Children's Hospital of Philadelphia

Annual Progress Report: 2008 Nonformula Grant

Reporting Period

July 1, 2010 – June 30, 2011

Nonformula Grant Overview

The Children's Hospital of Philadelphia received $4,708,555 in nonformula funds for the grant award period June 1, 2009 through May 31, 2013. Accomplishments for the reporting period are described below.

Research Project: Project Title and Purpose

CHOP/Penn Center of Excellence for Autism Research - This grant establishes a Center of Excellence for Autism Research at Children's Hospital of Philadelphia (CHOP), in collaboration with the University of Pennsylvania, Temple University, Lincoln University and the Philadelphia Public School system. The Center will engage in a set of closely inter-related studies aimed at improving our understanding of the biological causes of the autism spectrum disorders (ASD), so that more effective treatments can be created. We have new evidence of the first gene for autism, and now need to study this gene in more detail. We will study this gene in a group of 400 children, performing detailed clinical evaluations to understand how this gene affects their brains and behavior. We are also performing a study on the effectiveness of a behavioral treatment, and will test to see if genotype is correlated with treatment response.

Anticipated Duration of Project

6/1/2008 - 5/31/2013

Project Overview

This funding will support a Center of Excellence for Autism Research located at Children's Hospital of Philadelphia (CHOP), with partnerships at Lincoln and Temple Universities, the University of Pennsylvania, and the Philadelphia Public School System. This project's central theme is understanding the relationship between genetic risk factors, brain and behavior (including treatment response) in persons with an autism spectrum disorder (ASD). We will conduct a series of integrated subprojects that follow upon several recent breakthroughs in the genetics of ASD. We have identified the first common genetic variant for ASD. Our data also suggest that rare sequence and structural variants serve as significant risk factors for ASD. The genes appear to have a common biological function related to synaptic connectivity. We aim to study 400 children with ASD, and to investigate the relationship between genetic risk factors and clinical presentation, brain function, and response to treatment. Individual subprojects in the Center will focus on our common variant finding (Project I), rare genetic variant findings

(Project II), animal knockout models of risk variants replete with social behavioral and brain assays (Project III), comprehensive assessment ("deep phenotyping") of the ASD sample to assess the clinical impact of genetic risk factors, including treatment responsiveness (Project IV), and measures of the "social brain" using magnetic resonance imaging and magnetoencephalography (Project V). Neuroimaging will include several measures of connectivity (including diffusion tensor imaging, functional MRI connectivity, and coherence analysis with magnetoencephalography), as we aim to relate connectivity to genetic risk factors. Participants with an ASD from Project IV will be enrolled in projects I, II, and V, so as to provide a set of tightly inter-related experiments. Our animal models will knock out the rare and common genetic risk factors, and perform behavioral and imaging studies that parallel the human studies.

In addition, we will create undergraduate and graduate level training programs for underrepresented minorities in collaboration with the University of Pennsylvania, Temple University and Lincoln University (Project VI). Students will work within the labs of researchers on this grant, and will receive additional group and individual teaching and mentorship to prepare them for professional work in the area of autism.

Principal Investigator

Robert T. Schultz, PhD
Director, Center for Autism Research
The Children's Hospital of Philadelphia
Joseph Stokes Jr. Research Institute
3615 Civic Center Boulevard
Philadelphia, PA 19104-4318

Other Participating Researchers

Chris Edgar, PhD, Hakon Hakonarson, MD, PhD, John Herrington, PhD, Judith Miller, PhD, Timothy Roberts, PhD - employed by The Children's Hospital of Philadelphia
Edwin Abel, PhD, Edward Brodkin, MD, Maja Bucan, PhD, James Connell, PhD, Christos Davatzikos, PhD, David Mandell, ScD, Steve Marcus, PhD, Ragini Verma, PhD, - employed by University of Pennsylvania.
Nicole Stephens, PhD - employed by Lincoln University

