Elsevier Editorial System(tm) for Behavioural Brain Research

Manuscript Draft

Manuscript Number: BBR-D-16-00860R1

Title: Genetic control of social behavior: lessons from mutant mice

Article Type: SI:Development of Attachment

Keywords: Gene; environment; social behavior; autism; neurodevelopmental disorder; mouse

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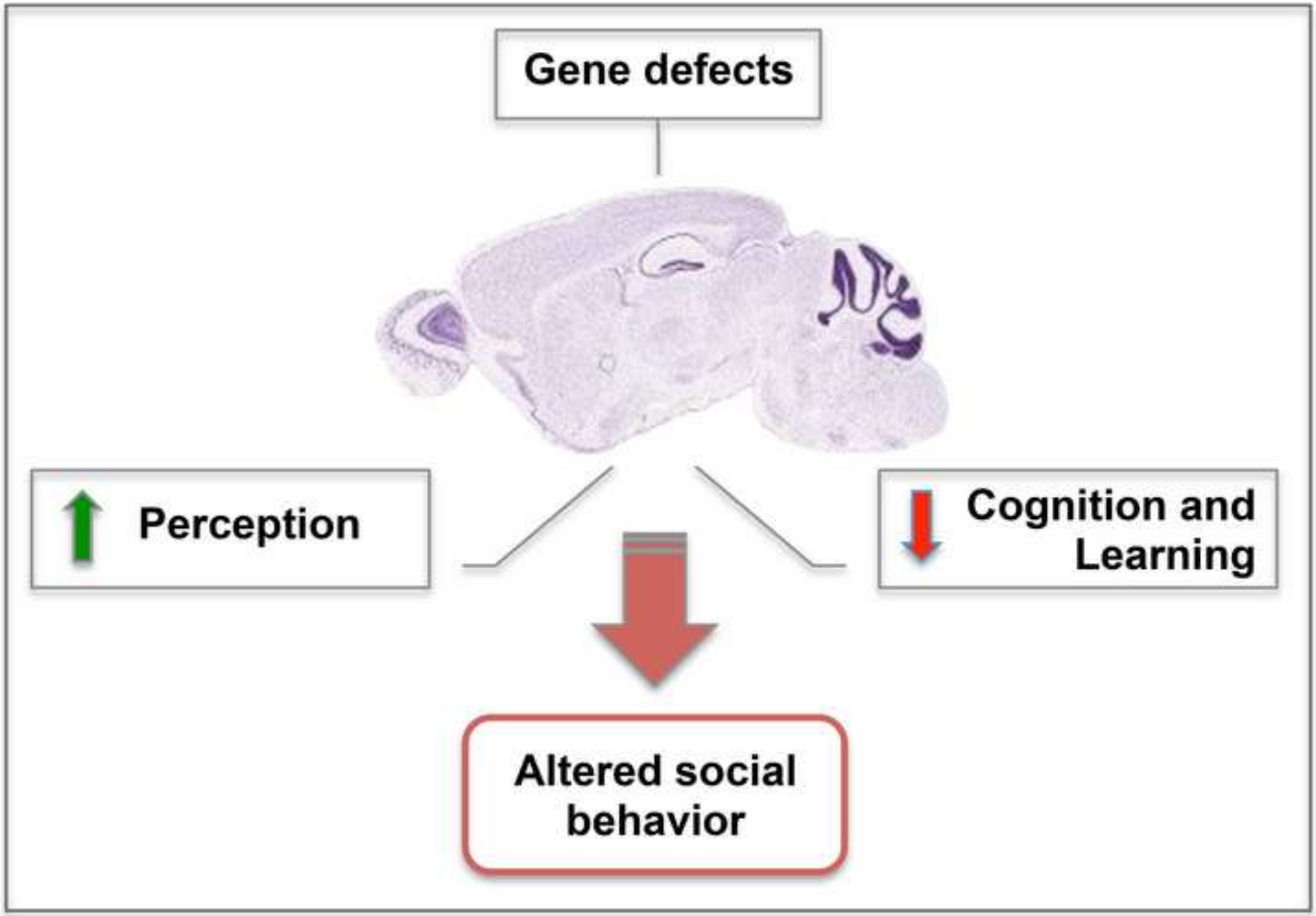
Abstract: Social behavior is evolutionary conserved, and is thought to be evolved since it increased reproductive and survival fitness of living species. In humans, disturbances of social behavior are a peculiar pathological trait of neurodevelopmental disorders, namely autism spectrum disorders (ASD). ASD are defined by deficits in two core domains (social interaction/communication and repetitive/restrictive behaviors), which emerge during early postnatal development. ASD have a strong genetic component: copy number variations, de novo and familial mutations, as well as epigenetic modifications have been reported in a huge number of genes. Recent studies in mice demonstrate that mutations in a wide variety of ASD-associated genes can cause neurodevelopmental defects, which subsequently result in social behavior disturbances during early postnatal age and adulthood. From these studies, it clearly emerges that functionally interrelated cellular mechanisms underlie social behavior and its disturbances in ASD. Indeed, most of ASD-associated genes control neuronal differentiation and migration, growth of neuronal connections and synaptic function. Here we will present the recent advances in understanding the genetic determinants of social behavior, as they emerge from the study of ASD mouse models, and discuss the importance of these studies for the development of novel therapeutic approaches to overcome social disturbances in ASD.

