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Altered fucosyltransferase expression in the superior temporal gyrus of elderly patients with schizophrenia

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ABSTRACT

Glycosylation is a post-translational modification that is an essential element in cell signaling and neurodevelopmental pathway regulation. Glycan attachment can influence the tertiary structure and molecular interactions of glycosylated substrates, adding an additional layer of regulatory complexity to functional mechanisms underlying central cell biological processes. One type of enzyme-mediated glycan attachment, fucosylation, can mediate glycoprotein and glycolipid cell surface expression, trafficking, secretion, and quality control to modulate a variety of inter- and intracellular signaling cascades. Building on prior reports of glycosylation abnormalities and evidence of dysregulated glycosylation enzyme expression in schizophrenia, we examined the protein expression of 5 key fucose-modifying enzymes: GDP-fucose:protein *O*-fucosyltransferase 1 (POFUT1), GDP-fucose:protein *O*-fucosyltransferase 2 (POFUT2), fucosyltransferase 8 (FUT8), fucosyltransferase 11 (FUT11), and plasma α-L-fucosidase (FUCA2) in postmortem superior temporal gyrus of schizophrenia (N = 16) and comparison (N = 14) subjects. We also used the fucose binding protein, Aleuria aurantia lectin (AAL), to assess α-1,6-fucosylated *N*-glycoprotein abundance in the same subjects. In schizophrenia, we found increased expression of POFUT2, a fucosyltransferase uniquely responsible for *O*-fucosylation of thrombospondin-like repeat domains that is involved in a non-canonical endoplasmic reticulum quality control pathway. We also found decreased expression of FUT8 in schizophrenia. Given that FUT8 is the only α-1,6-fucosyltransferase expressed in mammals, the concurrent decrease in AAL binding in schizophrenia, particularly evident for *N*-glycoproteins in the ~52–58 kDa and ~60–70 kDa molecular mass ranges, likely reflects a consequence of abnormal FUT8 expression in the disorder. Dysregulated FUT8 and POFUT2 expression could potentially explain a variety of molecular abnormalities in schizophrenia.

1. Introduction

The role of posttranslational protein modifications in the pathophysiology of schizophrenia has become a target of investigation in this complex neuropsychiatric illness. One modification, glycosylation, has come under study due to the role glycan adornment plays in modulating a wide variety of inter- and intracellular processes. Glycosylation is the enzyme-mediated modification of protein, lipid, or carbohydrate substrates by glycans (sugars) and several unique glycosylation pathways exist with a variety of cellular functions (Varki et al., 2009). Abnormalities of *N*-linked protein glycosylation (Bauer et al., 2010; Mueller et al., 2014; Stanta et al., 2010; Tucholski et al., 2013a, b), sphingolipid metabolism (Narayan et al., 2009), chondroitin sulfate proteoglycan processing (Berretta, 2012; Pantazopoulos et al., 2013) and polysialylation of cell adhesion molecules in schizophrenia (Barbeau et al., 1995; Gilabert-Juan et al., 2012; Sato and Kitajima, 2013; Varea et al., 2012) have been reported. Our lab recently identified altered transcript levels of 36 carbohydrate active enzymes (CAzymes) in schizophrenia dorsolateral prefrontal cortex (unpublished data). A subset of these enzymes, fucosyltransferases and fucosidases, catalyze the attachment or cleavage of the deoxyhexose monosaccharide fucose on a wide variety of substrates (Luo et al., 2006b; Ma et al., 2006; Miyoshi et al., 2008; Varki et al., 2009), and we found increased mRNA expression of four of the six fucose-modifying CAzymes assessed: GDP-fucose:protein *O*-fucosyltransferase 1 (*POFUT1*), fucosyltransferase 8 (*FUT8*), fucosyltransferase 11 (*FUT11*), and plasma α-L-fucosidase (*FUCA2*).

