Chapter 3: Identification of the Plasticity-Relevant Fucose-α(1-2)Galactose Proteome

Background

The human genome project led to the striking revelation that the number of proteins necessary for human function and development is only 20-25,000 genes, much less than the anticipated 100,000 genes thought to be discovered.[1] While genomics has produced a wealth of information on the genes encoded by DNA, it provides little information on the protein products themselves. Proteomics has emerged as an important new field to annotate the functions of proteins and provide valuable information that expedites the characterization of these proteins. Numerous large-scale approaches have been developed to characterize proteins, such as protein microarray technologies,[2, 3] large-scale yeast two-hybrid screens,[4] and high-throughput protein production and crystallization.[5] In these approaches, mass spectrometry has emerged as the main method for analysis of protein functions from native biological systems.[6-9]

Mass spectrometry is a powerful approach to analyze complex proteomes because of its unparalleled ability to acquire quantitative information from samples of enormous complexity. Recent advances in instrumentation, data acquisition, and analysis have yielded this methodology amenable to a variety of proteomics techniques such as the characterization of functional protein complexes, protein pathways, and proteins modified by post-translational modifications. Furthermore, the ability to directly sequence peptides has facilitated the high-throughput identification and characterization of phosphorylation and glycosylation sites, problems that would normally take weeks of

molecular cloning, site-directed mutagenesis, and analysis through in-direct methods to establish the modified sites. More recently, mass-spectrometry-based proteomics has yielded the ability to quantitatively analyze changes in proteins during disease states.[10, 11] Such experiments enable the identification of biomarkers for disease progression such as in cancer, diabetes, and other metabolic diseases.

With the acceleration of mass-spectrometry-based proteomics methods, it is no wonder the current literature is full of proteomic identifications and characterizations. However, despite this wealth of information, the ability to characterize proteomes by post-translation modifications (PTMs) has remained technically challenging. The lability of phosphorylation and glycosidic linkages on the mass spectrometer has hindered efforts to identify proteomes with these modifications. In addition, PTMs are often present in the cell in substoichiometric abundance, further complicating their analysis. Despite these limitations for identification of glycoproteomes, we report a successful mass-spectrometry-based proteomics method involving lectin affinity chromatography to identify the first comprehensive Fucα(1-2)Gal proteome from adult rat cortex and murine neonatal olfactory bulb.

We employed the use of the Fucα(1-2)Gal-binding lectin *Ulex europeaus* agglutinin (UEAI), which is reported to interact specifically with Fucα(1-2)Gal epitopes.[12, 13] The key binding interactions between UEAI and Fucα(1-2)Gal disaccharides involve the hydroxyls at C2, C3, and C4 of the α-L-Fuc unit and the C3 hydroxyl of the β-D-Gal unit.[13, 14] The key polar interactions come from the C3 and C4 of α-L-Fuc and are necessary for the interaction.[13] The carbohydrate recognition domain of UEAI is comprised of residues Glu44, Thr86, Asp87, Arg102, Ala103, Gly104,

Gly105, Tyr106, Ile129, Val133, Asn134, Trp136, Tyr219, and Arg222.[15] In addition to the key polar interactions with hydroyxl groups, Thr86 is reported to make an important hydrophobic interaction with the C5-methyl of α-L-Fuc. Thus, due to the high specificity of UEAI for Fucα(1-2)Gal disaccharides, we chose to exploit this lectin to identify the Fucα(1-2)Gal proteome.

We identify four major functional classes of Fucα(1-2)Gal glycoproteins from murine olfactory bulb: the cell adhesion molecules, ion channels and solute transporters/carriers, ATP-binding proteins, and synaptic vesicle-associated proteins. We demonstrate that these proteins involved in neurotransmitter release, neurite outgrowth, and synaptic plasticity. Their identification suggests extended roles for protein fucosylation in mediating neuronal communication processes.

Results

Identification of Brain Fractions Enriched in Fucα(1-2)Gal Glycoproteins

Identification of proteins modified by carbohydrates is especially challenging from a proteomics perspective. Lectins and antibodies that are used to capture proteins often suffer from low binding affinities, precluding the isolation of large quantities of proteins for LC-MS studies. For example, a recent proteomics study necessitated the use of a 39-foot lectin column to enrich desired glycoproteins.[16] In addition, fucose is present in low cellular abundance, further complicating the isolation and identification of proteins modified by this monosaccharide. Despite these limitations for protein capture and identification, we have developed a novel method to identify the first comprehensive

Fucα(1-2)Gal proteome from murine olfactory bulb using lectin affinity chromatography (LAC).

Previous studies have indicated that Fucα(1-2)Gal are present in the adult and developing hippocampus.[17] However, we first sought to address whether other brain regions might be further enriched in expression of Fucα(1-2)Gal glycoproteins to facilitate isolation of glycoproteins for proteomics studies. Postnatal day 3 (P3) and adult rat brains were isolated and dissected into major brain regions including the cortex, cerebellum, hippocampus, hypothalamus, olfactory bulb, striatum, and thalamus. Brain substructures were analyzed by SDS-PAGE and Western blotting with UEAI lectin

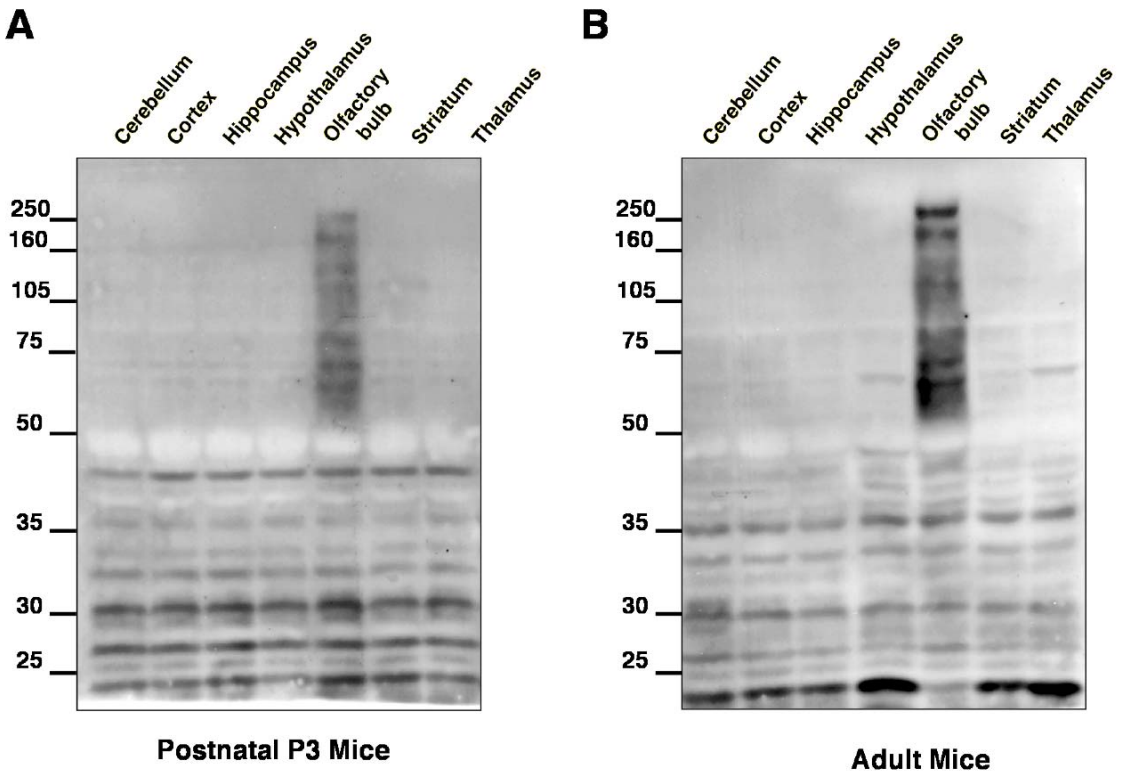

Figure 3.1. Fucα(1-2)Gal glycoproteins are enriched in mammalian olfactory bulb. Western blot of neonatal rat brain homogenates demonstrates enrichment of Fucα(1-2)Gal glycoproteins in postnatal (A) and adult (B) olfactory bulb. Blots were probed with UEAI.