Graphical Abstract



Highlights

- Altered social behavior is a pathological trait autism spectrum disorder (ASD)
- In mice, mutations in ASD genes result in altered social behavior
- Mouse models are a crucial tool to develop novel treatments for ASD

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Trento, October 30, 2016

Prof. Gianluca Esposito
Editor, Behavioural Brain Research
Special Issue "Development of Attachment"

Dear Gianluca,

please find attached the revised version of our Invited Review BBR-D-16-00860_R1 entitled "Genetic control of social behavior: lessons from mutant mice" that Giovanni Provenzano, Gabriele Chelini and myself are submitting for publication in the BBR Special Issue "Development of Attachment".

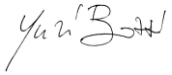
We would like to thank you both reviewers for their constructive comments about our first version of the manuscript. In this revised version, we have addressed all comments by Reviewer 3 and added a few references, as detailed in our point-to point response (see below). All changes have been are in red in our revised version. In addition, we have included a Graphical Abstract (which was absent in our previous version) and we slightly modified Figure 2 (we changed the backgound color of the brain image) to render it more suitable for printout or web reproduction.

We believe that our revised manuscript is now very much improved and suitable for publication in this Special Issue of BBR.

Looking forward to having your positive feedback soon,

best regards,

Yuri



Response Reviewers' comments:

Reviewer #2: *The manuscript by Provenzano, Chelini and Bozzi provides a very good overview on the genetic basis of social behavior in ASD mouse models. The paper deals with all the most recent advances in the field including the role of hyperexcitability, low cognitive elaboration and synaptic dysfunction, and the most common behavioral assay to study the phenotyping of rodent ASD models. The paper is very clearly written and comprehensive and it is suitable for publication in BBR journal.*

R: We thank this reviewer for her/his very positive comments.

Reviewer #3: *This manuscript by Bozzi and colleagues nicely summarizes the current state of analysis of mouse models of ASD. The manuscript would be improved by incorporating the following specific points.*

R: We thank this reviewer for her/his constructive comments. We have addressed all these concerns in our revised version.

1) The assertion that most ASD genes regulate neuronal differentiation and migration, growth of neuronal connections and synaptic functions is not new and has an interpretative pitfall. It is one thing to say mutation of a gene causes a certain neuronal phenotype, but it is quite another to claim that that is the cause of ASD. The argument that LTP, LTD and E/I deficits have anything to do with social behavior deficits has little solid basis. These neuronal phenotypes are just correlates and could be epiphenomena.

R: we agree with this reviewer that the assertion that most ASD genes regulate neuronal differentiation/ migration, growth of neuronal connections and synaptic functions is not new. Indeed, in our review we acknowledge that this concept is not novel (page 5, beginning of paragraph 4). To make it even clearer, we now state (page 5, paragraph 3) that "As expected, and already reported in previous review studies [5], many of the corresponding mutated or deleted genes encode proteins relevant for synaptic formation".
As regarding the interpretative pitfall, we agree with this reviewer that it is important to distinguish between the fact that a mutation causes a certain neuronal phenotype, and the claim that a mutation is the cause of ASD. Nevertheless, it is very well known that synaptic genes (e.g. Neuroligin 3, 4, Shank3) are mutated in hereditary cases of ASD. The crucial point is to understand how ASD may arise from disruption of synaptic function following such mutations. And this is the exact goal of this review. As an example, at the beginning of paragraph 4 we state that "we will discuss how such mutations affect basic neurophysiological mechanisms at the cellular level, thus modifying the response of brain circuits involved in the expression of social behaviors".
As regarding the argument that LTP, LTD and E/I deficits have anything to do with social behavior deficits, we slightly disagree with this reviewer. Certainly these neuronal phenotypes just correlate with social behavior deficits, and could be epiphenomena. This is certainly true for human ASD, in which a causal relationship between these deficits and the disease condition is very difficult, if not impossible, to be established. Nevertheless, as we state and discuss at the end of paragraph 4.2 ("Mouse models of ASD offer the possibility to directly test this hypothesis…" and following sentences), mouse models offer the real possibility to alter E/I balance and see whether such alteration results in ASD-like phenotypes in mice.

2) The term "social behavior" is too broad to be relevant to ASD. In mice, three-chamber sociability, natural reciprocal social interaction, nest building and whisker trimming and other tasks have been used, but not all readouts are relevant to ASD.

R: in our revised version (beginning of paragraph 2) we address this point and acknowledge that not all social tests in mice are relevant to ASD. Indeed, we have restricted our analysis only to "the most relevant types of assays for detecting social interaction abnormalities, which ensure a pure measure of simple interest in reciprocal approach excluding sexual, mating, aggressive, agonistic and parental care components, and whose readouts are considered to be relevant for ASD [6]" (see paragraphs 2.1-2.4 and Table 1).

