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

RUTGERS
Ronald E. McNair Postbaccalaureate Achievement Program

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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** (methylmercury, 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.

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

Why the Hippocampus?

Underlies functions such as **regulation of social-stress and depression**, which are commonly affected domains in individuals with ASDs. This circuitry develops in adolescence (around 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

GOALS AND OBJECTIVES

In order to understand how 16p11.2 heterozygosity affects hippocampal neurogenesis in early adolescence (P21), I will compare the relative rates of **proliferation** (cell growth) and **apoptosis** (cell death) in control (WT) and experimental (Hets) subjects. **(Fig 3,4)**

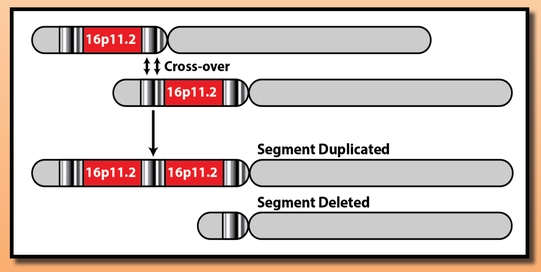
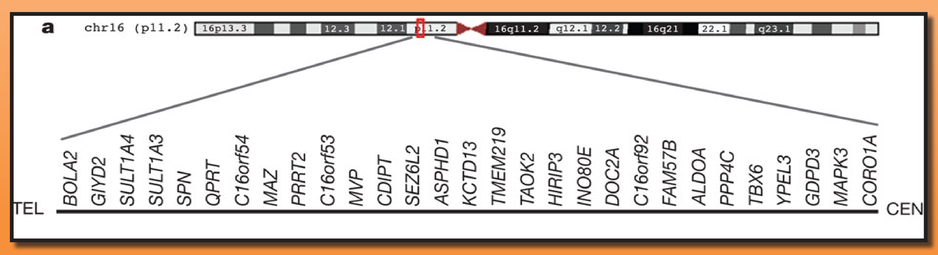


Figure 1 (left): 16p11.2 CNVs occur via non-allelic homologous recombination (NAHR)

Figure 2 (below): Human chromosome 16; Expansion shows 29 genes contained within 16p11.2 locus; TEL (telomere), CEN (centromere) [7]



METHODOLOGY

1) **BrdU injections** at P21 (2hr, 24hr, 48hr, and 3wks prior to perfusion)
→ Incorporated into proliferating (S-phase) cells

2) **Anesthetization & 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

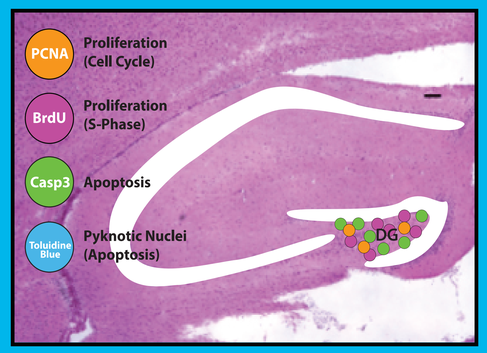


Figure 3: Schematic for how cell counts will be assessed within the **dentate gyrus (DG)** of the hippocampus.

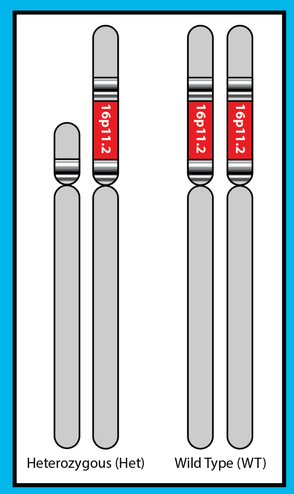


Figure 4: Diagram of chromosome 16 in wild type (WT) and heterozygous (Het) animals.

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)**

BrdU+ or PCNA+ → Proliferative Cell

caspase3+ or Pyknotic Nuclei (Toulidine Blue) → Apoptotic Cell

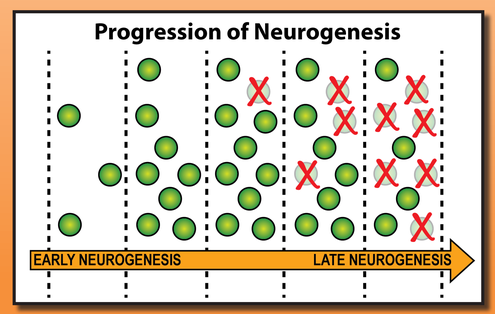
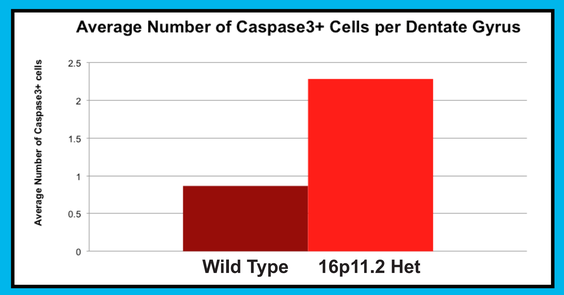


Figure 5: Simplified progression through neurogenesis; Proliferation (cell growth) and apoptosis (cell death) collectively contribute to overall cell survival; Earlier points are characterized by more proliferation, and later points are characterized by more apoptosis; Red X's indicate cells that have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

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

→ **Overall decrease** in number of cells in dentate gyrus

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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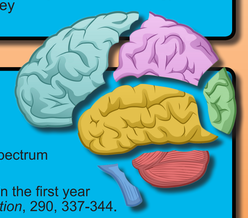

REFRENCES

[1] Centers for Disease Control and Prevention, 2015

[2] Buxbaum and Hof, (2013). The neuroscience of autism spectrum disorders (1st ed.). *Oxford: Elsevier.*

[3] Courchesne et al., (2003). Evidence of brain overgrowth in the first year of life in autism. *Journal of the American Medical Association*, 290, 337-344.

[4] Kirshenbaum et al., (2014). Adolescent but not adult-born neurons are critical for susceptibility to chronic social defeat. *Frontiers in Behavioral Neuroscience*, 8(289)

[5] Weiss et al., (2008). Association between microdeletion and microduplication at 16p11.2 and autism. *The New England Journal of Medicine*, 358(7), 667-675.

[6] Hanson et al., (2014). The cognitive and behavioral phenotype of the 16p11.2 deletion in a clinically ascertained population. *Society of Biological Psychiatry*

[7] Golzio et al., (2012). KCTD13 is a major driver or mirrored neuroanatomical phenotypes. *Nature*, 485 (7398), 363-367.