Glycoproteins can be fucosylated either by the direct attachment of fucose to a serine/threonine residue of a growing polypeptide in the endoplasmic reticulum (ER), called *O*-fucosylation, or by α-fucosylation of glycans on glycoprotein or glycolipid substrates in the Golgi apparatus (Ma et al., 2006; Varki et al., 2009). Types of α-fucose linkages in mammals include α-1,2-fucose on terminal galactose (Gal); α-1,3/4-fucose on *N*-acetylglucosamine (GlcNAc) within poly-*N*-acetyllactosamine chains of glycolipids or glycoproteins; or α-1,6-fucose on the proximal

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GlcNAc of the chitobiose core of protein *N*-glycans, called "core fucosylation" (for detailed discussion of fucose linkage types, see review by Ma et al., 2006). In addition to being a key determinant of human blood group antigens and facilitating forward trafficking and secretion of fucosylproteins, constellations of fucosylated structures are dynamically regulated by the repertoire of fucosyltransferases expressed to facilitate a variety of cell signaling and developmental processes (Ma et al., 2006; Varki et al., 2009).

To characterize dysregulation of fucosylation in schizophrenia, we measured the protein expression of fucose-modifying enzymes in the superior temporal gyrus (STG) of elderly schizophrenia and comparison subjects. The STG is a brain region of interest in schizophrenia pathophysiology, and abnormalities of *N*-glycosylation (Mueller et al., 2014) as well as abnormal molecular phenotypes in multiple cell types are reflected in this cortical area (Pietersen et al., 2014a,b; Steffek et al., 2008). In addition to four enzymes we previously found altered at the mRNA level (POFUT1, FUT8, FUT11, and FUCA2), the current study also examines the protein expression of GDP-fucose:protein *O*-fucosyltransferase 2 (POFUT2). POFUT1 and POFUT2 similarly modify correctly folded cysteine-rich regions of protein and respectively mediate *O*-linked fucosylation of epidermal growth factor or thrombospondin-like repeat (TSR) domains (Luo et al., 2006b). We also assayed the binding pattern of the α-1,6-fucose-specific glycan binding protein AAL (*Aleuria aurantia* lectin) in the same subjects (Matsumura et al., 2007; Monzo et al., 2007; Stelck et al., 1999; Wu et al., 2014). FUT8 is an α-1,6-fucosyltransferase that is uniquely responsible for the core fucosylation of *N*-glycoproteins and is the only α-1,6-fucosyltransferase expressed in mammals (Ihara et al., 2006; Kötzler et al., 2012). Interestingly, a *FUT8* knock-out mouse demonstrates a schizophrenia-like behavioral phenotype and has deficits in multiple neurotransmitter pathways (Fukuda et al., 2011; Gu et al., 2015). Using AAL binding as a proxy for α-1,6-fucosylprotein expression, we assessed total levels of core fucosylation as well as the expression of α-1,6-fucosylproteins within specific molecular mass ranges in these same subjects.

2. Methods and materials

2.1. Human subjects

Postmortem brain tissue was obtained from the Icahn School of Medicine at Mount Sinai NIH Brain and Tissue Repository, and detailed information regarding assessment is available at http://icahn.mssm.edu/research/labs/neuropathology-and-brain-banking/neuropathology-evaluation. Subjects used in this study included patients diagnosed with schizophrenia using DSM-III-R criteria excluding those with death not due to natural causes, previous history of drug/alcohol abuse, or coma longer than six hours prior to death (Mueller et al., 2015). Brains were evaluated micro- and macroscopically using CERAD guidelines. Schizophrenia subjects had no neuropathologies or signs of neurodegenerative disorders, including Alzheimer's disease, at assessment (Powchik et al., 1993; Purohit et al., 1993). Subjects with no documented history of psychiatric illness obtained from the same collection were assessed similarly and served as a comparison group (Table 1 and Supplementary Table S1).

Table 1
Summary of subject demographics.