Expected Research Outcomes and Benefits

Autism spectrum disorders are caused by a combination of genetic and nongenetic factors. The role of genetics is particularly strong. We have new genetic findings that we will explore in greater depth. The integrated set of studies in this project will focus on understanding how these newly discovered genetic risk factors affect the health of children with autism. We will carefully measure the clinical profile of each child in our study, and relate different genetic risk factors to differences in these profiles. We will also perform comprehensive brain imaging studies and relate genetic risk factors to underlying problems in brain structure and function. One immediate health benefit of the research process is that we will give expert clinical evaluations and

feedback free of charge to a large community of individuals with autism and their families. In addition, we expect to make scientific progress that will significantly contribute to the health of children by providing an improved understanding of the genetic factors that initiate this disorder; the findings will serve to identify molecular mechanisms critical to regulating the clinical presentation of ASD, and thereby facilitate the development of effective diagnostic tests. In turn, this could lead to earlier diagnosis and a good possibility for the development of effective new therapeutic interventions. Because a significant proportion of the study subjects will have multiple risk factors that will markedly amplify their genetic risk of developing autism, we will also be able to determine the cumulative risk of autism incurred from having more than one genetic risk factor.

Summary of Research Completed

Project 1 (PI: Hakonarson) made significant progress for each of its 3 Specific Aims. Aim 1 (to isolate the chromosomal region corresponding to the linkage disequilibrium block harboring an ASD risk locus identified through GWA plus the two flanking cadherin genes in 120 individuals using a sequence enrichment protocol) was completed using the RainDance capture method on 144 DNA samples from ASD subjects (including 500 Kb region combined) and using Agilent SureSelect method on 79 samples from ASD subjects, including exome and regulatory sequences from 100 candidate genes (cadherins and protocadherins gene networks), for a total sequence of 500kb. Aim 2 (to apply massively parallel sequencing to the regions obtained as a consequence of the isolation procedures in Aim 1 and to assemble the resulting sequence data with bioinformatic tools in order to catalogue all variation in the isolated DNA sequences) was completed with greater than 50 times coverage and a capture success of 99%. Over 1000 novel variants were identified (including 2 non-synonymous variants the cadherin10 gene). Work has begun on Aim 3 (to genetically validate the short-listed variants identified in Aims 1 and 2 by genotyping them in 3,000 ASD cases and 3,000 control subjects). A Veracode genotyping array has been designed with 384 novel variants, using haplotype-based approach to ensure all independent novel variants are genotyped. This includes all rare variants (less than 1% MAFs) and one or more representative common variants for each haplotype. The Veracode array is on target to be delivered in July, and we anticipate completing the genotyping for SA3 in the next year.

Project 2 (PI: Bucan**)** focused the last year on the identification of rare variants in exonic and non-coding regions of 100 ASD associated genes. Target capture sequencing was performed on 20 ASD subjects using an Agilent Sure Select Custom kit for in-solution hybrid capture of 3.5 Mb encompassing 100 ASD genomic regions (1.5 Mb exonic and UTRs and 2 Mb highly conserved elements in intergenic and intronic regions) followed by Illumina HiSeq sequencing of 20 individuals with ASD and 68 HapMap control subjects. This has resulted in identification of a large number of novel rare variants (MAF <1%) in ASD probands including non- synonymous mutations in multiple known ASD-associated genes. Figure 1 illustrates the findings in two families. During the last funding period, the researchers also developed a novel computational pipeline for identification of potentially disease-causing SNPs with non-coding regions. Specifically, publically available data on predicted and experimentally validated regulatory sequences (p300 and CBP binding sites, neuronal enhancers, histone H3K9Ac marks in 6 brain regions and DNAse 1 hypersensitive sites in fetal brain) were combined and mapped genome-

wide. A scoring system was developed that can classify SNPs into three categories - low (no score), moderate (0.1-0.4) and high regulatory (0.4-1.0) potential. Among 3,453 SNPs with target regions in 18 individuals, 1202 have score > 0.1 with 63 SNPs with high scores. For example, this analysis identified a high scoring novel SNP in the upstream region *BPRAP1* gene on chromosome 17 (Figure 2). Understanding the predicted effects of these alleles on gene function and evaluation of these alleles in functional assays will provide key insights into the mechanisms underlying neurodevelopmental anomalies associated with autism.