Significant labeling of proteins was observed in olfactory bulb homogenates of both adult and neonatal rats, suggesting that Fucα(1-2)Gal glycoproteins are highly enriched in this brain regions (Figure 3.1). Furthermore, Fucα(1-2)Gal staining suggests the presence of many Fucα(1-2)Gal glycoproteins between 50 and 200 kilodaltons (kDa) of rat olfactory bulb. The presence of low molecular weight proteins between 20 and 50 kDa of all brain substructures may reflect Fucα(1-2)Gal glycoproteins expressed to a similar degree in each brain subfraction. Alternatively, these proteins may be non-specifically recognized by the Fucα(1-2)Gal-binding lectin UEAI. In addition, there is an enriched Fucα(1-2)Gal glycoprotein expressed at approximately 60 kDa in the adult thalamus and hypothalamus. Darker exposures of brain subfractions demonstrate that the olfactory bulb appears to contain significantly more labeling than hippocampus, another brain substructure with high expression of Fucα(1-2)Gal glycoproteins (Figure 3.2). Finally, expression of Fucα(1-2)Gal was significantly enhanced in neonatal mice when compared to adult olfactory bulb (data not shown), consistent with our studies suggesting that Fucα(1-2)Gal glycoproteins are enriched during periods of neuronal growth.[17] Thus, the mammalian olfactory bulb of P3 neonatal mice contains the highest expression of Fucα(1-2)Gal glycoproteins in the brain.

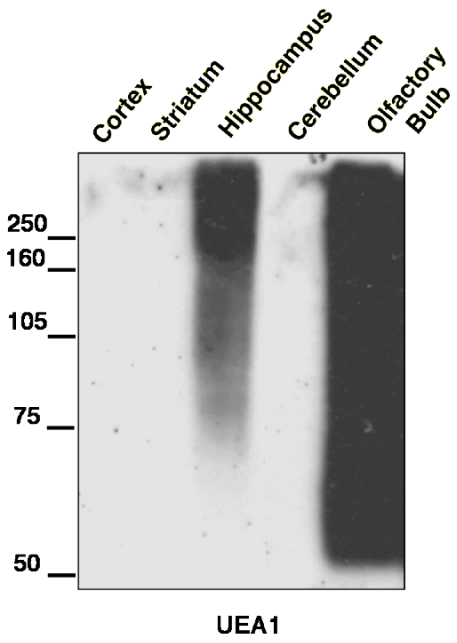

Figure 3.2. The mammalian olfactory bulb is highly enriched in expression of Fucα(1-2)Gal glycoproteins. Rat adult brain fractions were resolved by SDS-PAGE and analyzed by immunoblotting with lectin UEAI. The olfactory bulb contains the highest expression of Fucα(1-2)Gal glycoproteins while the hippocampus is the next region enriched.

Identification of the Fucα(1-2)Gal Proteome from Mouse Olfactory Bulb

As the olfactory bulb of neonatal mice contained significantly higher expression of Fucα(1-2)Gal glycoproteins, we optimized a method for the enrichment of these proteins from olfactory bulb lysates by UEAI lectin affinity chromatography (Figure 3.3).

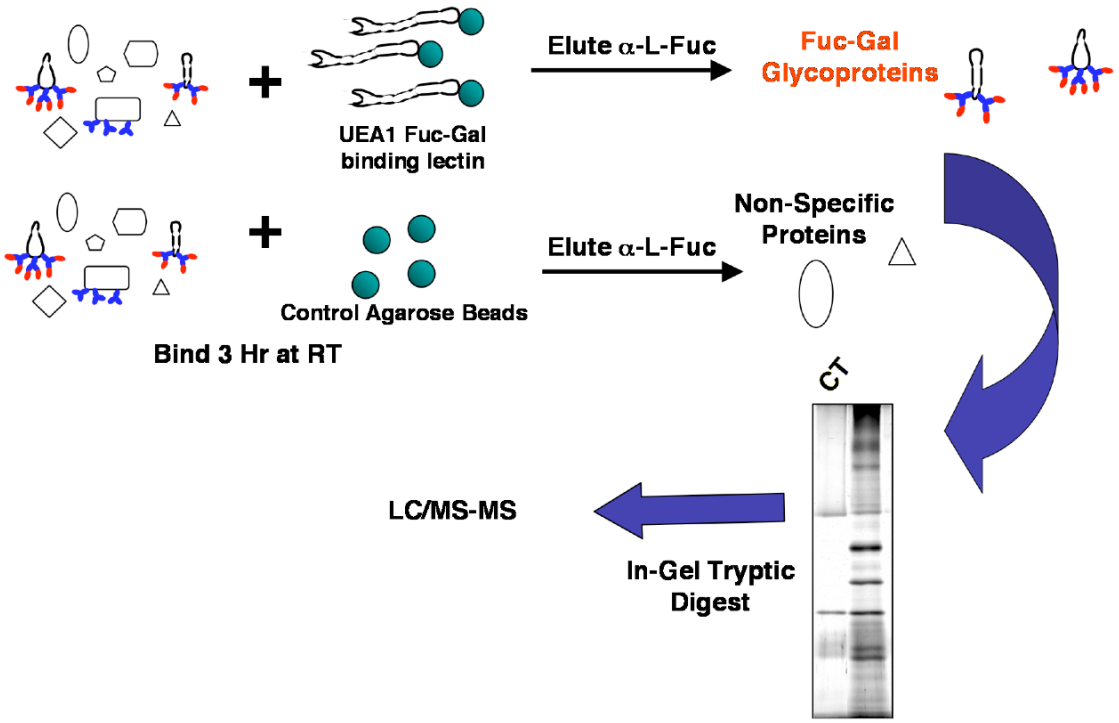

Figure 3.3. Strategy for the isolation and identification of Fucα(1-2)Gal glycoproteins from mouse olfactory bulb.

Our approach utilizes an empirically determined buffer that maximizes lectin-carbohydrate binding while minimizing non-specific interactions, and the counter-intuitive incubation of the columns with protein lysates at room temperature to enhance glycoprotein recognition. Furthermore, the use of control protein A-agarose columns

aided in the identification of non-specific proteins that bind to the agarose beads (Figure 3.3). Using these optimized conditions and Fucα(1-2)Gal-enriched olfactory bulb lysates, we identify a large number of proteins that were specifically captured in the UEAI lectin affinity column from C57BL/6 P3 mice with respect to the control column (Figure 3.4).

