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A Novel *Chd8* Mutant Mouse Displays Altered Ultrasonic Vocalizations and Enhanced Motor Coordination

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Abstract

Mutations in *CHD8* are among the most common autism-causing genetic defects identified in human genomics studies. Therefore, many labs have attempted to model this disorder by generating mice with mutations in *Chd8*. Using a gene trap inserted after Exon 31, we created a novel *Chd8* mutant mouse (*Chd8^{+/E31T}*) and characterized its behavior on several different assays thought to have face validity for the human condition, attempting to model both the core symptoms (repetitive behaviors and social communication impairments) and common comorbidities (motor deficits, anxiety, and intellectual disability). We found that *Chd8^{+/E31T}* mice showed no difference compared to wild-type mice in amount of self-grooming, reproducing the negative finding most other studies have reported. Unlike some of the other published lines, *Chd8^{+/E31T}* mice did not show deficits in the three-chamber test for social novelty preference. A few studies have examined ultrasonic vocalizations in *Chd8* mutant mice, but we are the first to report an increase in call length for adult mice. Additionally, we found that in contrast to previous published lines, *Chd8^{+/E31T}* mice displayed no anxiety-like behaviors or learning impairments but showed paradoxically significant improvement in motor function. The inconsistencies in behavioral phenotypes in the *Chd8* mutant mice generated by different laboratories poses a

challenge for modeling autism spectrum disorder and preclinical studies in mice going forward and warrants further investigation into the molecular consequences of the different mutations in *Chd8* and the functional impact on behavior.

Lay Summary:

Several different mouse models carrying mutations in the *Chd8* gene have been created to study the effects of these autism-causing mutations in the laboratory. The current study characterizes a novel *Chd8* mutant mouse model as well as summarizes data from previously published *Chd8* mutant mice. The inconsistencies between different studies are concerning, but future research into the reasons why these inconsistencies occur may help us understand why patients with various mutations have different degrees of symptom severity.

Keywords

ASD; autism; *CHD8*; mouse behavior; mouse models

Introduction

Mouse models are valuable tools for preclinical investigation into the underlying pathophysiology of neurodevelopmental disorders and for testing potential therapies. Modeling a disorder that is defined by behavioral symptoms rather than biomarkers is challenging, but to model autism spectrum disorder (ASD), behavioral assays have been developed for mice that test for phenotypes resembling the core symptoms, restricted/repetitive behaviors, and impaired social communication [Crawley, 2012; Silverman, Yang, Lord, & Crawley, 2010]. Assays that test for phenotypes resembling common comorbidities, such as impaired motor function, intellectual disability, anxiety, epilepsy, and sleep disturbances, have also been designed and implemented in studies utilizing mouse models of ASD [Bey & Jiang, 2014]. However, the phenotypes reported in such studies are not necessarily consistent across different models of ASD, even between mouse models that have the same gene disrupted.

Mutations involving *CHD8*, a gene encoding a chromatin regulator, are among the most common genetic defects reported in studies of ASD, and the vast majority of them are assumed to be loss-of-function mutations [An et al., 2020; Barnard, Pomaville, & O’Roak, 2015; Bernier et al., 2014; Douzgou et al., 2019; Du et al., 2018; Li et al., 2017; Merner et al., 2016; Neale et al., 2012; O’Roak et al., 2012; O’Roak et al., 2012; Prontera et al., 2014; Smol et al., 2020; Stoleran, Smith, Chaubey, & Jones, 2016; Terrone et al., 2014; Wang et al., 2016; Wang, Liu, Gao, Wang, & Jiang, 2018; Wu et al., 2018; Yasin et al., 2019]. This has led to the development of several different mouse models, harboring mutations in different coding regions of the *Chd8* gene, that have been generated and characterized in recent years [Gompers et al., 2017; Jung et al., 2018; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018]. These studies report many conflicting behavioral phenotypes and the reasons for the discrepancies are unclear, but this issue is concerning for the field moving forward.

In the current study, we report a new model created using a gene trap after Exon 31 of *Chd8* that is predicted to result in a truncated protein product. This mutation is clinically relevant because multiple different truncating mutations in this region have been reported in the literature [Douzgou et al., 2019]. The current study adds to the growing resource of *Chd8* mouse models. In this paper, we also review the current published lines of *Chd8* mutant mice, propose some potential reasons for differing phenotypes, and suggest avenues for future research.

Methods

Animals and Genotyping

Embryonic stem cells (SIGTR ES cell line AY0061) containing the gene trap was ordered from the Mutant Mouse Resource & Research Center (www.mmrrc.org). These cells were then injected into 129/R1 blastocysts for germline transmission as described [Jiang et al., 1998; Pappas et al., 2017]. The offspring with the mutation were identified by genotyping with PCR (Forward primer: TATCTGGAAGATCAGGATATGTGG, reverse primer: AACTGTTACCCGTAGGTAGTCACG, conditions: 58°C annealing temperature for 33 cycles, product: mutant band at ~200 bp) and were subsequently backcrossed onto a C57BL/6J background for more than five generations. Mice were identified only by their cage card number and location of ear punch (the tissue taken for genotyping) until all data were collected and analyzed. All experiments were conducted with protocols approved by the Institutional Animal Care and Use Committee at Duke University.