Project 3 (PI: Brodkin) made substantial progress on Aim 1 during the reporting period: GenOway will be generating a mouse in which exons 4 and 5 of *Cdh10* are flanked with loxP sites and then conditionally deleted. Although GenOway initially encountered serious difficulties generating these mice because of the GC-rich nature of the sequence around *Cdh10*, these technical challenges were surmounted, and, as of May 2011, 3 live chimera mice with floxed *Cdh10* had been generated, and the breeding of the chimeras has been initiated. If breeding proceeds well, the researchers will obtain live *Cdh10* conditional knockout mice for phenotyping during the next (3rd) project period. Generation of the *Cdh9* floxed mouse was initiated during Year 2 by the Knockout Mouse Project. Regarding Specific Aim 2, using the Social Choice Test, the researchers have identified a highly significant reduction in sociability in male, juvenile (~30-day-old) heterozygous protocadherin gene deletion mice (*Pcdh10*$^{+/-}$ mice) relative to wildtype littermates (WT) (n=9 WT males, n = 8 *Pcdh10*$^{+/-}$ males), suggesting that these mice are a model with both etiological validity and face validity for social withdrawal symptoms of ASD. These results appear specific to the social domain, as there are no significant group differences between male, juvenile *Pcdh10*$^{+/-}$ mice and male wildtype littermates in baseline locomotor activity or generalized novelty seeking behavior. A similar trend toward reduced sociability in female *Pcdh10*$^{+/-}$, but a less robust reduction relative to males, was identified (n = 13 WT females and 12 *Pcdh10*$^{+/-}$ females) (Figure 3). An additional set of 13 WT males, 13 *Pcdh10*$^{+/-}$males, 9 WT females, 9 *Pcdh10*$^{+/-}$ females at a juvenile age (~30-days-of age) have undergone assessments of motor coordination (rotarod), anxiety-related behavior (elevated zero maze), and olfactory acuity and discrimination (olfactory habituation-dishabituation), and there appear to be no differences between and *Pcdh10*$^{+/-}$ WT mice that could account for the reduced sociability of juvenile male *Pcdh10*$^{+/-}$ mice (e.g., see Figure 4). Another set of adult mice (~11 *Pcdh10*$^{+/-}$ and 11 WT) have undergone tests of learning and memory (contextual fear conditioning), olfactory acuity (latency to find buried food), and exploratory behavior (open field testing). These particular tests are being conducted in adults because the paradigms (e.g. contextual fear conditioning) are best validated in adults. The data so far suggest that *Pcdh10*$^{+/-}$ males specifically have a deficit in learning (contextual fear conditioning). Also, adult *Pcdh10*$^{+/-}$ mice as a group tended to show increased olfactory acuity (shorter latency to find buried food). The *Pcdh10*$^{+/-}$ males tended to show higher levels of locomotor activity in the open field test. Studies of brain phenotypes are underway, for example studies of synaptic function (LTP) and quantitative analysis of dendritic structure in brains of *Pcdh10*$^{+/-}$ vs. WT mice. Studies of brain phenotypes and behaviors will continue in *Pcdh10*$^{+/-}$ mice in Years 3 and 4.

Project 4 (PI: Schultz) has continued to make good progress in characterizing the phenotype in children with ASD, for use in Projects 1, 2, and 5. For the last reporting period, the researchers recruited, scheduled, and completed evaluations on 156 participants (115 male, and 41 female) (128 Caucasian, non-Hispanic, 7 African American, non-Hispanic, 6 Asian, non-Hispanic, 5

Biracial, 5 Caucasian, Hispanic, 2 African American, Hispanic and 3 Hispanic). Forty were typically developing controls (TDCs) and 116 carried a clinical diagnosis of an autism spectrum disorder (ASD). However, 19 did not meet more rigorous study specific diagnostic definitions and were therefore dropped. 1 TDC participant was excluded because of an Axis I disorder discovered during deep phenotyping. Three participants with ASD previously took part in the Philly AIMS study. The Mullen Scales of Early Learning has been added to the core battery for characterizing general cognitive ability in those who are not able to complete the Differential Abilities Scale. All participants received comprehensive written and oral feedback on psychological testing results and individually tailored treatment recommendations.