To determine protein identities, 33 bands from each lane were excised, digested

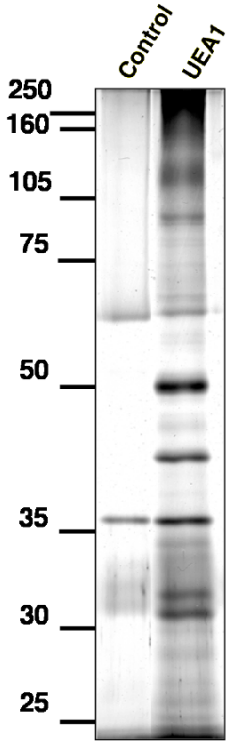

Figure 3.4. Silver stain of proteins isolated from the control protein A agarose-column (left lane) and the UEAI column (right lane).

in-gel by trypsin and subjected to LC-MS/MS for peptide identification. Peptides were searched using a SEQUEST 3.0 search algorithm against the European Bioinformatics Institute-International Protein Index (EBI-IPI) database. The search results were compiled and filtered using Scaffold 2.0. Each series of bands was analyzed for the

presence of the protein in the agarose control columns to eliminate false positives, and putative glycoproteins between columns were compared using the Scaffold viewer. Proteins were considered potential Fucα(1-2)Gal glycoproteins when at least 2 unique peptides were identified, which gives ≥ 99% probability that the protein is present in the sample. Proteins were considered non-specific if any peptides were isolated from the control protein A-agarose column. As lectin affinity chromatography can also suffer from the purification of protein complexes due to the mild lysis conditions, other proteins that associate with the Fucα(1-2)Gal glycoproteins may also be isolated. Thus, we implemented a stringent peptide cut-off of 7 peptides to reduce the number of non-specific proteins identified by this technique. The proteins meeting the search criteria were sorted by functions and are listed in Table 3.1. For a list of all proteins identified, see the supplemental table after the Materials and Methods section.

Proteins	Accession Number	MW	Peptides
Cell Adhesion Molecules			
Ncam1 neural cell adhesion molecule 1 isoform 1	IPI:IPI00831465.1	93474.6	35
Ncam1 Isoform N-CAM 180 of Neural cell adhesion molecule 1, 180 kDa isoform precursor	IPI:IPI00122971.1	119333.5	26
Igsf3 Immunoglobulin superfamily, member 3	IPI:IPI00420589.2	134605.2	24
L1cam L1 cell adhesion molecule	IPI:IPI00406778.3 IPI:IPI00785371.1 IPI:IPI00831568.1	140413.3	15
Cntn1 Contactin-1 precursor	IPI:IPI00123058.1	113372.8	11
Nrcam Isoform 6 of Neuronal cell adhesion molecule precursor	IPI:IPI00120564.5 IPI:IPI00338880.3 IPI:IPI00395042.1 IPI:IPI00403536.1	122736	8
Ncam2 Isoform Long of Neural cell adhesion molecule 2 precursor	IPI:IPI00127556.1 IPI:IPI00322617.1	93187.3	7

Ion Channels and Solute Transporters/ Carrier Proteins			
Slc12a2 solute carrier family 12, member 2	IPI:IPI00135324.2	130654.7	20
Cacna2d1 Isoform 2B of Dihydropyridine-sensitive L-type calcium channel subunits alpha-2/delta precursor	IPI:IPI00230013.3 IPI:IPI00319970.1 IPI:IPI00407868.1 IPI:IPI00410982.1 IPI:IPI00626793.3	122505.5	16
Slc25a12 Calcium-binding mitochondrial carrier protein Aralar1	IPI:IPI00308162.3	74553.8	12
Slc3a2 CD98 heavy chain	IPI:IPI00114641.2	58805.6	11
Slc7a5 Solute carrier family 7 (Cationic amino acid transporter, y+ system), member 5	IPI:IPI00331577.3	55856	7
ATP Synthase/ ATPase/ Transporters			
Atp1a1 Sodium/potassium-transporting ATPase subunit alpha-1 precursor	IPI:IPI00311682.5	112967.3	51
Atp5a1 ATP synthase subunit alpha, mitochondrial precursor	IPI:IPI00130280.1	59736.1	14
Atp1a3 Sodium/potassium-transporting ATPase subunit alpha-3	IPI:IPI00122048.2	111676.4	11
Atp1b1 Sodium/potassium-transporting ATPase subunit beta-1	IPI:IPI00121550.2	35771.2	10
Synaptic Vesicle Proteins			
Stxbp1 Isoform 1 of Syntaxin-binding protein 1	IPI:IPI00415402.3	67553.8	14
Syt1 Synaptotagmin-1	IPI:IPI00129618.1 IPI:IPI00750142.1	47400.9	10
Nsf Vesicle-fusing ATPase	IPI:IPI00656325.2	82598.5	8
Receptors			
Plxnb2 14 days pregnant adult female placenta cDNA, RIKEN full-length enriched library, clone:M5C1068G03 product:plexin B2, full insert sequence	IPI:IPI00405742.6 IPI:IPI00666301.1 IPI:IPI00752396.1	206212.3	16
Igf1r Insulin-like growth factor 1 receptor precursor	IPI:IPI00120225.1	155772.7	8
Cytoskeletal Remodeling/			

Binding/ Dynamics			
Dpysl3 Dihydropyrimidinase-related protein 3	IPI:IPI00122349.1	61918.9	11
Dpysl2 Dihydropyrimidinase-related protein 2	IPI:IPI00114375.2	62259.9	7
Heat Shock Proteins/ Chaperonins			
Hsp90ab1 Heat shock protein 84b	IPI:IPI00229080.7	83266.3	15
Endoplasmin precursor	IPI:IPI00129526.1	92460.6	7
Transcription/ Translation/ Ribosomal			
Eef1a1 Elongation factor 1-alpha 1	IPI:IPI00307837.5	50736.9	9
Eef2 Elongation factor 2	IPI:IPI00466069.3	95298	7
Rps3 40S ribosomal protein S3	IPI:IPI00134599.1	26656.5	7
GTPase-binding/ Cell Signaling			
Gnao1 Guanine nucleotide-binding protein G(o) subunit alpha 2	IPI:IPI00115546.4	40019.4	10
Rab3a Ras-related protein Rab-3A	IPI:IPI00122965.1	24952.3	9
Ywhaz 14-3-3 protein zeta/delta	IPI:IPI00116498.1	27753.9	8
Rab14 Ras-related protein Rab-14	IPI:IPI00126042.3	23879.6	7
Other			
Npepps Aminopeptidase puromycin sensitive	IPI:IPI00608097.1	103310.1	9
Psmd2 26S proteasome non-ATPase regulatory subunit 2	IPI:IPI00123494.3	100187.1	9
Phb2;Grcc10 Prohibitin-2	IPI:IPI00321718.4	33279.6	7
Phb;EG665511 Prohibitin	IPI:IPI00133440.1	29802.6	7
LOC100042349;LOC100041325; Gapdh;LOC100046067;LOC100048291;LOC100042025;LOC100040053;LOC100042746 Glyceraldehyde-3-phosphate dehydrogenase	IPI:IPI00273646.9 IPI:IPI00462008.5 IPI:IPI00620663.3	35792.1	7
Ldha L-lactate dehydrogenase A chain	IPI:IPI00319994.6 IPI:IPI00751369.1	36480.9	7

Table 3.1. Table of all putative proteins Fucα(1-2)Gal glycoproteins identified by LAC and LC-MS/MS with the number of peptides in the sample ≥ 7. Accession numbers are from the European Bioinformatics Institute-International Protein Index (EBI-IPI).