Behavioral Phenotyping

Two independent cohorts of 8-week-old *Chd8*^{+/E31T} mice and their wild-type littermates were used to determine the behavioral phenotypes present in this novel line. For the first cohort, female C57BL/6J mice were crossed with male *Chd8*^{+/E31T} mice to produce a roughly equal distribution of wild-type and *Chd8*^{+/E31T} offspring. For the second cohort, female *Chd8*^{+/E31T} mice were crossed with male C57BL/6J mice to rule out a potential parental origin effect. There were no cohort effects, so the data were pooled together. Both male and female mice of both genotypes were tested on all assays except for adult ultrasonic vocalizations (USVs). There were no sex differences, except in body weight. For pup USVs, mice from Cohort 1 were intercrossed and the offspring were tested on postnatal Day 4 (P4). The numbers of mice in each group and order of behavioral tests are presented in Table 1. Mice were given at least 48 h between tests, with the exception of the accelerating rotarod and steady speed rotarod, which were performed on consecutive days, and the open field and grooming recording, which were performed on the same day. All handling of mice and scoring of behavior was done blind to genotype. Mice were housed 4–5 per cage with same-sex but mixed genotype littermates on a 14 h light and 10 h dark cycle with all testing done during the light cycle.

Three-chamber Test for Sociability and Social Novelty

Mice were tested in the three-chamber assay as described previously with a few modifications [Moy et al., 2004; Wang et al., 2016]. Testing was conducted in a white acrylic apparatus with no walls dividing the chambers and two stainless-steel wire-mesh

cages (10 cm diameter $\times$ 11 cm high; Rolodex, Oak Brook, IL) each stabilized with a 28 g steel sinker (Cabella, Oshkosh, NE) to prevent the cage from being repositioned. Age and sex-matched C3H/HeJ mice (Charles River) were used as nonaggressive conspecific social stimuli. The chambers and cages were all cleaned between each test mouse with LabSan 256CPQ solution (Sanitation Strategies LLC, Williamston, MI). Testing was divided into three phases. During each test phase, the cages were placed in the center of the two outer thirds of the chamber. The first phase began when a test mouse was placed into the center of the chamber and given free exploration of the apparatus, and the two cages contained nonsocial stimuli. After 10 min, the test mouse was removed and a C3H stimulus mouse was placed into one of the wire-mesh cages, replacing one of the nonsocial stimuli. The second phase (sociability) began with reintroduction of the test mouse into the center of the chamber. After 10 min, the test mouse was removed and a novel C3H mouse replaced the remaining nonsocial stimulus. The test mouse was then reintroduced into the chamber for the third phase (social novelty), which also lasted 10 min. All tests were filmed, and the digital videos were analyzed subsequently using EthoVision software (Noldus) that included the frequency and duration of contacts with each cage. Preference scores were calculated, where time spent with one stimulus was subtracted from the time spent with the other stimulus and divided by the total time spent exploring both stimuli. Positive scores indicate a preference for the novel social stimulus relative to the nonsocial or familiar social stimulus, negative scores reflect preference for the nonsocial or familiar stimulus, and scores approximating “0” indicate no preference.

Adult USVs

Male mice were given breeding experience with age-matched female mice for at least 4 days (the duration of the estrous cycle) and then were individually housed for at least 24 h. For testing, the male mice were placed in a sound-attenuating styrofoam chamber equipped with an externally polarized condenser CM16/CMPA microphone with a frequency range of 10–200 kHz (Avisoft Bioacoustics Inc.) suspended 20 cm above the floor of the chamber. After the test mouse acclimated for 10 min, an 8–10-weeks-old virgin female C57BL/6J mouse was added to the chamber and vocalizations were recorded for 5 min as waveform audio files using Avisoft-RECORDER. The files were analyzed later using Avisoft SASLab Pro software. Mice that did not call were excluded from analysis.

Pup USVs

Postnatal Day 4 (P4) pups were briefly separated from their nest and placed in the chambers described in the previous section, modified to include nesting material to keep the pups warm. Before testing, the mother was moved to a new cage to minimize disturbance from gradually removing pups. Vocalizations were recorded and analyzed as before, but the neonatal mice did not require any acclimatization time and vocalizations were recorded for 1 min immediately after separation from the nest. All pups called and were therefore included in analysis.

Self-grooming

Individual animals were placed into clean home cages without bedding and acclimated for 5 min. Mice were filmed for 10 min and then videos were later manually scored for time spent grooming.

Elevated Zero Maze

Mice were placed in a closed portion of the elevated zero maze and were allowed 5 min of exploration under dim (40–60 lux) illumination. Activity was measured by Ethovision XT 10 (Noldus) using a high-resolution camera suspended two meters above the maze. Tracking profiles generated by Ethovision were used to calculate time spent in open and closed portions of the maze.

Dark–light Emergence

Mice were placed into a dark side of a two-chamber apparatus (Med-Associates, St. Albans, VT) consisting of a dark chamber and a light chamber and were allowed 5 min to freely explore the dark (<2 lux) and heavily lit (>750 lux) chambers. Infrared diodes within the test chamber tracked the location and activity of the mouse throughout testing. The behaviors tracked by the Med-Associates software included the latency to enter the well-lit chamber and the time spent in each of the dark and light chambers.