Biosamples were collected on all TDC participants and 90 of the 97 ASD participants (7 became overly distressed during blood drawing attempts). Biosample collection for these participants will be re-attempted through two means: 1) The researchers have recently hired a traveling phlebotomist who will attempt to collect these samples by going to the participants' homes; 2) if blood is not obtained through method 1, a saliva collection kit will be mailed to the families' home with instructions for providing the sample and a prepaid return shipping package. Experimental psychological test procedures are showing some promising results. For example, the researchers are finding significantly slower social orientation in children with ASD during the eye tracking experimental protocol.

Project 5 (PIs: Schultz, Roberts and Davatzikos) has continued to make good progress in acquiring brain imaging data and in preliminary analyses of those data. For the last reporting period, the researchers attempted the MRI study protocol [diffusion tensor imaging (DTI), functional MRI (fMRI) and structural MRI (sMRI)] on 55 participants (32 male) from Project 4 (including 1 Hispanic, 2 African-Americans, 2 Biracial, 46 Caucasian, non-Hispanic, 3 Asian, and 1 African-American, Hispanic). Forty two of the 55 participants completed all image acquisition procedures (1 of whom did not complete DTI and sMRI, but has been re-scheduled; 12 did not successfully complete the fMRI protocol and will not return). All 42 also have DNA biosample collected. Magnetoencephalograhy (MEG) data collection is behind because the project was waiting for the installation of the new MEG machine (completed in November 2010). Since that time, protocol data was successfully collected on 27 children (22 ASD, 5 TDC), all of whom also had the complete MRI protocol. Although the researchers will wait for the final sample (80 ASD, 80 TDCs) before completing data analyses, preliminary analyses are ongoing for all types of data with encouraging preliminary results. For example, using a Support Vector Machine pattern classification algorithm on the sMRI data and on the DTI data we derived a computational marker which quantifies the presence of ASD-like abnormality in an individual's brain, with several fiber pathways implicated in heuristic models of ASD, e.g. the inferior longitudinal fasciculus. The cross-validated accuracy of the classifier in the task of separating ASD from controls was 73% for the sMRI data and 79% for the DTI data. Preliminary fMRI analyses are finding significant (cluster corrected) hypoactivation of social perception areas, including the fusiform gyrus.

Project 6 (PI: Connell, replacing Doehring). Dr. Connell increased the media and print recruitment efforts at Lincoln and Temple Universities, and the University of Pennsylvania by developing an e-flyer which was circulated through university student association listservs (e.g., psychology student council, Psi Chi, psychology honors program), and printed for posting on

psychology program bulletin boards. Dr. Connell visited the local universities and described to students directly, the tobacco grant minority training program. Dr. Connell met individually with local university students, corresponded with national students via email and phone, and met via Skype with interested international students. As a result of these targeted and aggressive recruiting efforts, the two undergrad summer positions were filled for the 2011 summer session. The remaining two post-baccalaureate positions have been filled this period for fall, 2011. The two year-long undergrad positions have numerous applicants and both are expected to be filled for this coming academic year as well. Our objective is to have a cohort of four minority students (two undergrads and two post-baccalaureate) in our center to share their clinical, research and co-curricular. The team's first post-baccalaureate minority student was accepted into 4 PhD programs this past winter (and recently left the Center for the University of Florida).

