	0	0
		3^+	706.2	ND		
Total					$7.36\ E^3$	100

* ND: Not detected

Table S4. Identified papetides of human PrP^C from human brains after HILIC column.

Fragment	Obsd. m/z	Calcd. m/z	Charge	Score	*P*
QHTVTTTTK + Gln->pyro-Glu (N-term Q)	500.2595	998.5033	2	44	0.00025
ESQAYYQR	522.7402	1043.4672	2	39	0.00042
YPGQGSPGGNR	545.2570	1088.4999	2	42	0.00038
VVEQMCITQYER + Oxidation (M)	786.3609	1570.7120	2	29	0.0055

Table S5. LC-ESI-MS/MS analysis of GPI-anchor in human PrP^C from human brains.

Fragment	Calcd. m/z	Charge	Calcd. m/z	Peak intensity		%
				2^+ or 3^+	Total	Total
G-GPI core	1211.3^+	2^+	606.2	ND*	0	0
		3^+	404.4	ND		
G-GPI core + 4th Man	1373.4^+	2^+	687.2	ND	0	0
		3^+	458.5	ND		
G-GPI core + GalNAc	1414.4^+	2^+	707.7	ND	0	0
		3^+	472.1	ND		
G-GPI core + GalNAc + 4th Man	1576.4^+	2^+	788.7	$2.18\ E^2$	$2.18\ E^2$	8.5
G-GPI core + GalNAc + Gal		3^+	526.1	ND		
G-GPI core + GalNAc + Gal + 4th Man	1738.5^+	2^+	869.8	$1.29\ E^3$	$1.29\ E^3$	50.3
		3^+	580.2	ND		
G-GPI core + GalNAc + Gal + Neu5Ac	1867.5^+	2^+	934.3	$3.58\ E^1$	$3.58\ E^1$	1.4
		3^+	623.2	ND		
G-GPI core + GalNAc + Gal + Neu5Ac + 4th Man	2029.6^+	2^+	1015.3	$1.02\ E^3$	$1.02\ E^3$	39.8
		3^+	677.2	ND		
GS-GPI core	1298.3^+	2^+	649.7	ND	0	0
		3^+	433.4	ND		
GS-GPI core + 4th Man	1460.4^+	2^+	730.7	ND	0	0
		3^+	487.5	ND		
GS-GPI core + GalNAc	1501.4^+	2^+	751.2	ND	0	0
		3^+	501.1	ND		
GS-GPI core + GalNAc + 4th Man	1663.5^+	2^+	832.2	ND	0	0
GS-GPI core + GalNAc + Gal		3^+	555.2	ND		
GS-GPI core + GalNAc + Gal + 4th Man	1825.5^+	2^+	913.3	ND	0	0
		3^+	609.2	ND		
GS-GPI core + GalNAc + Gal + Neu5Ac	1954.6^+	2^+	977.8	ND	0	0
		3^+	652.2	ND		
GS-GPI core + GalNAc + Gal + Neu5Ac + 4th Man	2116.6^+	2^+	1058.8	ND	0	0
		3^+	706.2	ND		
Total					$2.56\ E^3$	100