	Comparison	Schizophrenia
n	14	16
Age	79.4 ± 9.3	75.8 ± 11.9
Sex	4M/10F	11M/5F
PMI (hours)	10.0 ± 7.3	11.4 ± 4.4
Freezer storage time (years)	15.2 ± 3.4	16.3 ± 3.1
Tissue pH	6.3 ± 0.2	6.4 ± 0.3
On/off Rx	0/14	11/5

Abbreviations: male (M), female (F), postmortem interval (PMI), antipsychotic medication (Rx).
Values are expressed as means ± standard deviation. Off Rx indicates patients that had not received antipsychotic medications for 6 weeks or more at time of death.

2.2. Tissue preparation

Whole brains were collected at time of autopsy and cut into 1 cm slabs. The full thickness of grey matter from the left hemisphere of STG (Brodmann area 22) was dissected, blocked into 1 cm cubes, and powdered using small amounts of liquid nitrogen and a mortar and pestle then stored at −80 °C. Samples were reconstituted in 1× isotonic extraction buffer (ER0100, Sigma-Aldrich, St Louis, MO) and homogenized on ice in a glass-teflon homogenizer. Homogenate was transferred into a nitrogen cavitation vessel (Parr Instrument Company, Moline, IL) and pressurized for 8 min at 450 psi, then collected through the vessel outlet during decompression (Mueller et al., 2015). Protein concentrations using a BCA protein assay kit (Thermo Scientific, Waltham, MA) were obtained for each of the homogenates prior to storage at −80 °C.

2.3. Antipsychotic treated rats

The Institutional Animal Care and Use Committee of the University of Alabama at Birmingham approved all procedures using animals. Male Sprague-Dawley rats were housed in pairs and treated with chronic administration of either 28.5 mg/kg haloperidol decanoate (HALO; N = 10) or vehicle (CTRL; N = 10) delivered once every 3 weeks over 9 months via intramuscular injection, for a total of 12 injections. This method of drug delivery, length of treatment, and dose have been previously described and validated (Harte et al., 2005; Kashihara et al., 1986; Kippe et al., 2015). Animals were euthanized by rapid decapitation following CO_2 administration; samples of frontal cortex were dissected on ice then snap frozen and stored at −80 °C until homogenization. Cortical homogenates were prepared in 320 mM sucrose in 5 mM Tris-HCL, pH 7.5, with protease and phosphatase inhibitor tablets (Complete Mini, EDTA-free and PhosSTOP, Roche Diagnostics, Indianapolis, IN), and protein concentration determined by BCA Assay (Thermo Scientific) prior to storage at −80 °C.

2.4. Western blot for protein expression

Human and rat homogenates were thawed on ice then prepared with 6× reducing buffer (4.5% sodium dodecyl sulfate (SDS), 0.02% bromophenol blue, 15% β-mercaptoethanol, and 36% glycerol in 170 mM Tris-HCl, pH 6.8) to a final 1× buffer concentration and heated at 70 °C for 10 min. For each subject, 10 μg of prepared sample was loaded in duplicate into NuPAGE Bis-Tris 4–12% gradient gels (Life Technologies, Carlsbad, CA) for SDS-PAGE using NuPAGE MOPS SDS running buffer (Life Technologies) and electrophoresed for 20 min at 50 V and then 1.5 h at 150 V. Proteins were transferred to nitrocellulose membranes using semi-dry transblotters (Bio-Rad, Hercules, CA) at 16 V for 30 min. Membranes were then blocked and probed using conditions optimized for each antibody (Supplementary Table S2). Membranes were washed with Tris-buffered saline with 0.1% Tween-20 (TBST) and probed with the appropriate IR-dye labeled secondary antibody in the same diluent as the primary antibody. Membranes were again washed with TBST, rinsed with sterile water then imaged with a LiCor Odyssey scanner (LiCor, Lincoln, NE). Image Studio Lite Version 4.0.21 (LiCor) was used to measure the signal intensity of each protein band with the median right-left background signal intensity (3 pixels wide) subtracted. Each target was normalized to the intralane signal intensity of valosin-containing protein (VCP), a ubiquitously expressed protein we have previously reported to be unchanged in these same subjects (Mueller et al., 2015). Expression levels were similarly assessed in HALO and CTRL rats for protein measures that were significantly altered in schizophrenia.

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