Figures

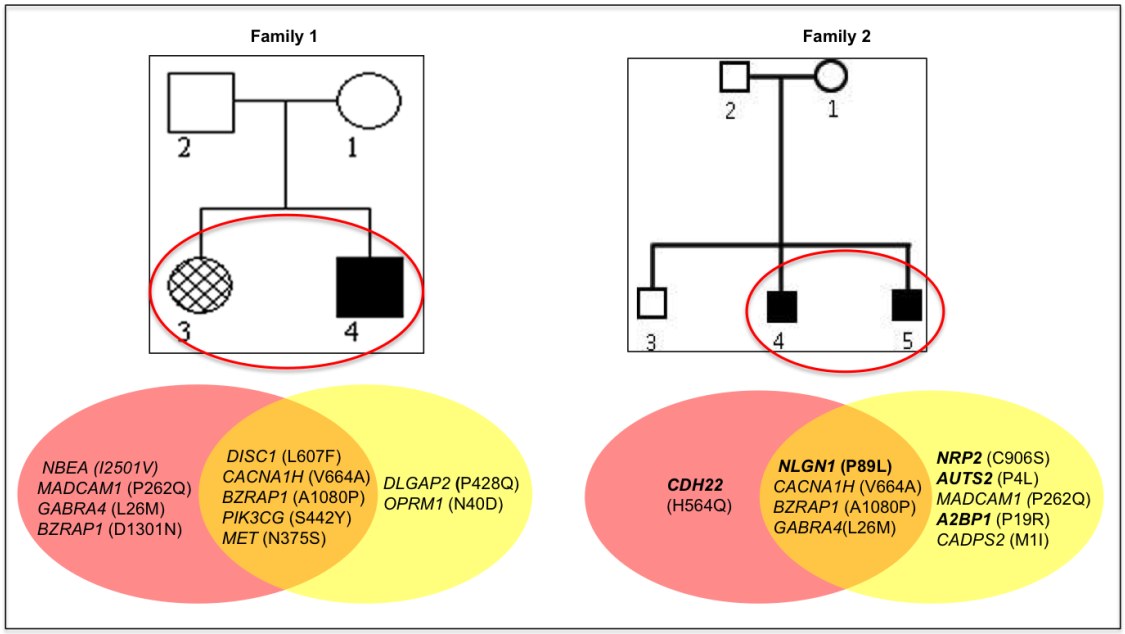


FIGURE1: Mutational load in affected siblings from 2 AGRE families
Exome sequencing identified common variants in genes *CACNA1H*, *BZRAP1* and *GABRA* in both families, and novel nonsynonymous mutations (in bold) in genes *NLGN1*, *CDH22*, *NRP2*, *A2BP1* and *AUTS2* in family 2. All listed genes have been previously associated with ASD susceptibility (www.sfari.org).

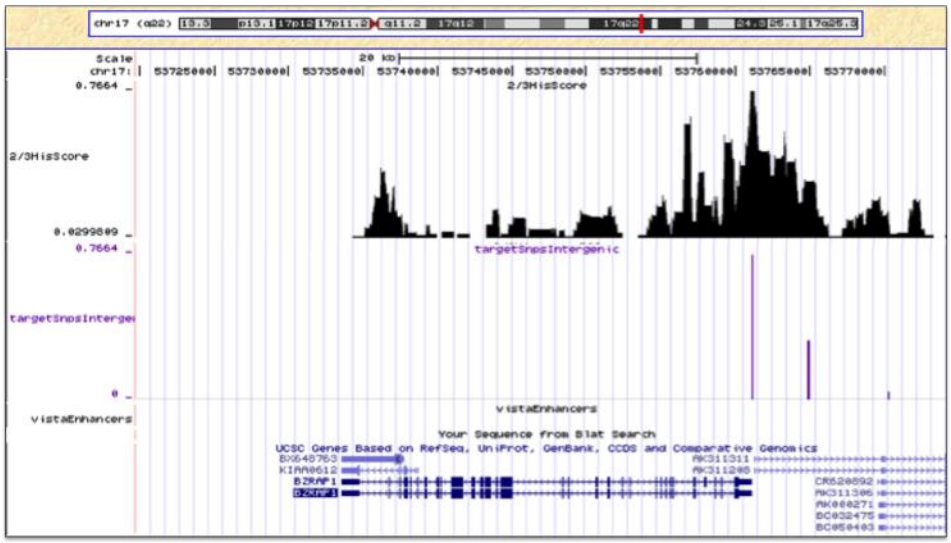
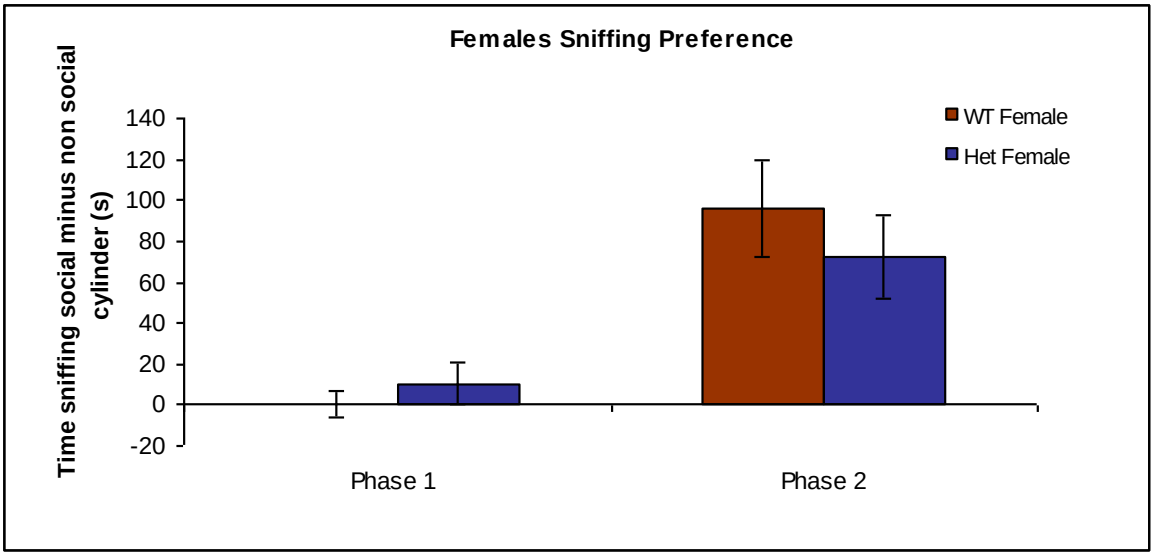