Based on these criteria, we identified 39 putative Fucα(1-2)Gal glycoproteins from the UEAI lectin enrichment column (Table 3.1). The major protein hit was the identification of 51 peptides corresponding to the Na^+/K^+ ATPase α1 subunit. Importantly, we also identified 35 peptides for NCAM, a protein previously reported to be modified by Fucα(1-2)Gal in mammalian olfactory bulb,[18] providing validation of our methodology for the identification of Fucα(1-2)Gal glycoproteins. In addition to this known Fucα(1-2)Gal glycoprotein, our approach identifies 38 novel proteins. The major protein hits (greater than 10 peptides) can be categorized into four major functional classes: the cell adhesion molecules, ion channels and solute carriers/transporters, ATP-binding proteins, and synaptic vesicle-associated proteins. Thus, these studies have significantly expanded the repertoire of known Fucα(1-2)Gal glycoproteins, from 2 to almost 40. Furthermore, identification of novel classes of Fucα(1-2)Gal glycoproteins such as ion channels, solute transporters, and ATP-binding proteins suggests an extended role for Fucα(1-2)Gal glycoproteins in mediating membrane excitability and ATP metabolism. In addition to these novel classes, the all of the cell adhesion molecules identified are members of the immunoglobulin superfamily (IgSF) of cell adhesion molecules, which may suggest an important functional role for fucosylation of these proteins in mediating olfactory bulb development.

Confirmation of Fucα(1-2)Gal Glycoproteins

We next sought to confirm the presence of the four major classes of Fucα(1-2)Gal glycoproteins identified in the MS by immunoblotting lectin column eluates. The presence of many cell adhesion molecules such as NCAM, NCAM L1, and NCAM2

(more commonly know as OCAM) was independently confirmed by immunoblotting UEAI column eluates with the appropriate antibodies (Figure 3.5) The proteins were specifically recognized by UEAI and were absent from the control column suggesting that these proteins are definitely present in the sample. We next examined eluates for the presence of synaptic vesicle proteins synaptotagmin I and munc18 (syntaxin-binding protein 1), which were also found to be specifically present (Figure 3.5). In addition, we demonstrate that the ATP-binding protein α1 subunit of the Na^+K^+ ATPase, which was the best protein hit by MS analysis, is conclusively present in the sample. We observed other interesting proteins with peptides less than 7 of the four major classes identified, and verified the presence of two of them by immunoblotting. The cell adhesion molecule N-cadherin is a calcium-dependent cell adhesion molecule that is not a member of the IGSF family.[19-22] However, the presence of N-cadherin in UEAI column eluates, suggests that other classes of cell adhesion molecules can be fucosylated. We also demonstrate binding of the voltage-dependent anion channel 1 (VDAC1), which is an ion channel normally found in the mitochondrial membrane that is present in post-synaptic density preparations with unknown function.[23] Therefore, we were able to validate a number of putative Fucα(1-2)Gal glycoproteins from the MS data, including proteins from all four major functional classes identified. Abundant cellular proteins such as tubulin and hsp/hsc 70 were found to bind non-specifically to the control columns, consistent with our MS data, and demonstrating the importance use of the control column in eliminating false positives (Figure 3.5).

NCAM and synaptotagmin were found to run as multiple bands, which is consistent with the presence of multiple fucosylated glycoforms. In fact, NCAM has

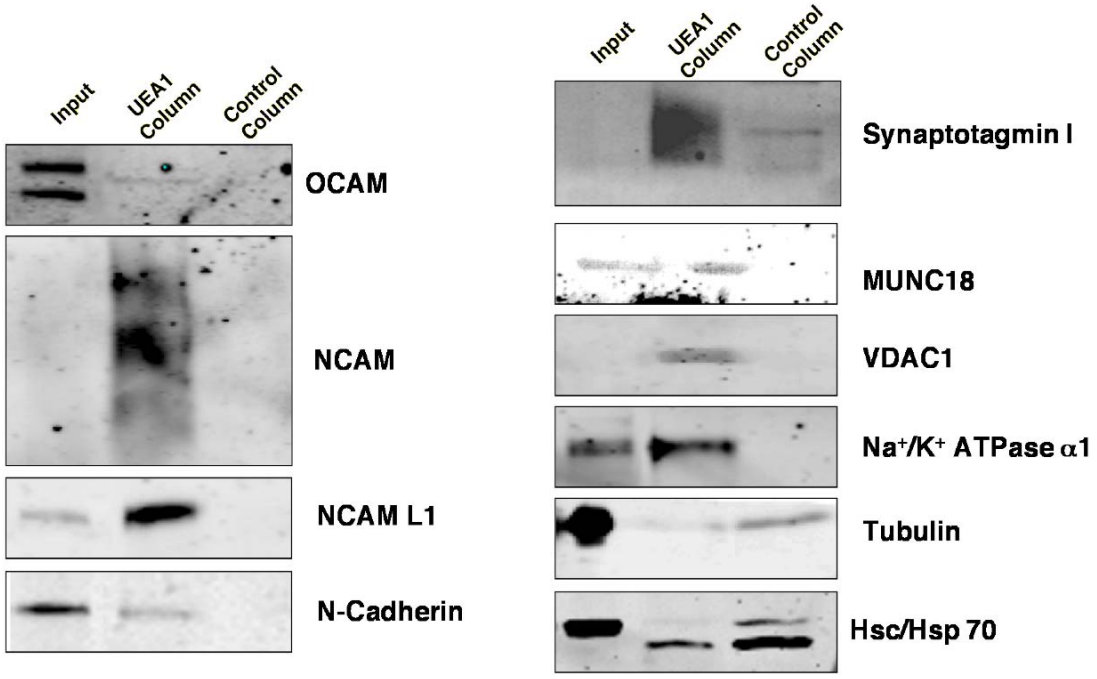

Figure 3.5. All four major classes of Fucα(1-2)Gal glycoproteins identified in MS data are confirmed by immunoblotting lectin column eluates. We demonstrate the presence of OCAM, NCAM, NCAM L1, N-cadherin, synaptotaming I, MUNC18, VDAC1, and the Na^+/K^+ ATPase alpha 1 subunit as specific in MS sample. Tubulin and Hsc/Hsp 70 were present in the control column eluates, consistent with MS data.

previously been reported to exist in multiple glycoforms,[24] thus raising the intriguing possibility that different combinations of glycans may differentially affect NCAM function. We also immunoprecipitated NCAM to confirm fucosylation by immunoblotting with UEAI and antibody A46-B/B10 (data not shown).

Discussion

Herein we report the first comprehensive proteome of the plasticity-relevant Fucα(1-2)Gal epitope. The proteins were identified by an optimized lectin affinity

chromatography approach coupled to mass spectrometry. This study isolates the largest number of Fucα(1-2)Gal glycoproteins reported, and indicates specific roles for the Fucα(1-2)Gal epitope in synaptic vesicle cycling, membrane excitability, and synaptic remodeling.

In contrast to previous studies which suggest that the cell adhesion molecule NCAM is the only Fucα(1-2)Gal glycoprotein present in murine olfactory bulb,[18] our results indicate the presence of numerous Fucα(1-2)Gal glycoproteins in both murine neonatal olfactory bulb and adult cortex. Expression of these proteins is enhanced during periods of development. We exploited this period of increased Fucα(1-2)Gal expression to identify the Fucα(1-2)Gal proteome from neonatal olfactory bulb. Our studies significantly expand the number of known Fucα(1-2)Gal glycoproteins from 2 to almost 40. Our approach identifies four major classes of these glycoproteins in murine olfactory bulb, including the cell adhesion molecules, ion channels and solute carriers/transporters, ATP-binding proteins, and synaptic vesicle-associated proteins.

The cell adhesion molecules represent the largest class of Fucα(1-2)Gal glycoproteins identified. They are known to be involved in development and synaptic plasticity. Of the 7 proteins discovered fitting our search criteria, all are members of the immunoglobulin (Ig) superfamily of cell adhesion molecules, suggesting an important functional role for fucosylation of this subclass of proteins in mediating olfactory bulb development. Many of these proteins are already known to regulate development of the olfactory bulb. For example, NCAM is responsible for axonal fasciculation and targeting of OSNs in the olfactory bulb.[25, 26] NCAM is differentially glycosylated, and it's possible that these glycoforms help distinguish subsets of axons and aid in neuronal pathfinding.

