How Autistic Brains Grow Differently: Hippocampal Neurogenesis in the 16p11.2 Heterozygous Mouse, a Model of Non-Syndromic Autism

BACKGROUND

Autism spectrum disorder (ASD) is a heritable neurodevelopmental disorder that is characterized by lifelong deficits in two key domains: (1) social communication and interactions and (2) the presence of restricted or repetitive interests and behaviors. It is estimated to affect 1 out of 68 (1.47%) individuals. [1]

What Causes Autism?

~20% of cases have a known etiology [2]
~80% of cases are idiopathic (cause is unknown)
→ Likely attributed to the interaction of numerous mutations across the genome
→ Sometimes linked to environmental factors (methymercury, valproic acid, etc.)

What Role Does Neurogenesis Play in Autism?

Neurogenesis is the birth, development, and integration of new neurons into the developing brain. This process underlies the establishment of neuronal architecture and connections between brain regions. Since most of these connections persist into adulthood, even small aberrations can lead to lifelong deficits.

Why the Hippocampus?

Adulthood, even small aberrations can lead to lifelong deficits. [3]

One Theory: Autism is caused by development-associated deficits in brain connectivity, implicating early neurogenesis as a major contributor to the disorder. [3]

GOALS AND OBJECTIVES

In order to understand how 16p11.2 heterozygosity affects hippocampal neurogenesis in early adolescence (P21 in mice), [4] (Fig 5)

Why Study 16p11.2?

(1) found in up to 1.0% of ASD cases, but only in ~0.04% of general population [5]
(2) 90% of individuals with 16p have a psychiatric or developmental disorder [6] → 16p11.2 CNVs are likely to confer risk for ASD and other psychiatric conditions

METHODOLOGY

1) BrdU injections at P21 (2hr, 24hr, 48hr, and 3wks prior to perfusion) → Incorporated into proliferating (S-phase) cells
2) Anesthesia & Perfusion → Subjects rendered unconscious
3) Dissection, collection, & storage (freezing) of brain tissue
4) Brain tissue sectioned
5) Immunostaining
   a) Stained with primary antibodies (Fig 3): anti-cleaved-caspase3 (apoptotic marker) anti-BrdU (proliferative marker) anti-PCNA (proliferative marker)
   b) Reveal staining (using DAB)
8) Counterstained with toluidine blue (for pyknotic nuclei; apoptotic marker)
9) Slices analyzed under microscope → biomarkers counted within dentate gyrus

ANALYSIS OF RESULTS

Cell counts and overall survival within the dentate gyrus (DG) will be compared in control (WT) vs. experimental (Hets) groups. (Fig 3,4)

CONCLUSION

1) 16p11.2 deletion conveys risk to autistic symptoms
2) Preliminary results predict a change in hippocampal neurogenesis in 16p11.2 heterozygous mice
3) Changes in hippocampal neurogenesis likely underlie social deficits in 16p11.2 heterozygous mice

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REFERENCES


PRELIMINARY RESULTS

Increase in apoptotic markers in 16p11.2 hets at P7
Limitation: Small sample size; apoptosis is minimal at P7

HYPOTHESIS

→ Increased apoptosis - according to preliminary results
→ Decreased proliferation - due to deletion of MAPK3/ERK1 (Fig 2), which typically promotes progenitor proliferation when it is in its active state

Figure 3: Schematic for how cell counts will be assessed within the dentate gyrus (DG) of the hippocampus.

Figure 4: Diagram of chromosome 16 in wild type (WT) and heterozygous (Het) animals.

Figure 5: Simplified progression through neurogenesis; Proliferation (cell growth) and apoptosis (cell death) collectively contribute to overall cell survival; Earlier points are characterized by more proliferation, and later points are characterized by more apoptosis; Red X’s indicate cells that have died via apoptosis