First, cohort to cohort variation is noted in data coming from the three-chamber apparatus and parametric analysis should not be applied, but many studies did not follow this advice. Their statistical analyses do not provide a solid conclusion. Second, "sociability" does not reflect reciprocal social interaction. ASD patients have social curiosity, but they are not skilled in reciprocity of social interaction. It is not clear if the three chamber task is sensitive enough to detect defective reciprocal social interaction. In fact, there are many studies of genetic ASD models that show deficits in reciprocal social interaction, but not in the three-chamber "sociability" task.

R: we agree with this reviewer that results from the three-chamber test may have a high variability, and we added a sentence to highlight this point (paragraph 2.2). In addition, we now state that "it is not clear if this test is sensitive enough to detect defective reciprocal social interaction, since many studies of genetic ASD models show deficits in reciprocal social interaction, but not in the three-chamber test (Table 1)". Finally, we removed the first sentence of paragraph 2.2 (which defined the three chamber test as the "the most sensitive test for assessing social approach behavior in mice").

3) Instead of just repeating the SFARI site for a list of mouse models of ASD, the authors might want to provide their own evaluation of all models.

R: this was exactly the aim of our review. We used the SFARI list (which contains several hundreds of ASD-related genes and respective mouse models) and identified only those models that were studied for a subset of ASD-relevant social behaviors. As described in paragraphs 2.1-2.4 and 3, we used the SFARI repository in order to compile our own list of ASD genetic mouse models displaying social behavior deficits (as defined by impaired performance in reciprocal social interactions, 3-chamber, partition or social preference test). A total number of seventy-four ASD mouse models showing impaired social behavior are listed in Table 1.

4) The c-fos mapping study (Kim et al., 2015) should be more cautiously discussed, because c-fos is not a genuine maker of neuronal activity.

R: we have smoothened our discussion of data provided by Kim et al. (2015), by adding some references to study that indicate that c-fos induction may not correlate with neuronal activity (page 5).

5) The extensive screening of morphological abnormalities of genetic mouse models of ASD (20) should also be discussed

more carefully or omitted entirely. Such a massive screening has many issues. First, perfusion was done by the investigators of each mouse line and scanning by the investigators in Toronto. Even if the same perfusion protocol is followed, considerable variation among individual mice, labs and investigators is expected. Second, some mouse strains were not even congenic (16p11.2), making it impossible to rule out the confounding effects of genetic background. Third, the controls of mutant mice used in this analysis were not their own wild-type littermates, thus making it unclear if the observed anatomical phenotype truly reflects the ASD-related gene mutation or genetic backgrounds of different strains of mice. This is the worst kind of blind screening that adds yet another unreliable data to the literature

R: we agree with this reviewer that the study by Ellegood et al. [ref. 20] have some weak, crucial points, and we modified our discussion of this study by incorporating the reviewer's concerns (page 6 of our revised version).

6) Some of the "mouse models of ASD" might not be real models of ASD. For example, BTBR mice have no legitimate control to compare to and this mouse may not really impaired in social behaviors per se (Pobbe et al., 2011; Defensor et al., 2011).

R: we agree with this reviewer and we discuss this point at page 6; we also added the two references suggested by the reviewer.

7) ASD should be treated as a singular noun.
R: text has been corrected accordingly.

Genetic control of social behavior: lessons from mutant mice

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Abbreviations

ASD, autism spectrum disorders; BTBR, BTBR T^{+} *Itpr3tf/J* mouse strain; cKO, conditional knockout; CNS, central nervous system; CNTNAP2, contactin-associated protein-like 2; DREADD, designer receptors exclusively activated by designer drugs; DSM-5, Diagnostic and Statistical Manual of Mental Disorders, 5th edition; EEG, electroencephalogram; *En*, engrailed; *Fmr1*, Fragile X mental retardation gene 1; GABA, gamma-aminobutyric acid; GO, gene ontology; HE, hemizygous; HT, heterozygous; HM, homozygous; KI, knockin; KO, knockout; LTD, long term depression; LTP, long term potentiation; Mecp2, methyl-cytosine binding protein 2 ; mPFC, medial prefrontal cortex; mTOR, mammalian target of rapamycin; Nlgn, neuroligin; Nrxn, neurexin; Oxt, oxytocin; Oxtr, oxytocin receptor; PFC, prefrontal cortex; PV, parvalbumin; Scn1a, sodium channel 1a; SFARI, Simon Foundation Autism Research Initiative; Tg, transgenic; Tsc, tuberous sclerosis complex; WT, wild-type.

Abstract

Social behavior is evolutionary conserved, and is thought to be evolved since it increased reproductive and survival fitness of living species. In humans, disturbances of social behavior are a peculiar pathological trait of neurodevelopmental disorders, namely autism spectrum disorder (ASD). ASD is defined by deficits in two core domains (social interaction/communication and repetitive/restrictive behaviors), which emerge during early postnatal development. ASD has a strong genetic component: copy number variations, de novo and familial mutations, as well as epigenetic modifications have been reported in a huge number of genes. Recent studies in mice demonstrate that mutations in a wide variety of ASD-associated genes can cause neurodevelopmental defects, which subsequently result in social behavior disturbances during early postnatal age and adulthood. From these studies, it clearly emerges that functionally interrelated cellular mechanisms underlie social behavior and its disturbances in ASD. Indeed, most of ASD-associated genes control neuronal differentiation and migration, growth of neuronal connections and synaptic function. Here we will present the recent advances in understanding the genetic determinants of social behavior, as they emerge from the study of ASD mouse models, and discuss the importance of these studies for the development of novel therapeutic approaches to overcome social disturbances in ASD.