Two of these cell adhesion molecules, contactin and OCAM, have previously been reported to play important roles in development of the accessory olfactory bulb (AOB). Contactin is highly expressed in the AOB and is involved in neurite outgrowth,[27] while OCAM may be involved in setting up functional subdivisions and defining patterns of connectivity in the AOB.[28]

Several solute carriers/transporters were identified in this study, including the chloride transporter solute carrier family 12 member 2 protein (Slc12a2) and the calcium-binding mitochondrial carrier protein aralar1 (Slc25a12). Slc12a2 controls the accumulation of chloride in cells, and Slc12a2 knockout mice exhibit a loss of GABA-receptor-mediated currents in dorsal root ganglion neurons (DRGs).[29] In accordance with this observation, Slc12a2 KO mice exhibit alterations in locomotion and pain perception. The mitochondrial carrier protein aralar1 is expressed in neurons of the CNS where it undergoes enrichment during maturation of neuronal cultures[30] and may be involved in neurodegenerative conditions such as Alzheimer's disease.[31]

We identify the ion channel alpha2/delta subunit of the dihydropyridine-sesitive L-type calcium channel (Cacna2d1) as a putative Fucα(1-2)Gal glycoprotein. In addition, we observed a known glycosylated ion channel, the transient receptor potential cation channel (TRPV5), with less than 7 peptides in the MS data, and we discuss it here. Identification of Cacna2d1 and the TRPV5 suggests a role for protein fucosylation in regulating ion channel function. Calcium signaling plays a pivotal role in regulating intracellular signaling cascades that underlie synaptic plasticity,[32-35] and the identification of Cacna2d1 may suggest that protein fucosylation is involved in these processes. The TRP family of cation channels is expressed by sensory neurons and mediates a variety of

sensory functions such as thermal sensitivity and nociception.[36, 37] TRP channels of mice are also used in pheromone-sensing to distinguish male mice from females,[38] supporting a role for the Fucα(1-2)Gal epitope in regulating murine olfaction. *N*-linked glycosylation of the first extracellular loop of TRPV5 regulates channel function and targeting of TRP channels.[39, 40] As TRPV5 is a putative Fucα(1-2)Gal glycoprotein, it will be intriguing to learn whether fucosylation occurs on this *N*-linked glycan, and the specific roles of the Fucα(1-2)Gal epitope in regulating TRPV5 channel functions.

We also discovered 11 ATP-binding proteins, including 4 subunits of the Na^+/K^+-ATPase, 5 subunits of ATP synthases, and 2 other ATP-driven transport proteins. The Na^+/K^+-ATPase is critical for maintenance of the electrochemical gradient of these ions across the plasma membrane,[41, 42] and the beta1 subunit has previously been reported to contain *N*-linked fucosylated glycans.[43] While the precise fucose linkage was previously uncharacterized, our studies suggest that the beta1 glycans contain the plasticity-relevant Fucα(1-2)Gal epitope. In addition to ATPases, we also identify various ATP synthases, which implicates the Fucα(1-2)Gal epitope in cellular energy production.

Our identification of several synaptic vesicle-associated proteins suggests an important role for the Fucα(1-2)Gal epitope in regulating synaptic vesicle cycling. Our previous report identifies synapsin I as a Fucα(1-2)Gal glycoprotein,[17] and these studies suggest that other proteins such as munc18 and synaptotagmin are also fucosylated. Some of the proteins identified are on the cytosolic face of the membrane, and raises the intriguing possibility that protein fucosylation may occur in the cytoplasm. Although, no known cytosolic fucosyltransferases have been discovered in higher eukaryotes to date, cytosolic fucosylation exists in the lower eukaryote *Dictyostelium discoideum*.[44-46]

Identification of the Fucα(1-2)Gal glycoproteome reveals molecular insights into the functions of the Fucα(1-2)Gal epitope. We uncover proteins involved in development, neurite outgrowth, and synaptic plasticity. These results are consistent with an important role for the Fucα(1-2)Gal epitope in modulating the molecular mechanisms involved in neuronal communication and development.

Materials and Methods

Animals, Tissue Isolation and Homogenization

C57BL/6 wild-type animals and Sprague-Dawly rats were maintained in accordance with proper Institute of Animal Care and Use Committee (IACUC) procedures. Adult male mice ages 3-4 months and post-natal day 3 (P3) pups were anesthetized with CO_2 and dissected to remove the cerebellum, cortex, hippocampus, hypothalamus, olfactory bulb, striatum, and thalamus. For Western blotting, dissected tissues were cut into small pieces and placed immediately on ice, then lysed in boiling 1% SDS with sonication until homogeneous (5V:W). For lectin affinity chromatography, the olfactory bulbs from 30-50 P3 pups were isolated and homogenized in lectin binding buffer (100 mM Tris pH 7.5/ 150 mM NaCl/ 1mM $CaCl_2$/ 1 mM $MgCl_2$/ 0.5% NP-40/ 0.2% Na deoxycholate plus protease inhibitors) by passing through a 26G needle 5 times, then sonicated to homogeneity. Samples were clarified by centrifugation at 12,000g × 10 min. Lysates were between 6 to 10 mg/mL total protein concentration as determined by the BCA protein assay (Pierce) for lectin affinity chromatography. Alternatively, the olfactory bulbs from 12-16 rat pups were used for lectin affinity chromatography experiments followed by immunoblotting with different antibodies.

Lectin Affinity Chromatography and SDS-PAGE

One mL bed volume of *Ulex europaeus* agglutinin I (UEAI) conjugated to agarose (Vector Labs,) and control Protein A conjugated to agarose (Vector Labs) columns were packed ~333 μL into 3 minicolumns run in parallel (BioRad). The resin was equilibrated with 10 column volumes (CV) lectin binding buffer. 3 mLs of olfactory bulb lysate at 6-10 mg/mL was bound in batch at RT for 4 hours. Columns were repacked and the flowthrough was passed 3 additional times over the column. Columns were washed with 40 CV of lectin binding buffer, followed by 10 CV of lectin binding buffer lacking detergent (NP-40 and Na deoxycholate). Proteins were eluted in 10 CV of lectin binding buffer lacking detergent supplemented with 200 mM α-L-Fuc and protease inhibitors.

Protein eluates were concentrated in 10,000 molecular weight cut-off (MWCO) centricons (Millipore) followed by 10,000 MWCO microcons (Millipore) to 100 μL. Following concentration, samples were boiled with 35 μL of 4X SDS loading dye and loaded onto 10% SDS gels for electrophoresis as described previously.[47]

