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Studying the role of DLGAP1 transcripts in autism using human neural progenitor stem cells

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Abstract: This communication provides a brief review of autism spectrum disorder (ASD), including psychological perspectives and biological insights from whole brain, as well as cellular and genomic studies. Our progress towards conducting a functional study on the role of DLGAP1 RNA transcripts in human glutamatergic neurons derived from ASD and control, non-autistic, induced pluripotent stem cells is also described.

Key words: *autism (ASD), induced pluripotent stem cell (ipsc), neural progenitor cell (npc), DLGAP1*

Introduction

Autism Spectrum Disorder (ASD) is a neurological condition characterized by two core features: (1) impairments in social interaction and communication and (2) the presence of restricted interests and/or repetitive behaviors. Spectrum refers to ASD's numerous clinical presentations, including variations in intelligence, learning disabilities, compulsive behavior, and speech deficits [1]. Studies in the USA, Canada, South America, Asia, and Europe identified ASD in 1-2% of their populations [2]. ASD is 4.5 times more common among males than females [3].

Psychology research proposes that behavior commonly associated with ASD results from alterations in several cognitive processes, including social cognition and global processing [4]. The Social Cognition Deficit theory describes aspects of ASD as altered processing of human stimuli, such as other people's emotions, in contrast to processing of non-human stimuli, such as objects. The Weak Central Coherence theory argues the general population is primed for central coherence, integrating details in the context of a global, meaningful whole. In contrast, ASD individuals orient their cognitive processing in a more detail-focused, decontextualized, local manner. Lastly, the

Complexity Theory suggests individuals with ASD experience cognitive overload when processing tasks of certain complexity.

From a biological perspective, several models have emerged describing the neuropathology of

autism [5]. Studies on brain volume and gray/
white matter volume have led to a theory that the brain of autistic individuals undergoes accelerated growth during early childhood followed by a deceleration in growth later in life. MRI studies on functional connectivity support the premise of global hyperconnectivity in the brains of ASD individuals. Indeed, altered neurotransmission, caused by an imbalance of excitatory and inhibitory signaling during development, is hypothesized to be an underlying cause of autism.

In the brain, excitatory and inhibitory signaling are primarily mediated by two neurotransmitters: glutamate and GABA. Postnatally, during excitatory signaling, glutamate transmission results in depolarization and excitation of the post-synaptic neuron. Postnatally, GABA is inhibitory. Its post-synaptic transmission results in hyperpolarization, so a stronger signal is required for future neural excitation. This inhibitory role for GABA is a shift from its prenatal function as an excitatory transmitter. Since neurite outgrowth is dependent on neuronal activity, deficits in prenatal excitatory GABA signaling in an individual with ASD could result in an initial period of reduced neurite outgrowth. A postnatal period of excitatory glutamate signaling could then lead to an increased number of "local" circuits, which are hyperactive, due to deficient postnatal GABA inhibitory signaling. These reinforced local circuits would later prevent the elimination of superfluous synapses during synaptic pruning [6].

On a genetic level, over 700 loci are implicated in autism, highlighting ASD's complexity and heterogeneity [7]. Based on these findings, approximately 25% of current autism cases have an identifiable genetic cause. Disruptions in several genes involved in synaptic development and function, as well as cortical neuron identity, have been associated with autism [5,6].

A functional study of DLGAP1

Recently, whole transcriptome sequencing was performed on cultured cortical neurons, derived from the induced pluripotent stem cells (ipscs) of individuals with autism and control, non-autistic individuals [6,8]. Three Discs large homolog-associated protein 1 (DLGAP1) transcripts were differentially expressed. DLGAP1 is a scaffolding protein, expressed in a structure termed the post-synaptic density, in glutamatergic synapses [9]. A 1.7 fold increase in DLGAP1, a 1.93 fold decrease in DLGAP1 antisense 1, and a 1.51 decrease in DLGAP1 antisense 2 were observed in ASD neurons compared to controls [6].

The aim of our research is to examine the role of DLGAP1 in autism biology. We will study how reducing DLGAP1 antisense 1 and 2 transcripts affects ASD and control, non-autistic glutamatergic neurons. While the functions of these transcripts are unknown, one hypothesis is that these non-coding RNAs negatively regulate DLGAP1 protein-coding mRNA expression. Based on the transcriptome data, we hypothesize that reducing DLGAP1 antisense transcripts in control neurons will cause them to develop more like autistic neurons. Conversely, decreasing DLGAP1 protein coding mRNA in ASD neurons will ameliorate ASD phenotypes.

Currently, we are culturing ASD and control human neural stem/progenitor cells differentiated from ipscs, reprogrammed from skin biopsies. A protocol, which mimics the steps of neurogenesis during cortical development, was used to terminally differentiate the cells into glutamatergic neurons [6]. Visible differences were observed in the morphology of ASD neurons compared to control neurons. While we are still characterizing these differences, our findings support a previous report showing significant changes in axon and dendrite length, branch count, and process count between ASD and control cultures [6].

To reduce DLGAP1 antisense 1 and 2 levels in ASD and control neurons, we designed six different short hairpin RNAs (shRNAs). shRNAs reduce protein expression levels by targeting complementary RNA for degradation. Each shRNA was molecularly cloned into a lentiviral vector, and the insertion was verified

by DNA sequencing. Lentiviruses carrying the shRNAs and a control shRNA, with no known RNA target, were produced and titered. Procedures were established to amplify DLGAP1 antisense 1 and 2 cDNA in preparation for quantifying shRNA-mediated transcript reduction utilizing real time reverse transcriptase PCR.

Conclusion: Future experiments will transduce the shRNA lentiviruses into ASD and control cortical stem/progenitor cells. The cellular morphology, differentiation, maturation, and electrophysiology, as well as synaptic structure and function, of glutamatergic neurons will be examined and quantified. This data will provide valuable insight to the role of DLGAP1 antisense 1 and 2 in glutamatergic neurons during normal development as well as in cases of ASD.

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References

1. Hus V.; Pickles A.; Cook E.H. Jr.; Risi S.; Lord C. *Biol Psychiatry*. **2007**, 61, pp.438-448.
2. Centers for Disease Control and Prevention. www.cdc.gov/ncbddd/autism/documents/asdprevalencedatatable.pdf. **2016**.
3. Christensen D.L.; et al. *MMWR Surveil Summ.* **2016**, 65, pp.1-23.
4. Ben-Yosef, D.; Anaki, D., Golan, O. *Autism Res.* in press, doi: 10.1022/aur.1676.
5. Ardhanaresswaran, K; Volmar, F. *Yale J Biol Med.* **2015**, 88, pp. 5-16.
6. DaRosa, B.A. *Open Access Dissertations.* **2015**, http://scholarlyrepository.miami.edu/oa_dissertations/1531.
7. Ziats M.N.; Rennert, O.M. *Front Genet.* **2016**, 7, doi: 10.3389/fgene.2016.00065.
8. DaRosa, B.A.; et al. *Neurosci Lett.* **2012**, 516, pp.9-14.
9. Sheng, M.; Hoogenraad, CC. *Annu Rev Biochem.* **2007**, 76, pp.823-847.