Keywords

Gene; environment; social behavior; autism; neurodevelopmental disorder; mouse

1.Introduction

Social behavior is defined as interactions among individuals, normally within the same species, which usually provide mutual benefits to all individuals involved. Social behavior likely evolved because it increased the individual's fitness, providing the species with a higher chance to reproduce and survive [1]. Deciphering the genes that influence the development and function of brain circuits mediating social behavior represents a fascinating and complex challenge.

In humans, disturbances of social behavior are a characteristic trait of developmental disorders of the central nervous system (CNS), namely autism spectrum disorder (ASD). ASD is a group of complex neurodevelopmental disorders whose diagnosis is exclusively made on a behavioral basis. According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), ASD is defined by deficits in two core domains (social interaction and communication, and repetitive/restrictive behaviors), which emerge in early phases of postnatal development [2].

It has long been known that ASD has a very high degree of heritability. However, only the recent advances in genetics and genomics have allowed the identification of an increasingly high number of gene variants associated to ASD: copy number variations, de novo and familial mutations, epigenetic modifications have been reported in candidate as well as novel genes [3-5]. Strong evidence indicates that functionally interrelated cellular mechanisms underlie the disorder. Indeed, most of ASD-associated genes code for proteins involved in synaptic signaling, transcriptional/post-transcriptional mechanisms and cell adhesion, which crucially operate in CNS development, controlling the birth and differentiation of neuronal subtypes, neuronal migration, synapse formation and maintenance of neuronal connections [3,5]. Thus, knowledge from genetic studies of ASD has provided new insights to enhance our understanding of the molecular mechanisms underlying social behavior abnormalities.

Most of these data resulted from the analysis of mouse models of ASD. The mouse (*Mus musculus*) is a highly social species, which shows a wide variety of social behaviors (such as reciprocal interactions, play, territorial marking, aggressive behaviors, sexual behaviors and parental cares). These peculiar behavioral traits pushed many research laboratories to investigate social behavior disturbances in mice bearing mutations or deletions of ASD-associated genes. Several ASD mouse models have been generated, providing a powerful tool to investigate the molecular, neuroanatomical and behavioral consequences of gene dysfunction in ASD. From these studies, it clearly emerges that social behavior disturbances in ASD have marked genetic determinants of neurodevelopmental origin. Another important asset of these studies is the possibility to test novel treatments to rescue social and other ASD-related behaviors in the laboratory animal [6,7].

In this review we will discuss recent findings from ASD mouse models, which greatly contributed to unravel the genetic basis of social behavior. We will focus on specific sets of genes involved in neural development and synaptic activity, describing how they may contribute to shape the structure and function of brain areas involved in social behavior. Finally, we will provide examples of ASD gene mutations resulting in altered social behavior in mice, and discuss the importance of these studies for the development of novel therapeutic approaches for ASD.

2. Behavioral assays to study social interactions in mice

Investigating social behavior in mice entails a constellation of behavioral assays, which have been used to unravel social disturbances in ASD mouse models; however, not all readouts of these tests are relevant to ASD. Here, we will focus only on the most relevant types of assays for detecting social interaction abnormalities, which ensure a pure measure of simple interest in reciprocal approach excluding sexual, mating, aggressive, agonistic and parental care components, and whose readouts are considered to be relevant for ASD [6]. In sections 2.1-2.4 we will briefly describe these assays (reciprocal social interactions, three-chamber, partition and social preference tests). For a detailed discussion of these experimental approaches, the reader is referred to the comprehensive review by Silverman et al. [6].

2.1 Reciprocal social interactions

This test is straightforward and conducted in an open field or home cage environment, where two unfamiliar mice are placed together. Parameters relevant to social interactions such as approaching, following, sniffing, climbing and allogrooming are routinely evaluated using a video-tracking system [6].

2.2 Three-chamber social approach test

In this test, the subject mouse is placed in a plexiglas box partitioned into three chambers. One side chamber contains an unfamiliar mouse (target) enclosed in a small wire cage with widely spaced wire bars, which permit visual, olfactory, auditory and some tactile contact but prevent aggressive and sexual interactions. The opposite side chamber contains the novel object (an inverted wire pencil cup), while the center chamber is completely empty. During the test, the subject mouse is placed in the center chamber, and given the choice to spend time with the non-social novel object or target mouse. Measures are taken of the amount of time spent in entries into each side of the test box, as well as time spent sniffing the wire cages [8]. It should be pointed out that a certain cohort to cohort variation has been noted in data coming from the three-chamber test. Moreover, it is not clear if this test is sensitive enough to detect defective reciprocal social interactions, since some studies of genetic ASD models show deficits in reciprocal social interactions, but not in the three-chamber test (Table 1).

2.3 Partition test

In this test, two unfamiliar mice are placed into a standard cage divided in two compartments by a perforated transparent partition, allowing the subject mouse to see, hear, and smell the target mouse through the holes in the plastic transparent divider precluding any direct physical contact. Time spent at the partition and numbers of approaches represent the amount of interest in the social partner [9].