Silver Staining, Peptide Extraction, and In-Gel Tryptic Digests

All silver staining reagents were prepared fresh. Gels were fixed in 50% MeOH/ 10% acetic acid for 30 min at RT, followed by 5% MeOH/ 1% for 15 min. Gels were rinsed 1x in 50% MeOH for 1min, then washed 6 × 10 min in ddH_2O. Following fixation, gels were sensitized in 20 mg/100 mL $Na_2S_2O_3 \bullet 5H_2O$ for 90 s, rinsed 3 x 30 s in

ddH_2O and stained in 200mg/100 mL $AgNO_3$ for 30 min. Gels were washed 3 x 60 s in ddH_2O, and developed in 6g in 100 mL Na_2CO_3/ 50 μL in 100 mL 37% formaldehyde/ 2mL of 20 mg/100 mL $Na_2S_2O_3 \bullet 5H_2O$ for up to 10 min, as required. Staining was stopped by 6% acetic acid for 10 min and then washed in ddH_2O. Gels were destained in 0.4 g $K_3Fe(CN)_6$/ 200 mL $Na_2S_2O_3 \bullet 5H_2O$ (0.2g/L) for 15 min or until no bands were visible then washed 6 × 15 min in ddH_2O to overnight. Gel pieces were excised and reduced in 150 μL of 8 mM TCEP in 80 mM ammonium bicarbonate buffer, pH 7.8/ 150 μL CH_3CN. Gel pieces were reduced for 20 min at RT. The solution was discarded and cysteines were alkynyled in 150 μL of 10 mM iodoacetamide in 80 mM ammonium bicarbonate buffer, pH 7.8/ 150 μL CH_3CN. Reactions were incubated in the dark for 20 min at RT. The supernatant was discarded and gel pieces were rehydrated in 500 μL of 50 mM ammonium bicarbonate for 10 min at RT. The supernatanat was removed and gel pieces were speed vac'd for 15 min. Gel pieces were resuspended in 40 μL H_2O/ 5 μL 500 mM ammonium bicarbonate, pH 7.8/ and 5 μL of 0.2 mg/mL trypsin (promega), and left on ice for 30 min. Tubes were then incubated overnight at 37 °C. The following day, excess trypsin solution not absorbed was removed and saved in a new tube. Gel pieces were washed with 500 μL of H_2O by vortexing for 20 min. The solution was removed and combined with the tyrptic digests. Peptides were extracted at 2 × 200 μL of 5% formic acid/ 50% CH_3CN by vortexing for 20 min and combined with the tryptic and wash fractions. Samples were concentrated in a speed vac down to 20 μL for MS analysis.

Orbitrap LC-MS Analysis

Approximately 50% of gel extractions were loaded onto a 360 μm O.D. X 75 μm precolumn packed with 4 cm of 5 μm Monitor C18 particles (Column Engineering) as described previously.[48]

Western Blotting

10% SDS gels were transferred to PVDF, blocked in 3% HIO_4^- BSA,[17] and incubated with HRP-conjugated UEAI (Sigma**)** at 50 μg/mL in TBST for 2 h at RT. Membranes were washed 3 × 10 min in TBST, then developed as described previously.[17] For immunoblotting to confirm proteome hits, proteins from lectin affinity chromatography were separated on Bis-Tris 4-12% NuPage gradient gels (Invitrogen) according to the manufacturer's protocol in MOPS running buffer. Gels were transferred to PVDF, blocked in infrared blocking buffer (Rockland), and incubated with the following primary antibodies overnight in TBST: mouse anti-tubulin at 1:50,000 (Sigma), mouse anti-hsc/hsp 70 at 1:2000 (Stressgen) , rabbit anti-munc18 at 1:2000 (Synaptic Systems), goat anti-VDAC1 at 1:1000 (Santa Cruz), mouse α5 anti-Na^+/K^+ ATPase alpha subunits at 5 μg/mL (Iowa Hybridoma Bank**)**, mouse anti-OCAM at 1:100 (R&D Systems), mouse 5e anti-NCAM at 10 μg/mL (Iowa Hybridoma Bank), mouse ASCS4 anti-NCAM L1 at 2 μg/mL (Iowa Hybridoma Bank), mouse anti-N-cadherin at 1:1000 (Chemicon), and rabbit anti-synaptotagmin I at 1:2000 (Synaptic Systems). Following incubation, blots were washed in TBST 3 times by 10 min, then incubated with secondary antibodies for 1h at 1:5000 in TBST plus 0.02% SDS. The following secondary antibodies were used: Alexa 680-conjugated donkey anti-mouse (Molecular Probes), Alexa 800-conjugated

donkey anti-rabbit (Rockland Immunochemicals), and Alexa 680-conjugated donkey anti-goat (Molecular Probes). Membranes were washed in TBST 3 times by 10 min, and imaged on the Odyssey Infrared imager (Licor).

Antibody Purification

500 μL of mouse ascites from the α5 Na^+/K^+ ATPase alpha subunit, 5e NCAM, and ASCS4 NCAM L1 were purified over 300 μL Immunopure Protein-A agarose columns following the manufacturer's protocol (Pierce). Eluted antibodies were dialyzed into PBS, concentrations were determined by the A_{280}, and normalized to 1 mg/mL.

Protein name	Accession	MW	C57	UEA1FUT1 KO
LHW_HM_P and Q Samples			Uncategorized Sample	Uncategorized Sampl
Atp1a1 Sodium/potassium-transporting ATPase subunit alpha	IPI:IPI00311682.5	112967.3	51	0
Ncam1 neural cell adhesion molecule 1 isoform 1	IPI:IPI00831465.1	93474.6	35	0
Tubb5 Tubulin beta-5 chain	IPI:IPI00117352.1	49652.6	28	0
Ncam1 Isoform N-CAM 180 of Neural cell adhesion molecule 1	IPI:IPI00122971.1	119333.5	26	0
Igsf3 Immunoglobulin superfamily, member 3	IPI:IPI00420589.2	134605.2	24	0
Tuba1a Tubulin alpha-1A chain	IPI:IPI00110753.1,IPI	50117.7	24	3
Hspa8 Heat shock cognate 71 kDa protein	IPI:IPI00323357.3,IPI	70854.7	22	6
Slc12a2 solute carrier family 12, member 2	IPI:IPI00135324.2	130654.7	20	0
Actb Actin, cytoplasmic 1	IPI:IPI00110850.1,IPI	41719.8	20	3
Alb Serum albumin precursor	IPI:IPI00131695.3	68674.9	18	14
Plxnb2 14 days pregnant adult female placenta cDNA, RIKEN	IPI:IPI00405742.6,IPI	206212.3	16	0
Cacna2d1 Isoform 2B of Dihydropyridine-sensitive L-type calc	IPI:IPI00230013.3,IPI	122505.5	16	0
Tuba1b Tubulin alpha-1B chain	IPI:IPI00117348.4	50133.7	16	3
L1cam L1 cell adhesion molecule	IPI:IPI00406778.3,IPI	140413.3	15	0
Hsp90ab1 Heat shock protein 84b	IPI:IPI00229080.7	83266.3	15	0
Tubb2c Tubulin beta-2C chain	IPI:IPI00169463.1	49812.7	15	0
Stxbp1 Isoform 1 of Syntaxin-binding protein 1	IPI:IPI00415402.3	67553.8	14	0
Atp5a1 ATP synthase subunit alpha, mitochondrial precursor	IPI:IPI00130280.1	59736.1	14	0
Tubb2b Tubulin beta-2B chain	IPI:IPI00109061.1,IPI	49935.1	14	0
Hspa5 78 kDa glucose-regulated protein precursor	IPI:IPI00319992.1	72405.6	13	0
Slc25a12 Calcium-binding mitochondrial carrier protein Aralar	IPI:IPI00308162.3	74553.8	12	0
Atp5b ATP synthase subunit beta, mitochondrial precursor	IPI:IPI00468481.2	56283.2	12	0
Tubb3 Tubulin beta-3 chain	IPI:IPI00112251.1	50400.7	12	0
Cntn1 Contactin-1 precursor	IPI:IPI00123058.1	113372.8	11	0
Atp1a3 Sodium/potassium-transporting ATPase subunit alpha	IPI:IPI00122048.2	111676.4	11	0
Dpysl3 Dihydropyrimidinase-related protein 3	IPI:IPI00122349.1	61918.9	11	0
Slc3a2 CD98 heavy chain	IPI:IPI00114641.2	58805.6	11	0
Syt1 Synaptotagmin-1	IPI:IPI00129618.1,IPI	47400.9	10	0
Gnao1 Guanine nucleotide-binding protein G(o) subunit alpha	IPI:IPI00115546.4	40019.4	10	0
Atp1b1 Sodium/potassium-transporting ATPase subunit beta-	IPI:IPI00121550.2	35771.2	10	0
Npepps Aminopeptidase puromycin sensitive	IPI:IPI00608097.1	103310.1	9	0
Psmd2 26S proteasome non-ATPase regulatory subunit 2	IPI:IPI00123494.3	100187.1	9	0
Eef1a1 Elongation factor 1-alpha 1	IPI:IPI00307837.5	50736.9	9	0
LOC100042651 similar to Tubulin, beta 4	IPI:IPI00458204.4	49767.5	9	0
Rab3a Ras-related protein Rab-3A	IPI:IPI00122965.1	24952.3	9	0
Gap43 Neuromodulin	IPI:IPI00128973.1	23614.3	9	5
Igf1r Insulin-like growth factor 1 receptor precursor	IPI:IPI00120225.1	155772.7	8	0
Nrcam Isoform 6 of Neuronal cell adhesion molecule precurso	IPI:IPI00120564.5,IPI	122736	8	0
Nsf Vesicle-fusing ATPase	IPI:IPI00656325.2	82598.5	8	0
Tpm3 Isoform 2 of Tropomyosin alpha-3 chain	IPI:IPI00230044.5	29003.2	8	2
Ywhaz 14-3-3 protein zeta/delta	IPI:IPI00116498.1	27753.9	8	0
Eef2 Elongation factor 2	IPI:IPI00466069.3	95298	7	0
Ncam2 Isoform Long of Neural cell adhesion molecule 2 precu	IPI:IPI00127556.1,IPI	93187.3	7	0
Hsp90b1 Endoplasmin precursor	IPI:IPI00129526.1	92460.6	7	0
Dpysl2 Dihydropyrimidinase-related protein 2	IPI:IPI00114375.2	62259.9	7	0
Slc7a5 Solute carrier family 7 (Cationic amino acid transporte	IPI:IPI00331577.3	55856	7	0
Ldha L-lactate dehydrogenase A chain	IPI:IPI00319994.6,IPI	36480.9	7	0
LOC100042349;LOC100041325;Gapdh;LOC100046067;LOC1	IPI:IPI00273646.9,IPI	35792.1	7	0