Open Field Locomotion

Activity in the open field was measured for 1 h in an automated Omnitech Digiscan apparatus (AccuScan Instruments, Columbus, OH). AccuScan software calculated the total distance traveled and time spent in the center of the arena.

Rotarod

Performance on the rotarod (Med-Associates) was assessed over four trials lasting 5 min each. The intertrial interval was 30 min. In the accelerating condition, the rod started at four rotations per minute (rpm) and slowly increased to 40 rpm over the 5 min trial. In the steady-state condition, the rod maintained 32 rpm.

Operant Conditioning

Mice were food restricted until they reached approximately 85% of their original body weight. Individual operant conditioning chambers (Med-Associates) were equipped with a retractable lever and a liquid dipper that delivered a drop of sweetened condensed milk as a food reward. The Med-PC-IV program was used to create the reinforcement schedule and record the data. The paradigm consisted of 7 days of continuous reinforcement (one lever press earned one drop of sweetened condensed milk every time). Each session began with illumination of the chamber and presentation of the lever and ended with extinguishing the light and retracting the lever. Trials ended after either one of two conditions had been satisfied: 60 min had passed or the mouse had pressed the lever 100 times. We utilized this simple shaping task, rather than more complex discrimination learning tasks because we previously a profound deficit in the shaping task in another mouse model of ASD [Wang, Bey, et al., 2016].

Data Analysis

Graphs were produced, and statistical analysis was performed in GraphPad Prism 8. For the rotarod and operant conditioning data, a repeated-measures analysis of variance (ANOVA) was performed. For all other tests, an unpaired two-tailed *t* test was performed. Statistical significance was defined as $p < 0.05$.

Results

Generation of *Chd8*^{+/-E31T} Mice

The gene structure of *Chd8*, the location of mutations in mouse models previously generated in the literature [Gompers et al., 2017; Jung et al., 2018; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018], and the targeting vector used for the current study are depicted in Figure 1(a). Consistent with previously published lines, homozygous mutants were embryonic lethal, but heterozygous mutants, which are haploinsufficient for the full-length protein (Figure 1(b)) were used for subsequent behavioral phenotyping. No gross abnormalities were observed and there was no effect of genotype on body weight (Figure 1(c)). Reduced body weight has been reported in two previous lines of *Chd8* mutant mice [Platt et al., 2017; Suetterlin et al., 2018]. However, two other previously reported lines have no difference in body weight compared to wild-type littermates [Jung et al., 2018; Katayama et al., 2016].

***Chd8*^{+/-E31T} Mice Do Not Display Repetitive Self-grooming or Social Interaction Deficits but Display Altered Communication Through USVs**

Self-grooming is often elevated in mouse models of ASD and is thought to represent a repetitive behavior [Lewis, Tanimura, Lee, & Bodfish, 2007]. However, most lines of *Chd8* mutant mice are no different than wild-type mice on self-grooming assays [Gompers et al., 2017; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018]. One line of *Chd8* mutant mice has elevated rates of self-grooming, but only under certain conditions: it only occurs after prolonged social isolation and it only occurs in male mice [Jung et al., 2018]. Like previously published *Chd8* mutant mice, our line did not show elevated rates of self-grooming under our standard protocol (Figure 2(a)).

A commonly used assay to test for social deficits in mice is the three-chamber test for sociability and social novelty [Crawley, 2004]. Previous characterizations of *Chd8* mutant mice revealed no deficits in sociability on this assay [Gompers et al., 2017; Jung et al., 2018; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018]. Some groups reported a deficit in recognizing social novelty [Katayama et al., 2016; Platt et al., 2017], whereas other groups reported no changes in this phase of the three-chamber test [Gompers et al., 2017; Jung et al., 2018]. Our *Chd8* mutant mice perform statistically the same as wild-type in both the sociability (Figure 2(b)) and social novelty (Figure 2(c)) phases of the three-chamber test.

Another way to test for social communication abnormalities in mice is through recording USVs [Simola & Granon, 2019]. USVs in adult *Chd8* mutant mice have been reported by two previous groups and in both cases, there were no significant differences between

mutants and wild-type littermates [Gompers et al., 2017; Jung et al., 2018]. We observed no difference between groups in the number of calls made (Figure 2(d)). However, we report a novel finding of increased call duration in *Chd8*^{+/E31T} mice (Figure 2(e)).

In pups, we observed a similar phenotype. *Chd8* mutant pups had an increased number of calls compared to wild-type littermates (Figure 2(f)), but no significant difference in the average call length (Figure 2(g)). There was, however, a strong trend toward increased call length, with a borderline significant p value. Two previous studies of *Chd8* mutant mice reported pup USVs. One study reported a similar finding of increased numbers of USVs, but only under certain conditions: only male P5 pups showed this phenotype [Jung et al., 2018]. The other study reported no changes in pup USVs at any developmental stage [Suetterlin et al., 2018]. While both of these other studies examined pups at multiple ages, we decided to examine P4 pups because we previously reported a phenotype at this age in another mouse model of ASD [Wang, Bey, et al., 2016].

