MET: A link to Autism & GI disorders

Characterizing and understanding autism spectrum disorders (ASD) represent great challenges facing neuroscientists today. Accumulating evidence suggests that alterations in the patterning of specific brain structures and circuitry during development may contribute to ASD. The Met tyrosine kinase receptor is important for cell differentiation and organ development. In the developing CNS, Met is thought to facilitate a number of processes including neuronal migration, axon guidance and dendritic arborization by mediating cellular responses to its endogenous ligand, hepatocyte growth factor (HGF). In a paper recently published in The Journal of Comparative Neurology, Judson and colleagues followed up on previous reports relating autism susceptibility to alterations in Met signaling by characterizing Met expression patterns in the developing mouse brain.

The authors used *in situ* hybridization and immunohistochemistry to localize Met transcript and protein, respectively, within the developing murine forebrain. They showed that Met is primarily expressed in specific cortical projection neurons and in certain limbic system components, and that the protein localizes to axonal projections and is particularly enriched in major axon tracts such as the corpus callosum. In addition to characterizing the spatial expression pattern, the temporal pattern of developmental expression was analyzed by quantitative western blot. They found evidence that Met expression levels are highest in the early postnatal developmental period from P0 to P21. This corresponds to the time of mouse brain development in which neurite outgrowth and synaptogenesis occur. This finding further supports a role for Met in the formation of neural circuitry, possibly by facilitating outgrowth and path-finding in forebrain axons. Using an $Emx1^{cre}$ line and a "floxed" Met allele, the authors analyzed mice with a selective ablation of Met in all cells arising from dorsal pallium, which includes projection neurons of the cerebral cortex, hippocampus and some amygdaloid nuclei. This analysis was useful for determining the source of Met expression in the forebrain and further supported the hypothesis that Met is most highly expressed in the axonal projections of neurons, particularly projection neurons of the cortex and components of limbic circuitry.

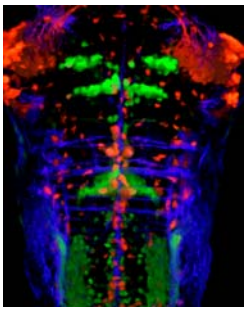
The highest levels of Met expression were observed in the cerebral cortex, and in limbic system associated structures thought to be important for emotional and social function, implicating Met in the establishment and organization of the neural circuitry responsible for maintaining normal emotional and social function. The manifestation of ASD often involves abnormal emotional and social behavior, possibly resulting from a physical disorganization of the circuits involved. This study provides evidence for a potential molecular substrate contributing to developmental abnormalities associated with ASD. Furthermore, it implies a significant role for Met receptor related signaling in normal development of the limbic system and forebrain.

Original Research Article:
MC Judson, **MY Bergman**, DB Campbell, KL Eagleson and P Levitt (2009). Dynamic Gene and Protein Expression Patterns of the Autism-Associated Met Receptor Tyrosine Kinase in the Developing Mouse Forebrain. *J Comp Neurol.* **513**: 511-531.

olig2 and Development

Centuries of painstaking contributions to the human brain atlas have resulted in a nearly gridlocked roadmap of neural networks. Relatively recent genetic characterizations in model organisms have shed new light on the developing brain. These developmental studies hold the capacity not only to decode the origins of neural complexity, but may in turn reveal the molecular nature of neurodegenerative diseases. In a recent paper highlighted on the cover of the *Journal of Neuroscience*, Zannino and colleagues identified neural and glial cell origins in the developing brain, ultimately demonstrating the impact of the *olig2* transcription factor on formation of oligodendrocyte progenitor cells (OPCs) and a specific type of motor neuron (MN) in the zebrafish hindbrain.

