Effects of PTEN Haploinsufficiency on ASD-relevant Behavioral Phenotypes

Hashna Manoharan and Dr. Damon Page
Department of Neuroscience - The Scripps Research Institute

Introduction

Mutations of *PTEN* (phosphate and tensin homolog), a tumor suppressant gene that interacts with the PI3K (Phosphoinositide 3 kinase) signaling pathway, have been correlated to an array of neurological disorders with genetic components.

Previous research has suggested a correlation between ASD and mutations of the *PTEN* gene, as there is an increased frequency of *PTEN* haploinsufficiency (PTEN +/) in individuals diagnosed with autism in comparison to the general population (1% of diagnosed individuals as compared to 0.007% of the general population). Haploinsufficiency refers to a subclass of *PTEN* mutations in which individuals possess both a normal and mutated version of the *PTEN* gene.

This correlation is especially significant when considering the subset of ASD patients with macrocephaly (7%), suggesting a specific relationship between mutations of the gene and macrocephaly associated ASD.

Exploration of a possible causative relationship between *PTEN* haploinsufficiency and ASD relevant behavioral phenotypes may lead to increased understanding of the genetic basis for ASD, as well as the eventual development of reliable biomarkers for ASD diagnosis.

In addition, the tumor suppressant functions of the gene and the PI3K pathway through which it acts may lead to a possible neuropharmacological approach to the management and treatment of ASD and associated behaviors.

Method

An applied mouse model testing method with a within subjects design was developed.

Male C57BL strain mutant (*Pten +/)* mice and littermate non mutant controls *(Pten +/+)* were used. Both proximity testing and resident intruder testing paradigms were used.

In proximity tests, adult mice (P21 and beyond) in their natural environment (home cage with 4 5 littermates) were observed and videotaped at specific intervals in order to gather information about their social activity.

Figure 1: Home cage proximity testing

The resident intruder testing paradigm, used to test behaviors exhibited by mice in unfamiliar environments, such as exploration and aggression, involved removing mice from their home cage, and placing them in a cage with a "resident" gonadectomized mouse. After an initial period of acclimation, the mice were taped for fifteen minute testing periods.

A comprehensive observational scoring method was utilized in order to determine the amount and duration of ASD relevant behavioral phenotypes in the mice during both tests. As established by prior literature, ASD relevant behavioral phenotypes scored for during observational analysis included exploratory behaviors, social behaviors, aggression, overt attacks, and stereotypic (repetitive) behaviors, all of which are exhibited in increased amounts by human patients diagnosed with ASD.

A double blind method was used in order to eliminate scoring bias. At the conclusion of testing, the mice were perfused and brains harvested in order to conduct diameter and by weight measurements.

Results

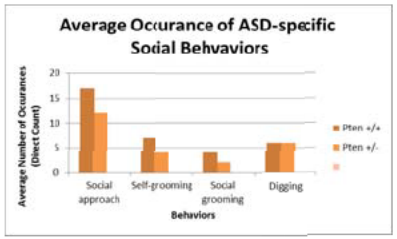

Figure 2: Preliminary (tally) scoring results for average occurrence of specific ASD-relevant social behaviors during home cage proximity testing

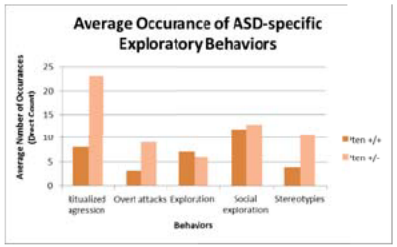

Figure 3: Preliminary (tally) scoring results for average occurrence of selected ASD-relevant behavioral phenotypes during resident-intruder testing

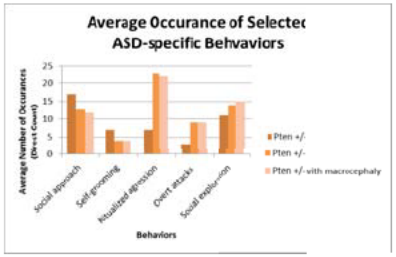

Figure 4: Tally scoring results for average occurrence of selected relevant behaviors (across both testing methods) based on genotype and macrocephaly

Discussion

Due to the ongoing nature of the current project, it is not yet possible to arrive at concrete conclusions. However, preliminary analysis of data suggests specific trends.

Overall, *Pten +/* mice displayed lower activity levels. *Pten +/* mice, on average, engaged in slightly fewer social behaviors as compared to *Pten +/+* mice during home cage proximity tests. They also displayed increased aggression, overt attacks, and stereotypies during resident intruder testing. The occurrence of exploratory behaviors was fairly equal across *Pten* genotypes.

There was not a significant difference in the trends for haploinsufficient mice with macrocephaly (as determined by extraction and weighing), although this may change as comprehensive analysis of collected data is conducted.

Preliminary analysis suggests a causative relationship between *Pten +/* and certain ASD relevant behavioral phenotypes in male mice cohorts, although there is not yet evidence of strengthened correlation in cases of macrocephaly associated haploinsufficiency.

Future directions include full observational analysis of the data, implementing a similar testing protocol to female mice, and adding additional relevant testing protocols.

References

Buxbaum, J. D., Chaste, P. (2007). Mutation screening of the Pten gene in patients with autism spectrum disorders and macrocephaly. *American Journal of Medical Genetics*, 484-91.

Page, D.T., Kuti, O., Mriganka, S. (2009). Haploinsufficiency for Pten and serotonin transporter cooperatively influences brain size and social behavior. *Proceedings of the National Academy of Sciences*, 106-6.

Page, D. T. (2011). A candidate circuit approach to investigating autism. *The Anatomical Record*, 294-10.

Undergraduate Research Symposium, April 5, 2013