***Chd8*^{+/E31T} Mice Do Not Present With Any Anxiety-like Phenotypes, Unlike Some Previously Published Lines**

The latency to enter the light chamber in the light–dark emergence task, a measurement resembling anxiety-like behavior [Crawley & Goodwin, 1980], was only reported in one previous study on *Chd8* mutant mice, but the mice in this study showed increased latency to enter the light chamber [Platt et al., 2017]. We observed a similar trend in the *Chd8*^{+/E31T} mice, but the difference between the groups was not significant (Figure 3(a)). Additionally, two previous lines displayed decreased time spent in the light chamber during this task [Katayama et al., 2016; Platt et al., 2017], but our mice showed no significant difference between genotypes for this measurement (Figure 3(b)).

The elevated zero maze [Kulkarni, Singh, & Bishnoi, 2007] and the conceptually similar elevated plus maze have also been used to test for anxiety-like behavior in *Chd8* mutant mice. One previously reported line of *Chd8* mutant mice spends less time in the open arms of the elevated plus maze compared to wild-type littermates [Katayama et al., 2016]. However, another previously reported line of *Chd8* mutant mice is not significantly different from wild-type mice on this test [Jung et al., 2018]. The *Chd8*^{+/E31T} mice also does not show deficits in the amount of time spent in the open areas of the elevated zero maze (Figure 3(c)).

Likewise, some previous lines spend less time in the center of the open field compared to their wild-type littermates [Katayama et al., 2016; Platt et al., 2017], whereas others show no thigmotaxis [Jung et al., 2018; Suetterlin et al., 2018]. Our *Chd8*^{+/E31T} mutant mice show no difference in time spent in the center of the open field compared to their wild-type littermates (Figure 3(d)).

***Spontaneous Motor Activity and Reward Learning are Unchanged in Chd8*^{+/E31T} Mice, but These Mice Perform Better than Wild-types at the Rotarod Task**

There have been conflicting reports of previous lines of *Chd8* mice in their spontaneous motor activity in the open field. Some groups have reported hypoactivity in their lines [Jung et al., 2018; Platt et al., 2017; Suetterlin et al., 2018], whereas others have reported no

changes in activity [Gompers et al., 2017; Katayama et al., 2016]. Our mice did not display hypoactivity and instead trended toward hyperactivity in the open field, although this difference was not significant (Figure 4(a)).

Most of the previous studies characterizing behavior in *Chd8* mutant mice also used the rotarod to assess motor function and reported no genotype effect on this behavior [Jung et al., 2018; Katayama et al., 2016; Suetterlin et al., 2018]. One study reported an increase in motor performance in the *Chd8* mutant mice on the rotarod [Platt et al., 2017]. We found the *Chd8*^{+/*E31T*} mice perform significantly better than wild-type mice on this task, to an even greater extent than what was previously reported in the other line. *Chd8* mutant mice outperformed wild-type mice on both the accelerating rotarod (Figure 4(b)) and on a steady speed of 32 rpm condition (Figure 4(c)).

There have been conflicting reports on cognitive impairments in *Chd8* mutant mice. One study reported that their mice were impaired in fear conditioning [Gompers et al., 2017], whereas multiple other studies found no impairment on this test in different lines [Jung et al., 2018; Platt et al., 2017]. Two studies also found no difference between their *Chd8* mutant mice and wild-types on the Morris Water Maze [Jung et al., 2018; Suetterlin et al., 2018]. Finally, one study reported an impairment on reversal learning in the T-maze, but no impairments in T-maze discrimination or on the Barnes maze test [Katayama et al., 2016]. We instead used an operant conditioning task, on which other mouse models of ASD show severe impairments [Wang, Bey, et al., 2016], but the *Chd8*^{+/*E31T*} mice were not significantly different than their wild-type littermates in their performance on this test (Figure 4(d)).

Discussion

In the current study, we characterized a new line of *Chd8* mutant mice that share some, but not all, behavioral phenotypes that have been reported in other *Chd8* mutant mouse lines in the literature [Gompers et al., 2017; Jung et al., 2018; Katayama et al., 2016; Platt et al., 2017; Suetterlin et al., 2018]. A summary of the findings from all these studies, including the current one, is provided in Table 2 and the significant findings are emphasized in Table 3.

The lack of a strong repetitive behavior phenotype or social impairment illustrates the challenge of modeling ASD in mice using the available behavioral paradigms. Although at least one study on mouse models of ASD has considered increased rotarod performance to resemble a repetitive behavior [Rothwell et al., 2014], humans with ASD tend to show motor impairments, rather than improvements in motor function [Licari et al., 2020]. It is therefore surprising that our model showed improvements in motor function and these findings directly contradict what has been reported in several other mouse models of ASD [Bey & Jiang, 2014]. One potential explanation is that our mutation caused a gain-of-function due to a truncated protein product, but because similar mutations are found in human patients that have motor difficulties, it is still unclear. It is also unclear why multiple other *Chd8* mutant mice show no phenotype on the rotarod [Jung et al., 2018; Katayama et al., 2016; Suetterlin et al., 2018]. *Chd8* is expressed relatively highly in the cerebellum [Xu et al., 2018; Zhao et

al., 2018] and another study showed volumetric increases in parts of the cerebellum in *Chd8* mutant mice [Suetterlin et al., 2018], so it is possible that our mutation caused alterations in cerebellar circuitry, but the mechanism underlying this counterintuitive increase in motor coordination should be further explored.

