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Review

Glycan susceptibility factors in autism spectrum disorders

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ABSTRACT

Idiopathic autism spectrum disorders (ASDs) are neurodevelopmental disorders with unknown etiology. An estimated 1:68 children in the U.S. are diagnosed with ASDs, making these disorders a substantial public health issue. Recent advances in genome sequencing have identified numerous genetic variants across the ASD patient population. Many genetic variants identified occur in genes that encode glycosylated extracellular proteins (proteoglycans or glycoproteins) or enzymes involved in glycosylation (glycosyltransferases and sulfotransferases). It remains unknown whether "glycogene" variants cause changes in glycosylation and whether they contribute to the etiology and pathogenesis of ASDs. Insights into glycan susceptibility factors are provided by studies in the normal brain and congenital disorders of glycosylation, which are often accompanied by ASD-like behaviors. The purpose of this review is to present evidence that supports a contribution of extracellular glycans and glycoconjugates to the etiology and pathogenesis of idiopathic ASDs and other types of pervasive neurodevelopmental disorders.

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1. Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by a wide range of symptoms that include abnormal social interactions, limited interests, and stereotypic and repetitive behaviors (American Psychiatric Association, 2013). Hallmark symptoms typically arise in the second or third year of life, following a period of normal development or accompanying prolonged developmental delay (Newschaffer et al., 2007).

Abbreviations: ASDs, autism spectrum disorders; CDGs, congenital disorders of glycosylation; CNVs, copy number variations; DMD, Duchenne muscular dystrophy; ECM, extracellular matrix; GPC, glypicans; HS, heparan sulfate; HSPGs, heparan sulfate proteoglycans; MPSIII, mucopolysaccharidosis III A-D; PSA-NCAM, polysialylated neural cell adhesion molecule; SDC, syndecan; SNPs, single nucleotide polymorphisms.

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Currently 1 out of 68 children are diagnosed with ASDs, with males having a four times greater risk than females. In the past decade the prevalence of ASDs has more than doubled, which emphasizes the need for improved early diagnosis and therapeutic intervention (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators and Centers for Disease Control and Prevention, 2012).

Idiopathic ASDs arise from an unknown cause, whereas syndromic ASD is secondary to a primary condition caused by a single gene mutation, for example Fragile X syndrome. ASD patients exhibit a wide range of behaviors, which is mirrored by equally impressive genetic heterogeneity. Recent findings support a significant genetic contribution to idiopathic ASD (Geschwind, 2011; Geschwind and State, 2015; Murdoch and State, 2013); however disease etiology and pathophysiology remain largely unclear. Efforts to associate genetic risk factors into common biochemical pathways and developmental processes have been made (Geschwind, 2008; Parikshak et al., 2013; Rubenstein and Merzenich, 2003; Subramanian et al., 2015). This approach has led to new theories on the etiology of ASD, which place alterations in developmental transcriptional regulation, brain growth, changes in the excitatory/inhibitory balance of the neural network, and abnormalities in neural plasticity at the crux of disease pathogenesis. It is also known that inflammation in the developing brain can lead to ASD-like behaviors (Kern et al., 2015). Thus genetic heterogeneity in the patient population may reflect a series of different genetic insults that converge on common neurodevelopmental processes that when perturbed have a similar impact on brain function.

The genetic heterogeneity of ASD introduces a significant challenge in understanding disease etiology. The complexity of the genetic architecture arises from numerous factors including (i) many chromosomal loci and common and rare genetic variants, which are either inherited or acquired *de novo*; (ii) genetic perturbations that range from single nucleotide substitutions to large chromosomal deletions/duplications; and (iii) genetic perturbations that range from single (monogenic) to multiple genes (polygenic). Despite these challenges the identification of genetic variants, including single nucleotide polymorphisms (SNPs) and copy number variations (CNVs), provide insight into the factors that may contribute to ASDs. Interestingly a number of these variants occur in genes ("glycogenes") that encode glycosylated extracellular proteins (proteoglycans or glycoproteins) and lipids (glycosphingolipids) or enzymes involved in glycosylation (glycosyltransferases and sulfotransferases).

Glycans and their conjugates (glycoproteins, proteoglycans and glycolipids) are major constituents of the neural extracellular matrix (ECM). In this context, glycans and glycoconjugates participate in nearly every biological process in the developing brain. A potential link between ASDs and changes in glycosylation was initially noted in patients with congenital disorders of glycosylation (CDGs) (Freeze et al., 2015). These disorders result from rare homozygous recessive mutations causing the loss-of-function of a specific glycoconjugate or glycosyltransferase. Studies in mouse models of CDGs and behavioral phenotypes observed in CDG patients support the idea that glycogene variants either cause or contribute to the development of idiopathic ASDs. The purpose of this review is to present evidence that supports a contribution of extracellular glycans and glycoconjugates to the etiology and pathogenesis of ASDs.

1.1. Organization and assembly of glycans and glycoconjugates in the brain

A glycan is defined generically as any sugar or assembly of sugars, in free form or attached to another molecule. Although some glycans are found as free chains (e.g. hyaluronan), most are found covalently linked to proteins or lipids, i.e. as glycoconjugates. These include glycoproteins, proteoglycans, and glycosphingolipids (Fig. 1). The assembly of glycans occurs in the endoplasmic reticulum and Golgi apparatus of cells by a series of glycotransferases. These enzymes catalyze glycan assembly using activated sugar nucleotide donor substrates (e.g. UDP-galactose, GDP-fucose, CMP-sialic acid) that are transferred to acceptor substrates. Many glycans are further modified by processing enzymes that catalyze removal of specific sugar residues, or sulfation, acetylation and phosphorylation (Fig. 1). These modifications fine-tune glycan structure and function. Regulation of glycan biosynthesis occurs at a variety of different levels, including the availability of high-energy nucleotide donors, enzyme expression levels, and competition among enzymes for common glycan precursors. The impact of reducing the expression or function of a glycosyltransferase gene, either through CNVs or a SNP, depends on the relationship between enzyme function and gene dosage. The majority of enzymes associated with ASDs show gene dosage effects, suggesting that they may be rate limiting in the formation of particular glycans.

Glycans and their glycoconjugates are abundant in the brain, in particular the ECM. All cell types including neurons, glia, and endothelial cells elaborate glycans and glycoconjugates. However, each cell type synthesizes a unique repertoire of glycan structures and glycoconjugates. For example, different antibody epitopes on different glycoforms of phosphacan label different types of cells in the developing cerebral cortex (Dwyer et al., 2015), supporting the idea that glycoform specialization may tailor protein function at the cellular level in the brain. Additional complexity arises from changes in the expression of different glycans and glycoconjugates in different brain regions and across developmental stages (Matthews et al., 2002; Morawski et al., 2012; Torii et al., 2014). The purpose of these differences is not fully understood.

The ECM of the brain can be divided into extracellular substructures comprising the pial basement membrane, interstitial neural extracellular matrix and cell surface glycocalyx (Fig. 2). The pial basement membrane covers the outermost surface of the brain and is comprised primarily of fibrillary proteins including laminin, fibronectin, collagen, and the secreted heparan sulfate proteoglycans agrin and perlecan (Fig. 2A). The predominant receptor for constituents of the pial basement membrane is the glycoprotein dystroglycan, which is expressed on the surface of radial glial cells and astrocytes comprising the limiting glial

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