Rab5c Ras-related protein Rab-5C	IPI:IPI00224518.2,IPI	23394.7	5	0
Prdx2 Peroxiredoxin-2	IPI:IPI00117910.3	21761.1	5	0
Atp7a Cation-transporting ATPase	IPI:IPI00830169.1	162013.2	4	0
Adcy3 Adenylate cyclase type 3	IPI:IPI00128323.3	129026.7	4	0
Pcdh17 Protocadherin 17	IPI:IPI00356667.4	126116.7	4	0
Slc12a7 Solute carrier family 12 member 7	IPI:IPI00331175.5	119465.7	4	0
Slc8a1 Sodium/calcium exchanger 1 precursor	IPI:IPI00109213.1	108019.5	4	0
Ctnna2 Isoform II of Catenin alpha-2	IPI:IPI00119870.2,IPI	105269.1	4	0
Pygb Glycogen phosphorylase, brain form	IPI:IPI00229796.3	96714.5	4	0
Trpv2 Transient receptor potential cation channel subfamily V	IPI:IPI00322698.6	86305.7	4	0
Ctnnb1 Catenin beta-1	IPI:IPI00125899.1,IPI	85452.9	4	0
Hsp90aa1 Heat shock protein HSP 90-alpha	IPI:IPI00330804.4	84773.2	4	0
Hadha Hydroxyacyl-Coenzyme A dehydrogenase/3-ketoacyl-C	IPI:IPI00223092.4	82627.3	4	0
Gars Glycyl-tRNA synthetase	IPI:IPI00112555.3	81861.1	4	0
Ndufs1 NADH-ubiquinone oxidoreductase 75 kDa subunit, mit	IPI:IPI00308882.4	79731.6	4	0
BC005764 CDNA sequence BC005764	IPI:IPI00117580.7	79143.5	4	0
Ncstn Nicastrin precursor	IPI:IPI00118674.7	78473	4	0
Trf Serotransferrin precursor	IPI:IPI00139788.2	76706.2	4	0
Slc24a2 solute carrier family 24 (sodium/potassium/calcium e	IPI:IPI00225530.1,IPI	74225.6	4	0
Lingo2 Adult male hippocampus cDNA, RIKEN full-length enric	IPI:IPI00341267.4	68054.1	4	0
Igsf8 Immunoglobulin superfamily member 8 precursor	IPI:IPI00321348.3,IPI	64991.6	4	0
Sucla2 Succinyl-CoA ligase [ADP-forming] beta-chain, mitoch	IPI:IPI00261627.1	50096.7	4	0
Tuba4a Tubulin alpha-4A chain	IPI:IPI00117350.1	49906.6	4	0
Idh1 0 day neonate lung cDNA, RIKEN full-length enriched lib	IPI:IPI00135231.2,IPI	47528.7	4	0
ENSMUSG00000075591;Got2 Aspartate aminotransferase, mi	IPI:IPI00117312.1,IPI	47394.3	4	0
Dnaja1 DnaJ homolog subfamily A member 1	IPI:IPI00132208.1,IPI	44850.6	4	0
Acat1 Acetyl-CoA acetyltransferase, mitochondrial precursor	IPI:IPI00154054.1	44798.4	4	0
Tardbp TAR DNA-binding protein 43	IPI:IPI00121758.1,IPI	44529.5	4	0
Kcnab2 Voltage-gated potassium channel subunit beta-2	IPI:IPI00315359.1	41004.4	4	0
Fdps Farnesyl pyrophosphate synthetase	IPI:IPI00120457.1	40565.2	4	0
Atp6v0d1 Vacuolar ATP synthase subunit d 1	IPI:IPI00313841.1	40284.8	4	0
Lgals9 Isoform Long of Galectin-9	IPI:IPI00114396.1,IPI	40017.4	4	0
Ahsa1 Activator of 90 kDa heat shock protein ATPase homolo	IPI:IPI00153740.1	38099.3	4	0
Gnb2l1 Guanine nucleotide-binding protein subunit beta 2-like	IPI:IPI00317740.5	35059	4	0
Slc25a4 ADP/ATP translocase 1	IPI:IPI00115564.5	32887.4	4	0
Vdac1 Isoform PI-VDAC1 of Voltage-dependent anion-selectiv	IPI:IPI00122549.1,IPI	32334.7	4	0
Ywhaq Isoform 1 of 14-3-3 protein theta	IPI:IPI00408378.4,IPI	27761.4	4	0
Etfb Electron transfer flavoprotein subunit beta	IPI:IPI00121440.4	27605.2	4	0
Rab8b Ras-related protein Rab-8B	IPI:IPI00411115.1	23586.9	4	0
Atp5o;LOC100047429 ATP synthase O subunit, mitochondrial	IPI:IPI00118986.1	23346.4	4	0
Snap25 Isoform SNAP-25b of Synaptosomal-associated protei	IPI:IPI00125635.1	23297.4	4	0
Prdx1 Peroxiredoxin-1	IPI:IPI00121788.1,IPI	22159.5	4	0
Basp1 Brain acid soluble protein 1	IPI:IPI00129519.3	22068.6	4	0
Fasn Fatty acid synthase	IPI:IPI00113223.2	272411.3	3	0
Sdk1 Isoform 1 of Protein sidekick-1 precursor	IPI:IPI00420682.5,IPI	240922.5	3	0
Cand1 TBP-interacting protein isoform 1	IPI:IPI00420562.5,IPI	158958.3	3	0
Atp6v0a1 Isoform A1-II of Vacuolar proton translocating ATPa	IPI:IPI00130187.1,IPI	96486.8	3	0
Gsn Isoform 1 of Gelsolin precursor	IPI:IPI00117167.2,IPI	85923.9	3	0
Sv2a Synaptic vesicle glycoprotein 2A	IPI:IPI00465810.3,IPI	82630.9	3	0
Sv2c similar to synaptic vesicle glycoprotein 2c isoform 1	IPI:IPI00751719.1,IPI	82463.2	3	0
Sv2b Synaptic vesicle glycoprotein 2B	IPI:IPI00221456.1	77442	3	0
Cnga2 Cyclic nucleotide-gated olfactory channel	IPI:IPI00330727.2,IPI	76194.7	3	0