* ND: Not detected

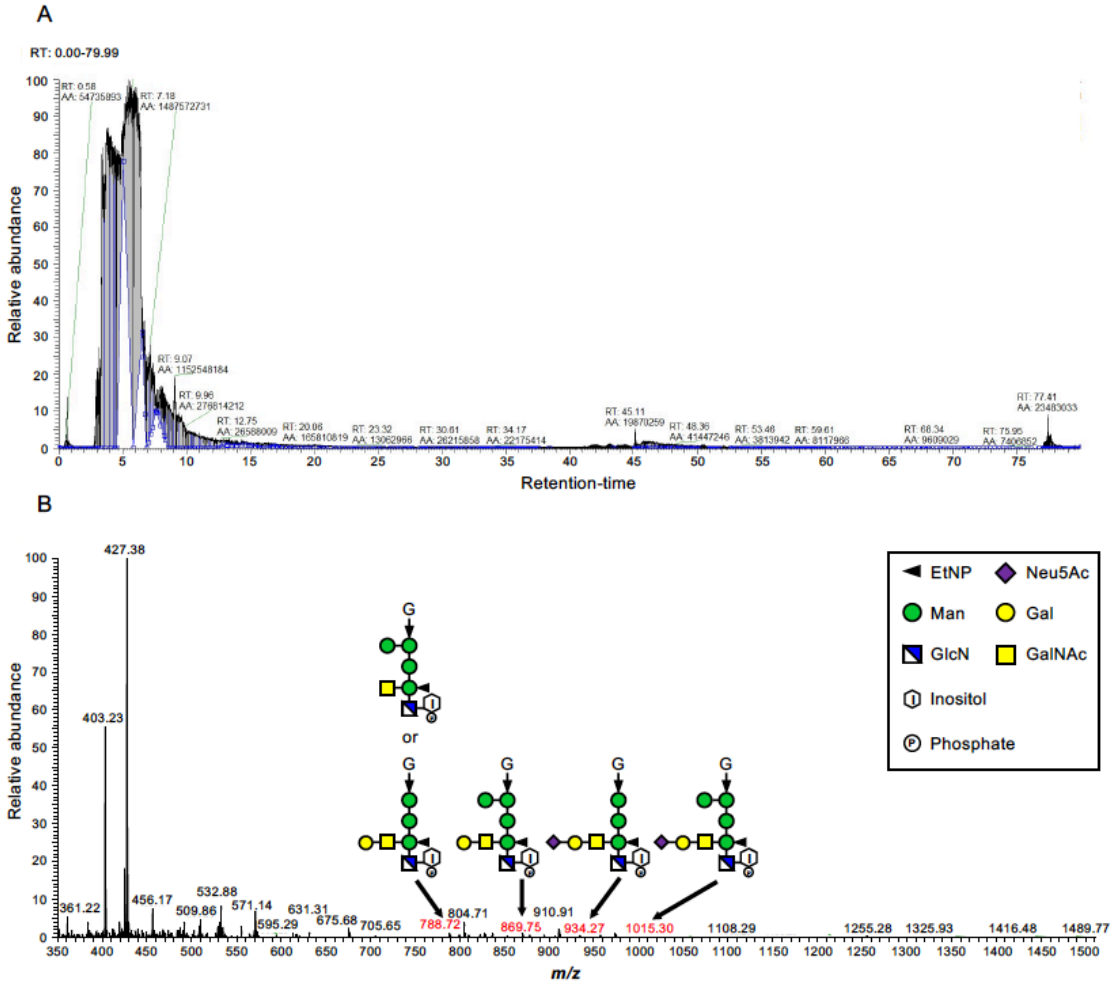

Figure S1: LC-ESI-MS analysis of purified human PrP^C from KI mouse brains.

A. Total ion chromatogram (TIC) of LC-ESI-MS analysis of purified human PrP^C from KI mouse brains. B. Total MS spectrum of purified human PrP^C from KI mouse brains. An average MS spectrum was obtained based on retention-time from 49.75 min to 77.01 min. The m/z value of the spectrum ranged from 350 to 1500. The peptides containing GPI-glycan were highlighted by red and corresponding structures were shown by schematics. Glycan symbols are according to Symbol Nomenclature for Glycans (42).