2.4 Social preference test

Social preference is a useful assay to evaluate social affiliation, social recognition and social memory in mice. The degree of sociability is scored the animal's preference for social novelty, defined as the amount of time spent by the subject mouse with a novel over a familiar mouse. This test can be performed in a three-chamber, Y-maze or partition test apparatus [6].

3. Genes involved in social behavior

Most of genetic mouse models of ASD have been extensively tested in many of the behavioral paradigms mentioned in sections 2.1-2.4, with the aim to assess social interaction deficits. Research focusing on genes mediating social behavior in mice may help identify molecular mechanisms essential for normal social interaction. Here, we took advantage of the Simons Foundation Autism Research Initiative (SFARI) repository (https://gene.sfari.org/), in order to compile a list of all known ASD genetic mouse models displaying social behavior deficits. A total number of seventy-four ASD mouse models showing impaired social behavior are listed in Table 1. As expected, and already reported in previous review studies [5], many of the corresponding mutated or deleted genes encode proteins relevant for synaptic formation (neurexins, neuroligins, shanks, reelin and contactins) [5,10]. Moreover a number of genes are involved in conserved pathways such as protein synthesis, transcriptional/epigenetic regulation and neurotrasmission [5,11].

To explore more in detail the biologic processes mediated by these seventy-four ASD-associated genes, a gene-network analysis has been performed. Functional annotation using Gene Ontology (GO) categories shows that the most significantly enriched disease and mouse phenotypes respectively are autism spectrum disorder and abnormal social investigation/interaction, as expected (Fig.1A, B). The most represented GO molecular function categories are neurexin family proteins, cell adhesion molecules, kinase binding and glutamate receptor binding (Fig.1C). Most importantly, pathway analysis shows a statistically significant over-representation of key neurobiological processes such as BDNF signaling pathway, neurotransmitter release cycle, calcium signaling and mTOR pathway (Fig.1D). This suggests that genes involved in synaptic function, neurotransmission and intracellular signaling cascades, are crucially involved in shaping the structure and function of brain areas involved in social behavior; accordingly, social behavior is disrupted when these genes are mutated (Table 1).

4. Neuroanatomical and functional substrates of social behavior

As described in section 3, many ASD-associated genes code for proteins participating to neurotransmission and synaptic signaling, transcriptional/post-transcriptional regulation and cell adhesion function. All these processes are crucially involved in embryonic and early postnatal phases of CNS development, when genetic cues and environmental stimuli sequentially act (and then cooperate) to shape the structure and function of the nervous system. Studies performed on mouse models of neurodevelopmental disorders clearly demonstrate that mutations in ASD-associated genes result in abnormal social behavior (Table 1). Here, we will discuss how such mutations affect basic neurophysiological mechanisms at the cellular level, thus modifying the response of brain circuits involved in the expression of social behaviors.

Functional imaging studies performed in ASD human patients have described significant changes in brain areas' activation during social behavioral tasks. Areas involved in emotional processing and motivated behaviors (such as the amygdala and medial prefrontal cortex, mPFC), as well as primary sensory areas (S1 somatosensory and V1 visual cortices) are clearly affected in ASD individuals [12-14]. Studies from mouse models support these data [15-17]. A crucial role of the prefrontal cortex (PFC) also emerged from the comparative analysis of human and mouse studies [18]. More recently, Kim et al. used serial two-photon microscopy to map the induction of the immediate-early gene c-*fos* in response to social behaviors in mice [19]. This study aimed to identify the brain regions controlling "pure" social behaviors (i.e., areas that are

activated independent of sex interactions, and not in response to a nonsocial stimulus). This approach provided a map of brain areas activated by social behaviors, which include (but are not restricted to) olfactory and other cortical areas, hippocampus and hypothalamus [19]. It is important to point out that while c-*fos* induction has long been known as an indirect but reliable marker of neuronal activity [20], some studies showed that in certain cases it may not correlate with neuronal activation [21,22]. Thus, data obtained by Kim et al. [19] should be carefully interpreted.

Finally, a recent neuroimaging study performed on twenty-six different ASD mouse models consistently found anatomical abnormalities across models in the parieto-temporal lobe, cerebellar cortex, frontal lobe, hypothalamus and striatum [23]. These models separated into three distinct clusters (each showing similar brain anatomical abnormalities), respectively comprising i) *Nrxn1α*, *En2* and *Fmr1* knockout mice, ii) *Nlgn3* knockout and BTBR T^{+} *Itpr3tf/J* (BTBR) mice and iii) *Mecp2* knockout mice (BTBR are an inbred mouse strain, considered a reference standard ASD model [6]). Of note, this study revealed previously unknown neuroanatomical similarities between different ASD mouse models. There are however some crucial issues that should be carefully taken into consideration when interpreting the results of this study. First, in some cases, brain perfusion and scanning were performed in different laboratories; even if the same perfusion protocol was followed, a certain variability among laboratories is expected, which might affect the result of brain scans. Second, some mouse strains were not congenic (e.g. 16p11.2), making it impossible to rule out the confounding effects of genetic background. Third, the controls of mutant mice used in this study were not their own wild-type littermates, thus making it unclear if the observed anatomical phenotype truly reflects the ASD-related gene mutation or genetic background. Last, previous studies questioned the validity of some mouse strains as reliable ASD models (e.g., BTBR mice may not be really impaired in social behaviors *per se* [24,25]).