Alterations in USVs could represent social communication impairments and our findings of increased call numbers and/or call length does indicate an abnormal pattern of USVs. It should also be noted that our sample size for adult USVs was somewhat limited, so the results should be interpreted with caution, but the consistency across multiple behavioral cohorts and the significant p values do increase our confidence in these findings. Regardless, the interpretation of these results and how they relate to social communication is not straightforward. Most studies involving mouse models of ASD report decreases in call length or call number on USV paradigms which perhaps more closely resembles human ASD patients who are nonverbal or have significant language impairments. However, at least for the pup USVs, our findings of increased call number could instead represent increased distress from maternal separation [Takahashi et al., 2009]. Still, the translational value of USV measurements is unknown at this time.

From a molecular perspective, the *Chd8*^{+E31T} mice closely mimic mutations that have been reported in the human literature [Douzgou et al., 2019]. However, from a behavioral perspective, this new line does not seem to model the human disorder well. One potential reason is that behavioral presentations of individuals with *CHD8* mutations are more complex than what is described as simply impairment in social communication and increased repetitive behaviors. Alternatively, human behavior could be more sensitive to smaller changes in gene dosage of *CHD8*. This is supported by other mouse models of ASD, where heterozygous mutants show little or no behavioral phenotypes, but homozygous mutants model the human disorder with strong face validity, even though human patients carry heterozygous mutations [Hulbert & Jiang, 2016; Wang, Bey, et al., 2016].

Nevertheless, conflicting findings from the different *Chd8* mutant lines raises an important question about the utility of behavioral analyses for mouse models in the field of autism research. Namely, why does disrupting the same gene in mice have such different consequences for different laboratories? One potential explanation is that the methods and testing logistics for assessing behavioral phenotypes are not completely identical. Stricter standards for behavioral methods must be implemented going forward or side-by-side comparisons of different lines within the same laboratory must be employed to completely rule out this possibility.

However, differences in methods for assessing behavioral phenotypes unlikely account for all the inconsistencies in the literature. Other factors may include the genetic background of mutant mice, husbandry practices, and differences in facilities. Another possibility is that disrupting various portions of the gene has different molecular consequences due to the presence of more than one isoform of *CHD8* both in humans and mice. More comprehensive data regarding the genotype to phenotype correlation in humans may help to understand this difference. It is known that *CHD8* has more than one mRNA isoform in both humans and mice [Lee et al., 2020]. However, the number of isoforms produced by multiple promoters

and alternative splicing of coding exons has not been fully investigated. Our lab previously demonstrated that another gene implicated in ASD has multiple intragenic promoters and alternatively spliced exons, leading to the production of many different isoforms [Wang et al., 2011; Wang, Xu, Bey, Lee, & Jiang, 2014]. This supports the need for a more thorough investigation of the transcriptional regulation of *CHD8* and how different mutations may disrupt different sets of isoforms, which could in turn lead to distinct clinical phenotypes in both humans and mice.

The variability in behavioral phenotypes between the different *Chd8* mutant lines also calls into question the generalizability of the cellular and molecular phenotypes reported in other studies. However, important discoveries have been made that indicate a possible mechanism linking *Chd8* deficiency to altered behavior, including delayed neurodevelopment [Katayama et al., 2016], myelination defects [Zhao et al., 2018], increased proliferation of neuronal progenitors [Gompers et al., 2017], increased total brain volume and volumetric increases of several brain regions [Suetterlin et al., 2018], and changes in inhibitory synaptic transmission [Jung et al., 2018]. Side-by-side comparisons of the different mutant lines could be informative after a more complete understanding of the transcriptional regulation of *CHD8* is obtained.

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Figure 1.

Generation of *Chd8*^{+/E31T} mice. (a) The gene structure of *Chd8* (NM_201637), along with the location of mutations for previously reported lines is depicted at the top of the figure. For the current study, a pGT0lrx gene trap vector was inserted after Exon 31 of *Chd8*. Intron 1 of *Engrailed2* (En2 intr1) and the following splice acceptor (SA) site are recognized by transcriptional machinery and as a result, *LacZ* (β geo) is incorporated into the immature mRNA. At the end of the β geo cassette are three stop codons which terminate translation of the mRNA, which forms a CHD8/ β -gal fusion protein. (b) Western blot shows a reduction in expression of the full-length CHD8 protein (~290 kDa) in *Chd8*^{+/E31T} (+/-) mice compared to wild-type (+/+) controls. (c) *Chd8* mutant mice are similar in body weight to their sex-matched wild-type littermates. There is no effect of genotype on body weight (two-way analysis of variance [ANOVA], effect of genotype, $F(1,45) = 0.05395$, $p = 0.8174$). Individual data points are superimposed onto means $\pm$ SEM. $n = 15$ +/+ males, 14 +/+ females, 8 +/- males (5 m+/p-, 3 m-/p+), and 12 +/- females (10 m+/p-, 2 m-/p+)

Figure 2.