3110004L20Rik Adult male brain UNDEFINED_CELL_LINE cDN	IPI:IPI00380443.4	74309.8	3	0
Cnnm4 Ancient conserved domain protein 4	IPI:IPI00120783.3	73273.9	3	0
Yars Tyrosyl-tRNA synthetase	IPI:IPI00314153.4,IPI	62985	3	0
Cct6a T-complex protein 1 subunit zeta	IPI:IPI00116281.3	57987.5	3	0
Hmgcs1;LOC100040592 Hydroxymethylglutaryl-CoA synthase	IPI:IPI00331707.1	57552.2	3	0
Dars Aspartyl-tRNA synthetase, cytoplasmic	IPI:IPI00122743.1,IPI	57099.7	3	0
Kcna2 Potassium voltage-gated channel subfamily A member	IPI:IPI00129774.1,IPI	56684.5	3	0
Camkv CaM kinase-like vesicle-associated protein	IPI:IPI00122486.3	54801.8	3	0
Dld Dihydrolipoamide dehydrogenase	IPI:IPI00331564.2	54580.6	3	0
Vim Vimentin	IPI:IPI00227299.6	53670.7	3	0
Hnrpk Isoform 1 of Heterogeneous nuclear ribonucleoprotein	IPI:IPI00223253.1,IPI	50960.5	3	0
Tufm Isoform 1 of Elongation factor Tu, mitochondrial precurs	IPI:IPI00274407.1,IPI	49490.8	3	0
Psmc4 26S protease regulatory subunit 6B	IPI:IPI00108895.1,IPI	47264.7	3	0
Actr2 Actin-like protein 2	IPI:IPI00177038.1,IPI	44743.7	3	0
Cnp Isoform CNPI of 2',3'-cyclic-nucleotide 3'-phosphodieste	IPI:IPI00229598.4,IPI	44638	3	0
Psmc6 26S protease regulatory subunit S10B	IPI:IPI00125971.1	44156.9	3	0
Pa2g4 Proliferation-associated protein 2G4	IPI:IPI00119305.3	43680.7	3	0
Ckb Creatine kinase B-type	IPI:IPI00136703.1	42696.1	3	0
Actr1a Alpha-centractin	IPI:IPI00113895.1	42597.2	3	0
Ndufa10 NADH dehydrogenase [ubiquinone] 1 alpha subcomp	IPI:IPI00116748.1	40586.7	3	0
Cxadr Isoform 1 of Coxsackievirus and adenovirus receptor h	IPI:IPI00270376.6,IPI	39930.2	3	0
LOC100048637;Tmod2 Isoform 1 of Tropomodulin-2	IPI:IPI00402982.2	39493.9	3	0
Hnrpd Isoform 1 of Heterogeneous nuclear ribonucleoprotein	IPI:IPI00330958.2,IPI	38336.5	3	0
Mdh1 Malate dehydrogenase, cytoplasmic	IPI:IPI00336324.11	36494.1	3	0
Rpl5;LOC100043295 60S ribosomal protein L5	IPI:IPI00308706.4,IPI	34383.4	3	0
Set;LOC671392 Isoform 1 of Protein SET	IPI:IPI00111560.3,IPI	33360.4	3	0
Vdac2 Voltage-dependent anion-selective channel protein 2	IPI:IPI00122547.1	31715.6	3	0
Rpl7 60S ribosomal protein L7	IPI:IPI00311236.1	31403.6	3	0
Calb2 Calretinin	IPI:IPI00119346.1	31356.6	3	0
Capzb Isoform 2 of F-actin capping protein subunit beta	IPI:IPI00269481.7,IPI	30611.7	3	0
Rps3a 40S ribosomal protein S3a	IPI:IPI00331345.5,IPI	29867.3	3	0
Rps4x 40S ribosomal protein S4, X isoform	IPI:IPI00331092.7	29581.3	3	0
Tpi1 Triosephosphate isomerase	IPI:IPI00467833.5	26694.2	3	0
Slc25a22 25 kDa protein	IPI:IPI00756073.1	24622.5	3	0
Rps18 40S ribosomal protein S18	IPI:IPI00317590.5,IPI	17701.3	3	0
Mrc1 Macrophage mannose receptor 1 precursor	IPI:IPI00126186.1	165051	2	0
Acly Adult male testis cDNA, RIKEN full-length enriched librar	IPI:IPI00126248.3,IPI	120780.5	2	0
Ephb2 Isoform 1 of Ephrin type-B receptor 2 precursor	IPI:IPI00108870.2,IPI	110743.2	2	0
Tmprss6 type II transmembrane serine protease 6	IPI:IPI00173181.6	90959.4	2	0
Plaa Phospholipase A-2-activating protein	IPI:IPI00226234.5,IPI	87219.3	2	0
Aco2 Aconitate hydratase, mitochondrial precursor	IPI:IPI00116074.1	85447.9	2	0
Cntn4 Isoform 2 of Contactin-4 precursor	IPI:IPI00228295.2,IPI	78406.6	2	0
Rpn2 Dolichyl-diphosphooligosaccharide--protein glycosyltran	IPI:IPI00475154.1,IPI	69045.6	2	0
Kcna5 Ventricular potassium channel Kv1.5	IPI:IPI00163025.1	66563.6	2	0
Dclk1 Doublecortin-like protein	IPI:IPI00119762.4	58625.1	2	0
Sept3 53 kDa protein	IPI:IPI00761331.1,IPI	53260.6	2	0
Cadm1 Isoform 1 of Cell adhesion molecule 1 precursor	IPI:IPI00322447.4,IPI	49770.5	2	0
Eif4a1 Eukaryotic initiation factor 4A-I	IPI:IPI00118676.3,IPI	46137.3	2	0
Acta1 Actin, alpha skeletal muscle	IPI:IPI00110827.1,IPI	42034.1	2	4
Taldo1 Transaldolase	IPI:IPI00124692.1	37370.5	2	0
Slc25a5 ADP/ATP translocase 2	IPI:IPI00127841.3	32914.6	2	0
Rpl28;LOC100042670;LOC100047349 60S ribosomal protein	IPI:IPI00222547.6,IPI	15715.7	2	0
RP23-24J10.5;Uba52 Uba52 protein	IPI:IPI00108590.2,IPI	14710.9	2	0
Dclk1 Doublecortin-like protein	IPI:IPI00468380.4,IPI	0	2	0
Dsp desmoplakin isoform 1	IPI:IPI00553419.3,IPI	332901.9	0	6
Myh1 Myosin-1	IPI:IPI00380896.1	223329.7	0	7
Krt13 Isoform 1 of Keratin, type I cytoskeletal 13	IPI:IPI00136056.1	47737.8	0	3
Anxa1 Annexin A1	IPI:IPI00230395.5,IPI	38717.7	0	3

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