FIGURE 2: High scoring SNPs in the intergenic regions of ASD-associated genes
A genome browser picture (http://genome.ucsc.edu) of SNPs (purple) in ASD individuals, in the upstream region of ASD-associated gene *BZRAP1* that overlap with high scoring non coding elements based on DHHS and HMM data.



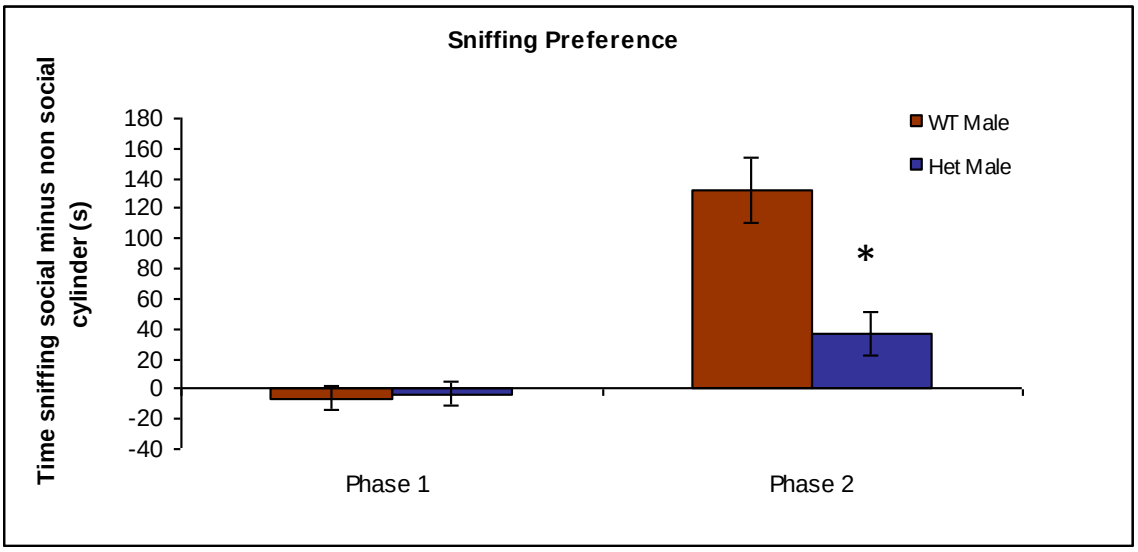


Figure 3. Reduced sociability of juvenile (~30-day-old) *Pcdh10*$^{+/-}$ mice in the social choice test. 3A. Males. Sniffing preference, a measure of sociability, is operationally defined as time spent sniffing the "social cylinder" (the cylinder into which a stimulus mouse was to be introduced) minus time spent sniffing the "non-social cylinder" (the cylinder into which a novel object was to be introduced). Phase 1 was the habituation phase, during which both cylinders were empty. Phase 2 was the social choice phase, during which the social cylinder contained a stimulus mouse and the nonsocial cylinder contained a paperweight. *Pcdh10*$^{+/-}$ mice showed lower sociability than wildtype littermate controls. (N = 9 WT males, N = 8 *Pcdh10*$^{+/-}$ males). 3B. Females. Female *Pcdh10*$^{+/-}$ mice showed a trend toward lower sociability than WT littermates(N = 13 WT, N = 12 *Pcdh10*$^{+/-}$ females).