Chd8^{+/E31T} mice show no differences in self-grooming or social interaction, but altered ultrasonic vocalizations as adults and as pups. (a) There was no significant difference in time spent grooming between *Chd8*^{+/E31T} mice and their wild-type littermates (unpaired *t* test, *t* = 0.1037, *df* = 47, *p* = 0.9179). (b) *Chd8*^{+/E31T} and wild-type mice display an equally strong preference for a social stimulus over a nonsocial stimulus (unpaired *t* test, *t* = 0.01031, *df* = 47, *p* = 0.9918). (c) *Chd8*^{+/E31T} and wild-type mice display an equally mild preference for a novel social stimulus over a familiar social stimulus (unpaired *t* test, *t* = 0.3929, *df* = 47, *p* = 0.6962). (d) There was no difference between *Chd8*^{+/E31T} mice and their wild-type littermates in the number of ultrasonic vocalizations (USVs) as adults (unpaired *t* test, *t* = 0.08294, *df* = 17, *p* = 0.9349). (e) As adults, *Chd8*^{+/E31T} mice had longer USV call lengths compared to their wild-type littermates (unpaired *t* test, *t* = 2.839, *df* = 17, *p* = 0.0113). (f)

As pups, *Chd8*^{+/E31T} mice called more frequently than their wild-type littermates (unpaired *t* test, *t* = 2.772, *df* = 29, *p* = 0.0096). (g) *Chd8* mutant mice and their wild-type littermates showed no difference in USV call length as pups, although there was a trend for mutant pups to have longer calls (unpaired *t* test, *t* = 1.838, *df* = 29, *p* = 0.0763). For all graphs, individual data points from each mouse are superimposed onto bar graphs with error bars representing means ± SEM. For self-grooming and three-chamber tests, *n* = 15 *+/+* males, 14 *+/+* females, 8 *+/-* males (5 *m+/p-*, 3 *m-/p+*), and 12 *+/-* females (10 *m+/p-*, 2 *m-/p+*). For adult USVs, *n* = 12 *+/+* males and 7 *+/-* males (4 *m+/p-*, 3 *m-/p+*). For pup USVs, *n* = 15 *+/+* and 16 *+/-* (10 *m+/p-*, 6 *m-/p+*)

Figure 3.

Chd8^{+/E31T} mice do not present with anxiety-like behavior. (a) Although there was a trend for the *Chd8*^{+/E31T} mice to exhibit increased latency to enter the light chamber in the light–dark emergence task, the difference between groups was not significant (unpaired *t* test, $t = 1.428$, $df = 47$, $p = 0.1599$). (b) There was no significant difference between *Chd8*^{+/E31T} mice and their wild-type littermates in time spent in the light chamber during the light–dark emergence task (unpaired *t* test, $t = 1.161$, $df = 47$, $p = 0.2517$). (c) In the elevated zero maze, *Chd8*^{+/E31T} mice and wild-type mice spend statistically equal amounts of time in the open areas (unpaired *t* test, $t = 0.8001$, $df = 47$, $p = 0.4277$). (d) In the open field, *Chd8*^{+/E31T} mice and wild-type mice spend statistically equal amounts of time in the center of the arena (unpaired *t* test, $t = 1.125$, $df = 47$, $p = 0.2663$). For all graphs, individual data points from each mouse are superimposed onto bar graphs with error bars representing means $\pm$ SEM. For all tests, $n = 15$ +/+ males, 14 +/+ females, 8 +/- males (5 m+/p–, 3 m–/p+), and 12 +/- females (10 m+/p–, 2 m–/p+)

Figure 4.

Chd8^{+/E31T} mice show strong improvements in motor function, but no differences in spontaneous activity or reward learning. (a) Although mutant mice tended to travel farther in the open field, the difference between genotypes was not significant (unpaired *t* test, $t = 1.335$, $df = 47$, $p = 0.1882$). (b) *Chd8*^{+/E31T} mice take significantly longer to fall off the rotarod in the accelerating (4–40 rpm) condition (repeated-measures analysis of variance [RMANOVA], effect of genotype $F(1,47) = 24.58$, $p < 0.0001$). (c) *Chd8*^{+/E31T} mice take significantly longer to fall of the rotarod in the steady speed (32 rpm) condition (RMANOVA, effect of genotype $F(1,47) = 27.98$, $p < 0.0001$). (d) *Chd8*^{+/E31T} mice perform statistically the same as wild-type mice on the operant conditioning task (RMANOVA, effect of genotype $F(1,45) = 0.3493$, $p = 0.5575$). For the rotarod and operant conditioning graphs, data represents means $\pm$ SEM. For open field activity, individual data points from each mouse are superimposed onto bar graphs with error bars representing means $\pm$ SEM. For rotarod and open field tests, $n = 15$ +/+ males, 14 +/+ females, 8 +/- males (5 m+/p-, 3 m-/p+), and 12 +/- females (10 m+/p-, 2 m-/p+). For operant conditioning, $n = 15$ +/+ males, 12 +/+ females, 8 +/- males (5 m+/p-, 3 m-/p+), and 12 +/- females (10 m+/p-, 2 m-/p+).

Table 1.