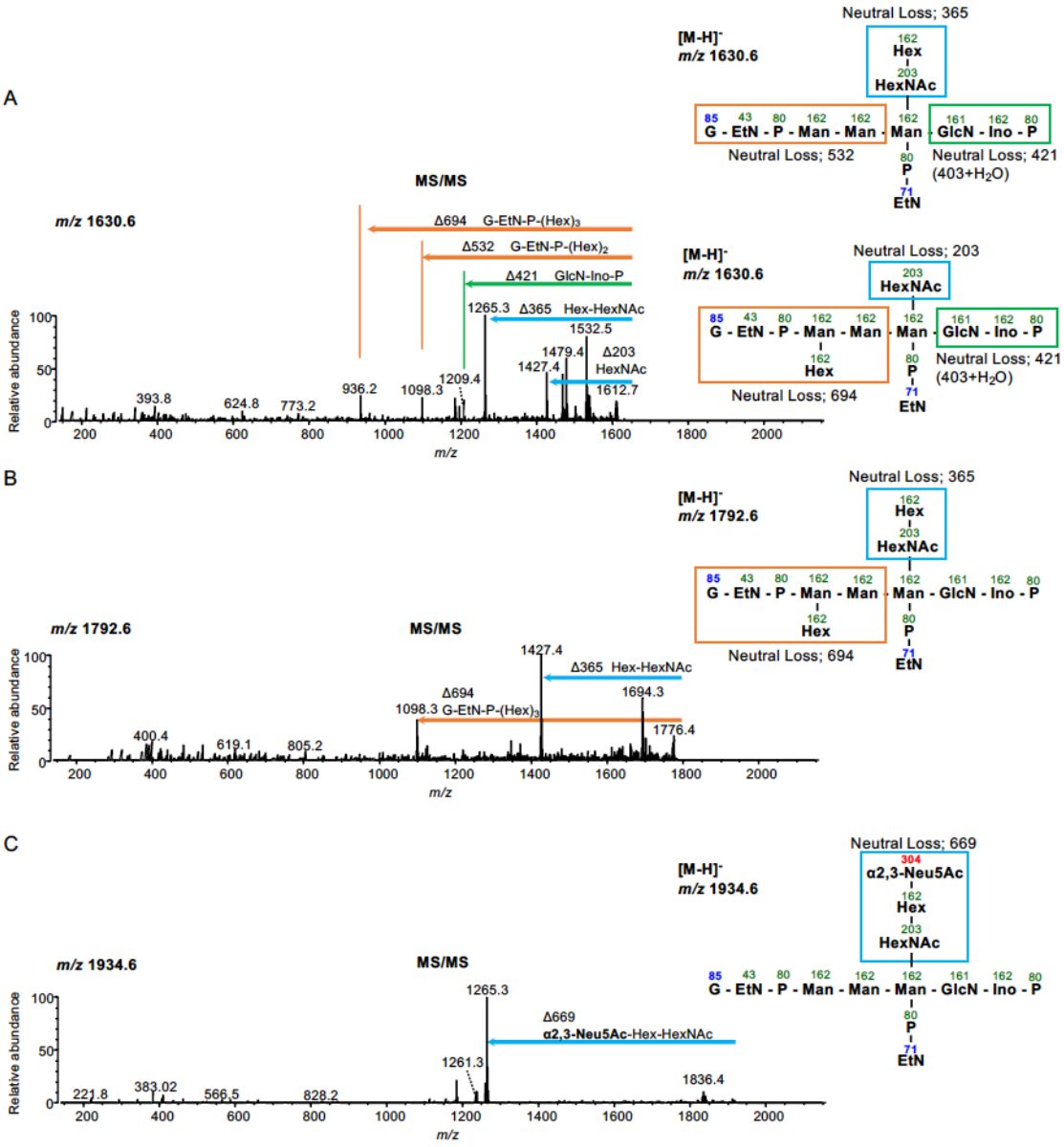

Figure S2: Linkage analysis of sialic acid on GPI-anchor by SALSA method using MALDI-MS. A. The MS/MS spectrum of a C-terminal peptide with GPI containing HexNAc and Hex is shown. A 365 Da loss indicates the loss of Hex (Gal) and HexNAc (GalNAc). A 203 Da loss indicates the loss of HexNAc (GalNAc). B. The MS/MS spectrum of a C-terminal peptide with GPI containing Hex and HexNAc-Hex is shown. 365 Da loss indicates the loss of Hex (Gal) and HexNAc (GalNAc). C. The MS/MS spectrum of a C-terminal peptide with GPI containing HexNAc-Hex-Neu5Ac is shown. 669 Da loss indicates the loss of α2,3-linked Sia, Hex (Gal) and HexNAc (GalNAc).