Nevertheless, these results indicate that social behaviors involve a wide range of brain structures, including areas responsible for motivation, emotions, sensory perception, learning and memory; accordingly, the structure and function of these areas is altered in both humans and mice showing altered social behaviors. However, despite the numerous brain areas participating to the expression of altered social behaviors, recent studies suggest that in neurodevelopmental disorders, similar dysfunctional mechanisms may take place at cellular level in different brain areas. In the following sections 4.1-4.4, we summarize these mechanisms and propose an integrative model of social behavior disturbance.

4.1 Hyper-excitability: a matter of balance

A widely accepted hypothesis of the neurophysiological basis of neurodevelopmental disorders such as ASD is the general reduction in the inhibitory tone across the brain, with consequent neuronal hyper-excitability [26,27]. Of note, a similar reduction of inhibitory tone has can be detected also in schizophrenia (SCZ) [28], another neurodevelopmental disorder of genetic origin, that shares profound similarities with ASD in terms of gene defects [29-31]. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the mammalian CNS, and GABA-releasing cells play the role of downscaling neuronal excitation, preventing brain hyper-excitability. Massive disruption of GABAergic function results in severe disease such as epilepsy, and is believed to occur in ASD as well [32]. Furthermore, the maintaining of a correct inhibitory to excitatory (E/I) balance is essential to promote development and plasticity, as clearly described for sensory areas of

the brain [33-35]. Interestingly, enhancement in perceptive, attentional and synesthetic tasks has been reported in humans affected by social behavior disturbances [36,37]. Notably, in ASD mouse models, similar improvements in sensorial perception have been described in association with impaired GABAergic function [30, 33]. Besides these positive effects on perceptual abilities, both human ASD patients [12, 13, 27] and mouse models of ASD (such as BTBR, *En2* and *CNTNAP2*) [35, 38-40] show severe impairment in the elaboration process of external stimuli as a consequence of reduced inhibitory activity. Based on these evidences, it has been hypothesized that loss of GABAergic function, with consequent neuronal hyper-excitability, slightly improves perceptual skills, but dramatically disrupts sensorial processing, triggering a hyper-arousal condition during the first step of internal elaboration.

4.2 Low cognitive elaboration: a matter of coherence

A particular sub-class of GABAergic cells, expressing the calcium binding protein parvalbumin (PV), has been shown to optimize cognitive functions and sensory responses by synchronizing brain oscillations (beta, gamma and theta rhythms), due to their peculiar intrinsic membrane properties that allow high frequency firing rate [41-43]. Among all interneuron subpopulations, PV-positive cells are the most recurrently damaged in neurodevelopmental disorders such as ASD and SCZ [28, 44]. On one hand, significant reduction in the number of PV-immunoreactive cells was reported in human post-mortem studies on ASD and SCZ patients [28, 45, 46]. In humans, cognitive dysfunction in SCZ has been linked to altered gamma-band oscillations in the cerebral cortex [47], which are generated by the interaction between pyramidal cells and PV+ interneurons [48]. Alterations in brain oscillations have been also described in ASD [49,50]. Specifically, electroencephalogram (EEG) recordings from ASD and SCZ subjects showed reduced gamma band power in primary and superior order sensory areas [51-53]. On the other hand, in animal studies, our group discovered reduced number of PV cells in the hippocampus and somatosensory cortex of autistic *En2* knockout mice [54,55]. Accordingly, other studies showed the same reduction of PV cells in the somatosensory cortex and insula of other ASD mouse models [respectively, Neuroligin 3 mutant (*Nlgn3* R451C) and BTBR mice] [35, 56]. Taken together, these evidences suggest that the reduced number of PV-positive interneurons, as well as their dysfunction, may drastically contribute to disrupt the coherence of brain oscillation, limiting the crosstalk of neuronal populations. Decreased coherence in brain communication may reflect the poor capability to modulate inward information observed in pathologies characterized by altered social behavior.

Mouse models of ASD offer the possibility to directly test this hypothesis. EEG profiling of different models might provide evidence of altered brain oscillations as a consequence of gene mutations affecting PV cell physiology. In addition, opto- and pharmaco-genetic modulation of PV cell activity in wild-type (WT) and ASD mutant mice could provide a causal demonstration of a physiological role of PV interneurons in modulating the function of brain circuits underlying social behaviors. To this aim, optogenetics [57] or “designer receptors exclusively activated by designer drugs” (DREADDs) [58] might be used to *in vivo* inactivate PV interneurons in precise brain structures of WT mice to see whether this results in altered brain oscillatory rhythms. An additional strategy might employ expression of the inward rectifying potassium channel Kir2.1 [59] to cell-autonomously reduce the activity of specific subsets of interneurons during development. Conversely, optogenetics could also be used to reactivate PV cell function in restricted brain

areas of ASD mouse models, with the aim to rescue circuit function and social behavior deficits.