Order of Behavioral Tests for Experimental Cohorts and Number of Mice Per Group

<i>Chd8</i> ^{m+/p-} Cohort	<i>Chd8</i> pup cohort	<i>Chd8</i> ^{m-/p+} cohort
+/, <i>n</i> = 19	+/+, <i>n</i> = 15	+/+, <i>n</i> = 10
+/-, <i>n</i> = 15	+/-, <i>n</i> = 16	+/-, <i>n</i> = 5
Open field	Pup USVs	Light-dark emergence
Grooming		Elevated zero maze
Elevated zero maze		Accelerating rotarod
Light-dark emergence		Steady speed rotarod
Accelerating rotarod		Open field
Steady speed rotarod		Grooming
Operant conditioning		Three chamber
Three chamber		Operant conditioning
Adult USVs (males only)		Adult USVs (males only)

Abbreviation: USV, ultrasonic vocalization.

Table 2.

Summary of Behavioral Phenotyping of *Chd8* Mutant Lines

Reference	Mutation	Sex, age, and genetic background of mice tested	Repetitive behavior	Social behavior	USV's	Anxiety-like behavior	Motor function	Learning and memory	Other
Current study	Gene trap after Exon 31	Male + female, except only males for adult USV's 8–12 weeks, except for pup USV's at P4 C57Bl/6J	↔ Self-grooming ($n = 20-29$)	↔ Three-chamber sociability ($n = 20-29$) ↔ Three-chamber social novelty ($n = 20-29$)	↔ Call number in adults ($n = 7-12$) ↑ Call length in adult (male) USV's ($n = 7-12$) ↑ Call number in P4 USV's ($n = 15-16$) ↔ Call length in P4 USV's ($n = 15-16$)	↔ Center time in open field ($n = 20-29$) ↔ Latency to enter light in LDE ($n = 20-29$) ↔ Time in light chamber in LDE ($n = 20-29$) ↔ Time in open arms of EZM ($n = 20-29$)	↑ Accelerating rotarod ($n = 20-29$) ↑ Steady speed rotarod ($n = 20-29$) ↔ Activity in open field ($n = 20-29$)	↔ Operant conditioning ($n = 20-27$)	↔ Body weight ($n = 23-26$)
Jung et al. (2018)	Frameshift knock-in at Exon 37	Male + female for pup and juvenile behaviors, males only for adult behaviors P5, P7, P9, and P11 for pup USV's P19 for maternal attachment P21–26 for open field, juvenile play, repetitive behaviors 8–9 weeks for isolation-induced grooming, Age for other tests in adulthood not specified. C57Bl/6J	↑ Self-grooming after social isolation in males only ($n = 24-26$) ↔ Self-grooming under standard conditions in juveniles ($n = 17-22$) ↔ Digging or jumping behavior in juveniles ($n = 17-22$)	↑ Maternal attachment at P19 in males only ($n = 14-22$) ↔ Juvenile play duration ($n = 6-11$ pairs) ↔ Three-chamber sociability ($n = 15$) ↔ Three-chamber social novelty ($n = 15$) ↔ Duration of contacts in direct social interaction ($n = 6$ pairs) ↔ Nest building ($n = 6$ cages)	↑ Call number in P5 USV's in males only ($n = 31-38$) ↔ Call number in P7, P9, or P11 USV's ($n = 31-38$) ↓ Latency to first call in P7, P9, and P11 USV's in males only ($n = 31-38$) ↔ Latency to first call in P5 USV's ($n = 31-38$) ↔ Call number in adults ($n = 13-14$) ↔ Call duration in adults ($n = 13-14$)	↔ Center time in open field for juveniles ($n = 34-40$) ↔ Time spent in open arms of EPMS ($n = 12$) ↔ Time in light chamber in LDE ($n = 12$)	↓ Activity in open field for juveniles ($n = 34-40$) ↔ Accelerating rotarod ($n = 12$)	↔ Novel object recognition ($n = 12$) ↔ Morris Water Maze acquisition ($n = 12$) ↔ Morris Water Maze reversal ($n = 12$) ↔ Contextual fear conditioning ($n = 9$)	↑ Brain volume ($n = 30$) ↔ Body weight ($n = 7-19$) ↔ Tail suspension test ($n = 12$)
Suetterlin et al. (2018)	Deletion of Exon 4 via Cre-loxP gene targeting	Male + female 9–22 weeks, except for motor development and USV's assessed in pups at P2, P4, P6, P8, and P12 C57Bl/6J	↔ Self-grooming ($n = 29-30$) ↔ Marble burying ($n = 29-30$)	↔ Three-chamber sociability ($n = 29-30$) ↑ Duration of contacts during direct social interaction ($n = 29-30$)	↔ Call number at P2, P4, P6, P8, and P12 ($n = 31-42$)	↔ Center time in open field ($n = 29-30$) ↔ Time in light chamber in LDE ($n = 29-30$)	↓ Righting reflex P2-P12 ($n = 31-42$) ↑ Locomotion at P12 ($n = 31-42$) ↓ Activity in open field ($n = 29-30$) ↓ Running wheel activity ($n = 29-30$)	↔ Morris Water Maze acquisition ($n = 29-30$) ↔ Morris Water Maze reversal ($n = 29-30$)	↑ Brain volume ($n = 11-12$) ↓ Body weight ($n = 27-46$) ↑ Brain weight at P35 ($n = 27-46$) ↑ Brain weight at P0 and P7 ($n = 14-18$)

