Converging pathways in autism spectrum disorders: interplay between synaptic dysfunction and immune responses

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Autism spectrum disorders (ASD) are highly heritable, yet genetically heterogeneous neurodevelopmental conditions. Recent genome-wide association and gene expression studies have provided evidence supporting the notion that the large number of genetic variants associated with ASD converge toward a core set of dysregulated biological processes. Here we review recent data demonstrating the involvement of synaptic dysfunction and abnormal immune responses in ASD, and discuss the functional interplay between the two phenomena.

Keywords: autism spectrum disorders, immune response, synapses, genomics, gene expression

INTRODUCTION

Autism spectrum disorders (ASD) are a spectrum of neurodevelopmental conditions characterized by language deficits, social impairments, and repetitive behaviors (Abrahams and Geschwind, 2008). Typically the disorder is diagnosed around 2–3 years of age and manifests with a regression in acquired language and behavioral skills. However, there are wide variations in the clinical presentation and disease progression. In addition to variable severity of the core symptomatology, ASD patients also present with a variable mix of co-morbid conditions: epilepsy, gastro-intestinal problems, intellectual disability, anxiety, and depression (Kim and Lord, 2013). Mirroring its clinical heterogeneity, ASD is also genetically very heterogeneous (State and Levitt, 2011). Based on the results of genome-wide association (GWAS) studies, candidate gene re-sequencing, and exome-sequencing studies, it is currently estimated that hundreds of genetic variants, including common and rare genetic variants, contribute to the disease (Murdoch and State, 2013). What are the molecular pathways that mediate the phenotypic expression of this myriad of genetic variants into a recognizable triad of symptoms? Here we review recent studies demonstrating a convergence of ASD genetic changes toward two main biological processes: synaptic function and immune responses, and discuss their functional interplay, with a focus on immune modulation of neuronal synapses (Figure 1).

FROM MANY GENES TO COMMON BIOLOGICAL PROCESSES

Results from genome-wide studies are beginning to confirm the long-held hypothesis that the wide variety of genetic variants associated with ASD ultimately converge on a core set of molecular pathways (Murdoch and State, 2013). It is worth noting that pathway enrichment analyses are inherently limited by our current knowledge of signaling pathways and molecular interactions, and thus the identification of distinct pathways by different studies might partially reflect yet uncharacterized complexity of molecular pathways.

Four recent studies undertook exome-sequencing in several hundreds of parent-child trios in order to identify de novo single nucleotide variants (SNVs) and copy number variants (CNVs) associated with the disease (Iossifov et al., 2012; Neale et al., 2012; O’Roak et al., 2012; Sanders et al., 2012). The study by O’Roak et al. found that the most disruptive de novo mutations converged onto a highly interconnected beta-catenin/chromatin remodeling protein network, which is involved in neuronal differentiation and synaptic formation (Ille and Sommer, 2005). Iossifov et al. found an enrichment of de novo variants in genes encoding proteins associated with the fragile X syndrome protein, FMRP, suggesting an involvement in synaptic plasticity. The study by Neale et al. demonstrated that genes carrying functional de novo variants were functionally related to each other and to synaptic genes previously implicated in ASD. Using a network-based analysis of genetic association data, Tulkowski et al. (2012) showed that rare de novo CNVs occurring in ASD cases affect primarily genes related to synapse development, axon targeting, and neuron motility. Collectively, these studies highlighted the fact that genes containing pathogenic DNA sequence variants in ASD patients affected primarily genes involved in various aspects of synaptic function.

While genetic association studies identify genetic loci potentially implicated in the disease, they do not assess the functional consequences of the associated variants. On the other hand, transcriptome analyses comparing disease and control groups assess
We found that the neuronal genes downregulated in ASD, but not the immune/inflammatory genes, showed an enrichment for genes involved in synaptic function. A second module was upregulated in ASD brain and contained primarily genes functioning in immune and inflammatory responses. To integrate the gene expression results with previously published GWAS data, we used the two co-expression modules as pre-defined “pathways.”

Thus immune cells could affect neuronal synaptic function either as a result of their activation during immune responses, or due to a failure of their non-immune roles in the brain (Figure 1).
Recent evidence supports the potential involvement of both of these mechanisms in ASD pathogenesis.

Active neuroinflammation has been consistently demonstrated in ASD brains. Prominent activation of microglia (Vargas et al., 2005; Morgan et al., 2010), as well as increased levels of inflammatory cytokines and chemokines [interferon-gamma, IL-1β, IL-6, tumor necrosis factor (TNF-α)] have been documented in post-mortem brain tissue and cerebrospinal fluid from ASD patients (Onore et al., 2012). Recently, activated microglia have also been observed by positron emission tomography in ASD subjects in several brain regions (Suzuki et al., 2013). While it is not clear what is the cause of microglial activation in ASD brain, the cytokines produced by activated microglia have been demonstrated to affect neuronal synaptic function (Onore et al., 2012). TNF-α regulates neuronal cell proliferation and synaptic pruning (Cacci et al., 2005), and modulates synaptic scaling (i.e., the adjustment of synaptic strength for all synapses on a neuronal cell in response to prolonged changes in electrical activity) (Stellwagen and Malenka, 2006). IL-1β regulates long-term potentiation and alters synaptic plasticity (Schneider et al., 1998), while IL-6 has been implicated in behavioral changes associated with maternal immune activation (Patterson, 2009). Mounting evidence suggests that maternal immune activation, particularly during the first and second trimester of pregnancy, may be an important environmental factor in ASD (Onore et al., 2012). Rodent models of maternal immune activation exhibit ASD-like behavioral changes (Patterson, 2009), and the behavioral effects observed in offspring after maternal immune activation appear to be mediated by microglia and IL-6 (Hsiao and Patterson, 2011). In some mouse models increased levels of IL-6 have been sufficient to induce behavioral changes (Onore et al., 2012). Unlike peripheral macrophages, microglia are long-lived, and thus it has been hypothesized that they could maintain an “immunological memory” of an early immune insult, leading to long-term neuronal deficits (Davis and Carson, 2013).