Oligodendrocytes are the myelinating cell type of the central nervous system. Through myelination of neural fibers in the CNS, oligodendrocytes contribute to rapid propagation of action potentials. Immature oligodendrocyte progenitor cells are specified from neuroepithelial precursor populations, which also give rise to neuronal cell types. The mechanism of specification and subsequent differentiation from precursor populations has been most intimately studied in the spinal cord, where the neural milieu is relatively restricted as compared to the brain, thereby facilitating the tracing of migratory behavior of cells and



their processes. Previous studies in the Appel laboratory demonstrated that the *olig2* gene is necessary for formation of OPC and spinal motor neurons from the pMN domain of the zebrafish spinal cord. Because oligodendrocytes are present throughout the central nervous system, they extended this hypothesis along the anterior axis to the hindbrain.

Using elegant transgenic strategies and lineage-specific antibody labeling, Zannino *et al*. first characterized neuronal and glial cells in the hindbrain. They witnessed *olig2* mRNA expression specifically in rhombomeres 5 and 6 (r5/r6) of the hindbrain, which was corroborated by enhanced green fluorescent protein (GFP) driven by *olig2* regulatory DNA in transgenic embryos: Tg(*olig2:eGFP*). Antibody staining for Zn8, a marker for somatic abducens motor neurons, was also specific for the 5th and 6th rhombomeres, unlike the broad motor neuron marker, Isl1. Thus hindbrain abducens motor neurons and some OPCs may be specified from a common precursor population. Through time-lapse imaging of Tg(olig2:eGFP) embryos, they next demonstrated that OPCs come from within neuroepithelial precursors in the 5th and 6th rhombomeres of the hindbrain, but that many also arise from *olig2*- precursors elsewhere in the hindbrain.

The investigators next show that the knockdown of the *olig2* gene by targeted antisense morpholino (MO) resulted in a specific effect on hindbrain cells. In morpholino-injected embryos, *olig2* RNA expression was maintained in rhombomeres r5 and r6; however, these cells appeared abnormal at 48 hours post-fertilization, in that most cells appeared to be undifferentiated neuroepithelial precursors and did not possess abducens morphologies. Additionally, BrdU staining for mitotically active cells continued in the hindbrain of MO-injected embryos long after control siblings, suggesting that these cells remain in an undifferentiated state. These results suggest that Olig2 function is necessary for formation of both hindbrain OPCs and somatic abducens motor neurons.

This paper provides several important characterizations of hindbrain cell fate decisions in early development. First, their confocal imaging provides evidence for multiple origins of hindbrain OPCs. In addition, this work shows that timing of *olig2* expression is essential: at early stages the gene is expressed only in the neuroepithelial precursors of rhombomeres r5 and r6, and at later stages only in cells that already possess OPC morphology. Finally, Zannino *et al*. found that *olig2* is also necessary for a class of abducens motor neurons to exit the cell cycle and begin neurogenesis. Ultimately, this work characterizes crucial cell-fate decisions in the developing brain, demonstrating the essential combination of gene regulation and temporal control toward proper specification of both glial and neural cell types in the vertebrate hindbrain.

Original Research Article:
DA Zannino and B Appel (2009). Olig2+ Precursors Produce Abducens Motor Neurons and Oligodendrocytes in the Zebrafish Hindbrain. *J Neurosci.* **29** (8): 2322-2333.

A note from the Director

In my first year as the Director of the Vanderbilt Brain Institute and the Vanderbilt Neuroscience Graduate Program, I have been continually impressed with the passion for science and dedication to the research endeavor that embodies each of our graduate students. This volume serves as a tangible testament to the exceptional nature of these individuals, and illustrates both the diversity and quality of the neuroscience research enterprise at Vanderbilt. As the first stage in their passage to doctoral candidacy, these reviews serve as springboards to the student's proposed thesis research, and I am delighted to say that each of our candidates demonstrated a strong breadth and depth of knowledge in their chosen research areas while defending these reviews. I am proud to serve in a leadership role for an organization that can join together to highlight its accomplishments in such a novel, impressive and attractive manner, and I am deeply indebted to those (most notably, Chris Ciarleglio) who have taken a leadership role in making this journal a reality.

Yours in science,

Mark T. Wallace, Ph.D.