4.3 Lack of learning: a matter of plasticity

Another recurrent feature observed in the presence of social behavior disturbances, evidenced both in human patients and mouse models, is the presence of immature or simplified spines on the dendritic branches of neurons in different brain areas such as the hippocampus [60,61]. The characterization of dendritic spines is largely used as an indirect measurement for the plasticity at the excitatory synapses. Indeed, a direct relationship between spines genesis and acquisition of new behavioral skills [62]. Thus, the complexity of the dendritic tree, enriched with stably maintained spines, can be used as a biomarker for learning, memory and plasticity. Reduced number and lack of maturation of dendritic spines in patients with ASD, together with findings on animal models, may reflect the inability of the subject to learn and produce social patterns that are usually acquired with the experience in healthy conditions. Indeed, according to DSM-5, most of neurodevelopmental disorders are associated with mental retardation and cognitive impairment [2]. Furthermore, recent findings on a mouse model of dysfunctional maturation of dendritic spines associated with ASD (*Shank1*$^{-/-}$ mice), revealed enhanced loss of excitatory synapses onto PV-positive cells in comparison with pyramidal excitatory neurons, further supporting the imbalance between excitation and inhibition occurring in ASD [63]. Whether these spine dysfunctions are due to impairment in spine stabilization or excessive active elimination has not been determined yet. However, long term potentiaton (LTP) and long term depression (LTD) experiments in ASD mice contributed to strengthen the idea that CNS plasticity is profoundly altered in the presence of social behavior deficits. For example significant reduction in induction or maintaining of hippocampal LTP has been detected in *Shank3*$^{+/-}$ or conditional *Nlgn1* mutant mice [64,65], while altered hippocampal LTD has been described in *Tsc2*$^{+/-}$ and *Fmr1* knockout mice [66].

4.4 Neural substrates of social behavior disturbances: a unifying view

A crucial issue in current ASD research is to understand how specific gene functions contribute to social behavior, and how genetic defects lead to behavioral dysfunctions. Indeed, social behavior is a complex psychosocial construct, hardly to be explained by direct gene-behavior relationships. However, several evidence described in sections 4.1-4.3 clearly demonstrate a substrate of neuroanatomical and neurophysiological dysfunctions that can be considered as risk factors for the occurrence of social behavior deficits in ASD and related neurodevelopmental disorders. Such mechanisms turned out to be recurrent across many brain areas and shared by different pathologies, suggesting a general malfunctioning in the whole brain network. According to this view, the disruption of information processing starts at the beginning of stimulus perception. As known, the emotional load of social stimuli is greater than non-social ones. Hypersensitivity to external cues may result particularly disabling in a social context, due to the emotional overload. However, cognitive functions, together with learning and memory, could act as late modulators appointed to control the emotional response. Remarkably, both these processes resulted frequently disrupted in ASD, in accordance with specific neuroanatomical and neurophysiological correlates (reduced GABAergic transmission, loss of function of PV-positive cells and lack of maturation of dendritic spines). Studies on mutant mouse models, reported in sections 4.1-4.3, have clearly shown that these

neuroanatomical and neurophysiological defects have a strong genetic basis (Fig. 2).

5. Rescue of social behavior deficits in mice

As discussed in section 1, an important asset of ASD mouse models is the possibility to test novel treatments to rescue social behavior deficits [6,7]. Several strategies have been followed, mostly pharmacological and (in a few cases) genetic. Table 2 reports the positive results of these different treatments, as obtained from interrogation of the SFARI database (https://gene.sfari.org/). Negative results (i.e., studies not reporting a rescue of social behavior deficits following pharmacological or genetic treatment) are not included in this list; the reader is referred to previous reviews for a comprehensive list of these studies [3, 7, 67].

Here we discuss three major treatment strategies that have been proved successful in different ASD mouse models: arginine-vasopressin/oxytocin, clonazepam and rapamycin. Treatment with arginine-vasopressin and oxytocin successfully rescued social behaviors in contactin-associated protein-like 2 knockout (*Cntnap2*$^{-/-}$) and oxytocin receptor knockout (*Oxtr*$^{+/-}$) mice (Table 2). Oxytocin (Oxt) and arginine-vasopressin are hypothalamic neurohormones that show structural and functional similarities, known for decades for promoting parturition, lactation, and social behavior [68]. Recent data suggest that oxytocin released during parturition is a crucial signal for shaping the correct E/I balance, which shapes brain plasticity and function after birth. Oxytocin-mediated GABA inhibition during delivery attenuates social behavior deficits and ASD-like phenotype in rodent offspring [69,70]. In line with these findings, recent results showed that *Oxtr* signaling is crucial to shape the inhibitory action of GABA during development [71]. Thus, Oxt- (and arginin-vasopressin?) mediated rescue of social behavior deficits in different ASD models might be due to its effect on E/I balance, which we proposed as a possible neural substrate of social disturbances (see section 4.1 and Fig. 2). In line with this view, rescue of E/I unbalance with the GABAergic agonist clonazepam has been proven to be effective to partially restore social behavior in *Scn1a*$^{+/-}$ mice, a model of ASD and epilepsy comorbidity [72] (Table 2). Finally, successful rescue of social behavior deficits was also achieved by treatment of tuberous sclerosis (*Tsc1/2*) mouse models with rapamycin, an immunosuppressant with the ability to inhibit the mammalian target of rapamycin (mTOR) signaling pathway [73]. In humans, *Tsc1/2* mutations cause tuberous sclerosis, a syndrome characterized by ASD and epilepsy symptoms [74]; in keeping with these findings, *Tsc1/2* mutations in mice result in social behavior deficits (Table 1). These deficits can be rescued by rapamycin (Table 2), which acts on mTOR, an upstream regulator of the TSC1/2 complex [73]. According to the view proposed in Fig. 2, inhibition of mTOR signaling by rapamycin would restore post-translational mechanisms involved in long term plasticity (see section 4.3) [66].