Reference	Mutation	Sex, age, and genetic background of mice tested	Repetitive behavior	Social behavior	USVs	Anxiety-like behavior	Motor function	Learning and memory	Other
Gompers et al. (2017)	CRISPR/Cas9-mediated frameshift deletion in Exon 6	Male + female, except only males for direct social interaction and USVs 6–16 weeks C57Bl/6N	↔ Self-grooming ($n = 20$) ↔ Marble burying ($n = 20$)	↔ Three-chamber sociability ($n = 19–20$) ↔ Three-chamber social novelty ($n = 19–20$) ↔ Duration of contacts during direct social interaction ($n = 9–10$)	↔ Call number ($n = 9–10$)	NR	↔ Activity in open field ($n = 20$) ↔ Accelerating rotarod ($n = 29–30$)	↓ Contextual fear conditioning ($n = 19–20$) ↓ Tone fear conditioning ($n = 19–20$) ↓ Novel object recognition ($n = 19–20$)	↑ Brain volume ($n = 18–19$)
Platt et al., 2017	CRISPR/Cas9-mediated frameshift deletion in Exon 2	Male only 10–14 weeks, except for juvenile play at P23–25 C57Bl/6J	↔ Self-grooming ($n = 17$) ↔ Marble burying ($n = 23–25$)	↔ Number of contacts during juvenile play ($n = 15–17$) ↑ Duration of contacts during juvenile play ($n = 15–17$) ↔ Three-chamber sociability ($n = 20–24$) ↓ Three-chamber social novelty ($n = 20–24$)	NR	↓ Center time in open field ($n = 55–64$) ↑ Latency to enter light chamber in LDE ($n = 19–25$) ↓ Time in light chamber in LDE ($n = 19–25$)	↑ Accelerating rotarod ($n = 10$) ↓ Activity in open field ($n = 55–64$)	↔ Contextual fear conditioning ($n = 16–21$) ↔ Tone fear conditioning ($n = 16–21$)	↓ Body weight ($n = 58–64$) ↑ Intraocular distance ($n = 8$) ↑ Brain volume ($n = 8$)
Katayama et al. (2016)	Deletion of Exons 12–14 (“L”) AND Deletion of Exons 2–10 (“SL”)	Male only 12–50 weeks C57Bl/6J	Self-grooming ($n = 40$)	↔ Nest building ($n = 40$) ↔ Three-chamber sociability ($n = 40$) ↓ Three-chamber social novelty ($n = 40$) ↓ Number of contacts in direct social interaction ($n = 40$) ↑ Duration of contacts in direct social interaction ($n = 40$) ↓ Duration of “active” contacts in direct social interaction ($n = 40$)	NR	↓ Center time in open field ($n = 40$) ↓ Time in light chamber in LDE ($n = 40$) ↓ Time in open arms of EPM ($n = 40$)	↔ Accelerating rotarod ($n = 40$) ↔ Wire hang ($n = 40$) ↔ Activity in open field ($n = 40$)	↔ T-maze discrimination ($n = 19–20$) ↓ T-maze reversal ($n = 19–20$) ↔ Barnes maze test ($n = 40$)	↑ Brain weight ($n = 15$) ↔ Body weight ($n = 15$) ↔ Forced swim test ($n = 40$) ↔ Hot plate reaction ($n = 40$) ↓ Intestine length ($n = 30$) ↓ Startle response, ↑ PPI ($n = 40$)

Note: ↑ represents a significant increase in a given behavior, whereas ↓ represents a significant decrease. ↔ represents no change or a nonsignificant difference in the reported behavior.

Abbreviations: EPM, elevated plus maze; EZM, elevated zero maze; LDE, light–dark emergence; USVs, ultrasonic vocalizations.

Table 3.Representation of Significant Findings from *Chd8* Behavioral Phenotyping

Reference	Self-grooming	Three-chamber sociability	Three-chamber social novelty	Adult USVs	Pup USVs	Latency to enter light chamber in LDE	Time spent in light chamber in LDE	Elevated zero or elevated plus maze	Cognitive tests (FC, MWM, or OC)	Rotarod	Open field activity	Open field center time	Body weight
Katayama et al. (2016)				NR	NR	NR							
Platt et al. (2017)				NR	NR			NR					
Gompers et al. (2017)					NR	NR	NR	NR		NR		NR	NR
Suetterlin et al. (2018)			NR	NR		NR		NR					
Jung et al. (2018)	^a				^a	NR							
Current study													

Note: Cyan represents a significant decrease in a given behavior, whereas magenta represents a significant increase. Gray boxes represent no change or a nonsignificant difference in the reported behavior. White boxes represent a behavior that was not reported in the manuscript.

Abbreviations: EPM, elevated plus maze; EZM, elevated zero maze; LDE, light-dark emergence; USVs, ultrasonic vocalizations.

^aThese phenotypes were only present under certain conditions (e.g., in only male mice, after stress).