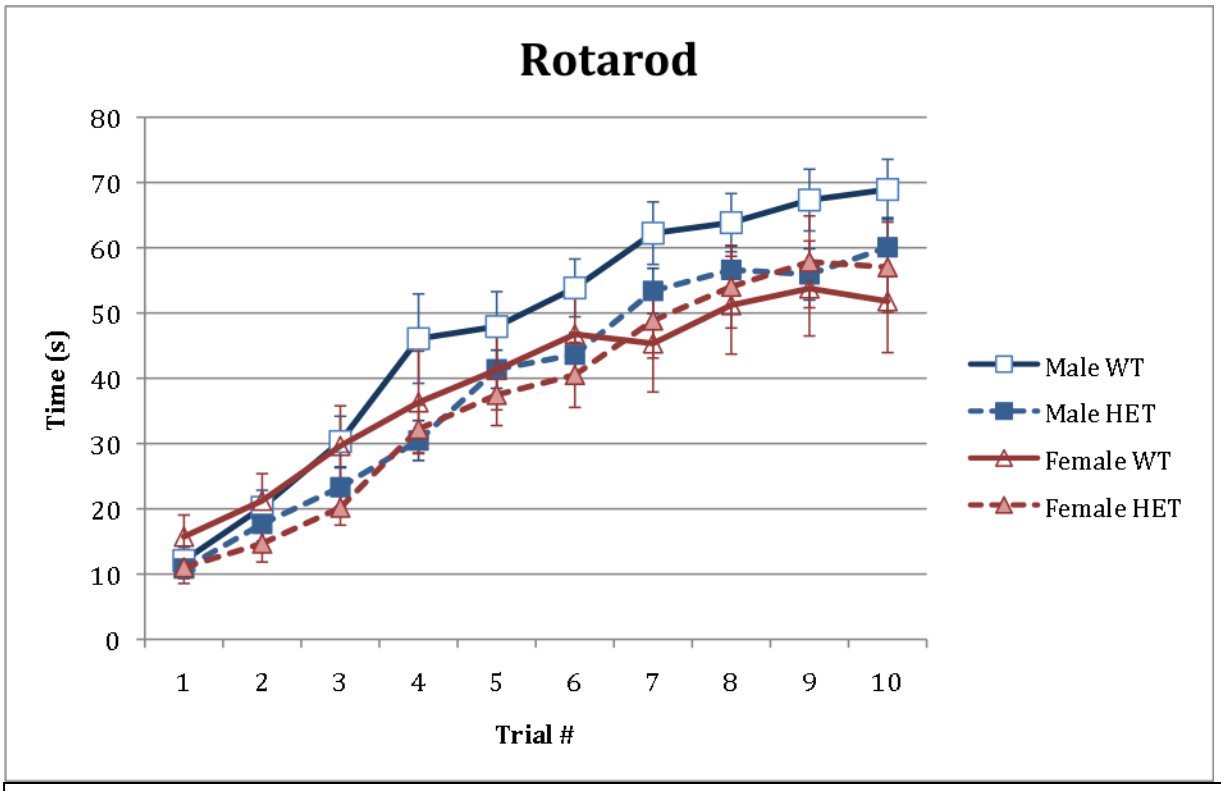


Figure 4. No difference in motor coordination between juvenile (30-day-old) *Pcdh10*$^{+/-}$ mice and wildtype littermates in motor coordination in the rotarod test. There does not appear to be a significant difference between *Pcdh10*$^{+/-}$ and WT in latency to fall off of an accelerating rotarod on repeated trials (N=13 WT males, 13 *Pcdh10*$^{+/-}$males, 9 WT females, 9 *Pcdh10*$^{+/-}$ females).