One of the first studies to demonstrate a direct causal relationship between microglial function and a behavioral phenotype, was a mouse model of obsessive-compulsive disorder (Chen et al., 2010). HOXB8 encodes a homeobox transcription factor expressed in the brain exclusively in bone-marrow-derived microglia. HOXB8-null mice exhibit excessive patho- logical grooming behavior similar to the obsessive-compulsive symptoms of trichotillomania. Chen et al. demonstrated that normal bone marrow transplant could rescue the excessive grooming and hair removal phenotype in the HOXB8 mutant mouse, and that selective disruption of HOXB8 in the hematopoietic lineage recapitulates pathological grooming. More recently, a role for non-immune functions of microglia has also been demonstrated in Rett syndrome, a pervasive developmental disorder, belonging to the wider group of ASD. Rett syndrome is caused by loss of function of the methyl-CpG binding protein 2 (MECP2) and is characterized by an initial period of normal development of about 5 months followed by deceleration of language development, psychomotor retardation, seizures and loss of social engagement skills (Chahrour and Zoghbi, 2007). It was initially believed that Rett syndrome is primarily due to loss of MECP2 function in neurons. However several recent studies clearly demonstrated that MECP2 loss in glial cells impairs neuronal function and contributes to the Rett syndrome symptomatology. MECP2 deficiency in astrocytes leads to impaired BDNF regulation, cytokine production, and neuronal dendritic arborization (Maezawa et al., 2009). Moreover, MECP2-deficient astrocytes are unable to support normal dendritic ramification of wild-type neurons (Ballas et al., 2009). Remarkably, astrocyte-specific expression of MECP2 in a MECP2-null mouse restored the normal neuronal dendritic morphology, improved locomotion, anxiety, and respiratory abnormalities (Lioy et al., 2011). A recent study by Derecki et al. (2012) demonstrated that not only astrocytes but also microglia contribute to the Rett syndrome phenotype. Using irradiation-mediated immune ablation in MECP2-null mice, followed by wild-type bone marrow transplantation, this study demonstrated that the wild-type microglia could arrest disease development. In addition, targeted expression of MECP2 in myeloid cells ameliorated the phenotype in MECP2-null mice. These results implicated microglia as important players in the pathophysiology of Rett syndrome, and suggested a potential therapeutic benefit of bone marrow transplantation in Rett syndrome.

CONCLUSION AND FUTURE DIRECTIONS

Understanding the core biological processes underlying the clinical and genetic heterogeneity of ASD is as yet in incipient stages. Further advances in elucidating the molecular underpinnings of ASD are expected to result from (a) larger cohort sizes of GWAS and exome-sequencing studies, (b) increased availability of archived post-mortem brain tissue for transcriptome studies, and (c) integrative analyses of genomic, transcriptomic, and epigenomic data.

At the same time, understanding the role of immune cells in regulating synaptic function is also a newly developing field. As discussed above, accumulating evidence supports the notion that immune cells play important roles in normal brain function, outside of neuroinflammation. Of particular relevance to ASD is the role of microglia in synaptic pruning during postnatal brain development, a period that coincides with the onset of ASD symptoms. While it has been demonstrated that increased numbers of activated microglia are present in brain parenchyma of ASD patients (Vargas et al., 2005; Morgan et al., 2010; Suzuki et al., 2013), these studies have not captured the early postnatal development window. Future studies, facilitated by early ASD diagnosis, could shed further light on microglial activation occurs during postnatal brain development and on potential changes in the magnitude of this phenomenon across development and adult life in ASD. Notably, abnormal synaptic density, which could result from a deficit of synaptic pruning, is a feature of several ASD animal models [e.g., increased synaptic density in Emtr1 KO mice, and decreased synaptic density in Rett syndrome mouse models (Delorme et al., 2013)], but it remains to be demonstrated whether it is also a feature of idiopathic ASD in human brain.

Since microglia and astrocytes have been shown to play a role in synaptic formation and maturation, and mutations in neuronal cell adhesion molecules have been associated with ASD, it is also tempting to speculate that ASD neurons might be particularly vulnerable to immune cell dysfunction in the brain.

Given the large amount of data supporting the role of immune responses in ASD and other neuropsychiatric disorders, advances in deciphering the functional interplay between immune cells and
neuronal synaptic function will likely provide vital insights into the mechanisms and potential therapy of neurodevelopmental disorders.

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ACKNOWLEDGMENTS

This work was supported by a Ramaciotti Establishment Grant and a NARSAD Young Investigator Award (IV), Converging pathways in autism spectrum disorders

Converging pathways in autism spectrum disorders
Talkowski, M. E., Maussion, G., Crapper, L., Rosenfeld, J. A., Blumenthal, I., Hanscom, C., et al. (2012). Disruption of a large intergenic non-coding RNA in subjects with neurodevelopmental disabilities. *Am. J. Hum. Genet.* 91, 1128–1134. doi:10.1016/j.ajhg.2012.10.016

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**Conflict of Interest Statement:** 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.

Received: 06 June 2013; accepted: 15 October 2013; published online: 07 November 2013.

Citation: Voineagu I and Eapen V (2013) Converging pathways in autism spectrum disorders: interplay between synaptic dysfunction and immune responses. *Front. Hum. Neurosci.* 7:738. doi: 10.3389/fnhum.2013.00738

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