It is important to note that some of the treatments described in Table 2 (such as clonazepam) are not able to completely rescue social behavior deficits, thus confirming the notion that dysfunctional mechanisms underlying lsocial disturbances are extremely complex. In this respect, the clustering approach using neuroanatomy described by Ellegood et al. [23] might be crucial to identify novel molecular and anatomical similarities among different ASD models, thus paving the way to common, effective treatments to be developed in multiple experimental settings.

6.Conclusions

In this review, we discussed evidence from studies demonstrating that mutations in ASD-associated genes result in altered social behavior in mice. These studies may help to elucidate the brain cellular and circuit mechanisms underlying the expression of social behaviors. Unraveling key cellular mechanisms involved in these processes (such as GABAergic neurotransmission and mTOR signaling) may contribute to identify novel therapeutic targets to restore social function in neurodevelopmental disorders.

Acknowledgements

Data reported in this review were identified by searches of SFARI database and PubMed (as of July 30, 2016). Abstracts and reports from meetings were not included, and only papers published in English were reviewed. However, due to the large amount of bibliographic material available on this subject, we apologize with those authors whose studies have not been cited in this review. We are grateful to the administrative staff of CIBIO (University of Trento) and CNR Institute of Neuroscience (Pisa, Italy) for assistance. This work was funded by grants from the Italian Ministry of University and Research (PRIN 2010-11 grant 2010N8PBAA_002 to YB) and University of Trento (CIBIO start-up to YB). GP is supported by a grant fellowship from the Fondazione Umberto Veronesi (Milan, Italy).

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Figure and Table legends

Figure 1. Overrepresented gene ontology and phenotype categories for ASD-associated genes linked to social behavior in mice. Genes listed in Table 1 were analyzed for functional enrichment in phenotype and gene ontology (GO) categories such as mouse phenotype (A), human disease (B), molecular function (C) and Pathways (D), using ToppGene (https://toppgene.cchmc.org/) with a corrected adjusted P value cutoff lower than 0.05. Categories are arranged from most significant and downwards, for each category the number of genes is indicated by the length of the horizontal bars (gene counts).

Figure 2. Neural substrates of social behavior deficits in mice. Figure schematically shows a multi-factorial model for the expression of social behavior disturbances in mice. Studies performed on mouse models of ASD (see section 2 and Table 1) clearly indicate that social behavior disturbances have marked genetic determinants of neurodevelopmental origin. Many ASD-associated genes code for proteins participating to neurotransmission and synaptic signaling, transcriptional/post-transcriptional regulation and cell adhesion function. Lack of function of these genes in brain areas involved in social behavior may result in altered synaptic function, E/I unbalance, altered plasticity and poor circuit functioning. These malfunctions may have two major consequences: perceptual ability may be enhanced (green arrow), while poor cognitive elaboration and impaired learning and memory (red arrow) prevent a correct emotional processing. Consequently, emotional control results disrupted, laying the foundations for social behavior disturbances. Experimental evidence suggests that similar mechanisms take place in the human brain (see section 4.1-4.4 for details). The mouse brain sagittal section is a Nissl stain taken from the Allen Mouse Brain Atlas (http://www.brain-map.org/).

Table 1. Genes affecting social behavior in ASD mouse models. Table shows all relevant mouse models carrying targeted inactivation of ASD-associated genes listed in SFARI database (https://gene.sfari.org/), which present social behavior abnormalities. This list was generated by filtering all ASD genetic mouse models annotated in the Animal Model Module of SFARI database (which contains a detailed phenotypic profile for each reported animal model). Filter applied were “phenotype category “ (social behavior, and more specifically social interaction) and “experimental paradigm” (reciprocal social interactions, social approach, partition and social preference tests). For each model, the table reports gene symbol, gene function and model construct for transgenesis/homologous recombination (chronologically ordered in the “Model Genotype” column using progressive numbers; e.g., ADNP_1_KO_HT, ADNP_2_KO_HT…). Table also reports the experimental paradigm tested and age at testing. Abbreviations for mouse model genotypes: cKO, conditional knockout; HE, hemizygous; HM, homozygous; HT, heterozygous; KI, knockin; KO, knockout; Tg, transgenic. For other abbreviations and detailed references to mouse models see https://gene.sfari.org/. List is updated at July 30, 2016.

Table 2 Pharmacological and genetic rescue of social interactions in mouse models of ASD. Table lists all pharmacological and genetic treatments decribed in ASD mouse model to rescue social behavior deficits. As for Table 1, data were retrieved from the Animal Model Module of SFARI database

(https://gene.sfari.org/), filtering the Mouse Rescue Models for “restored social interaction”. Abbreviations are as in Table 1. List is updated at July 30, 2016.

Figure 1

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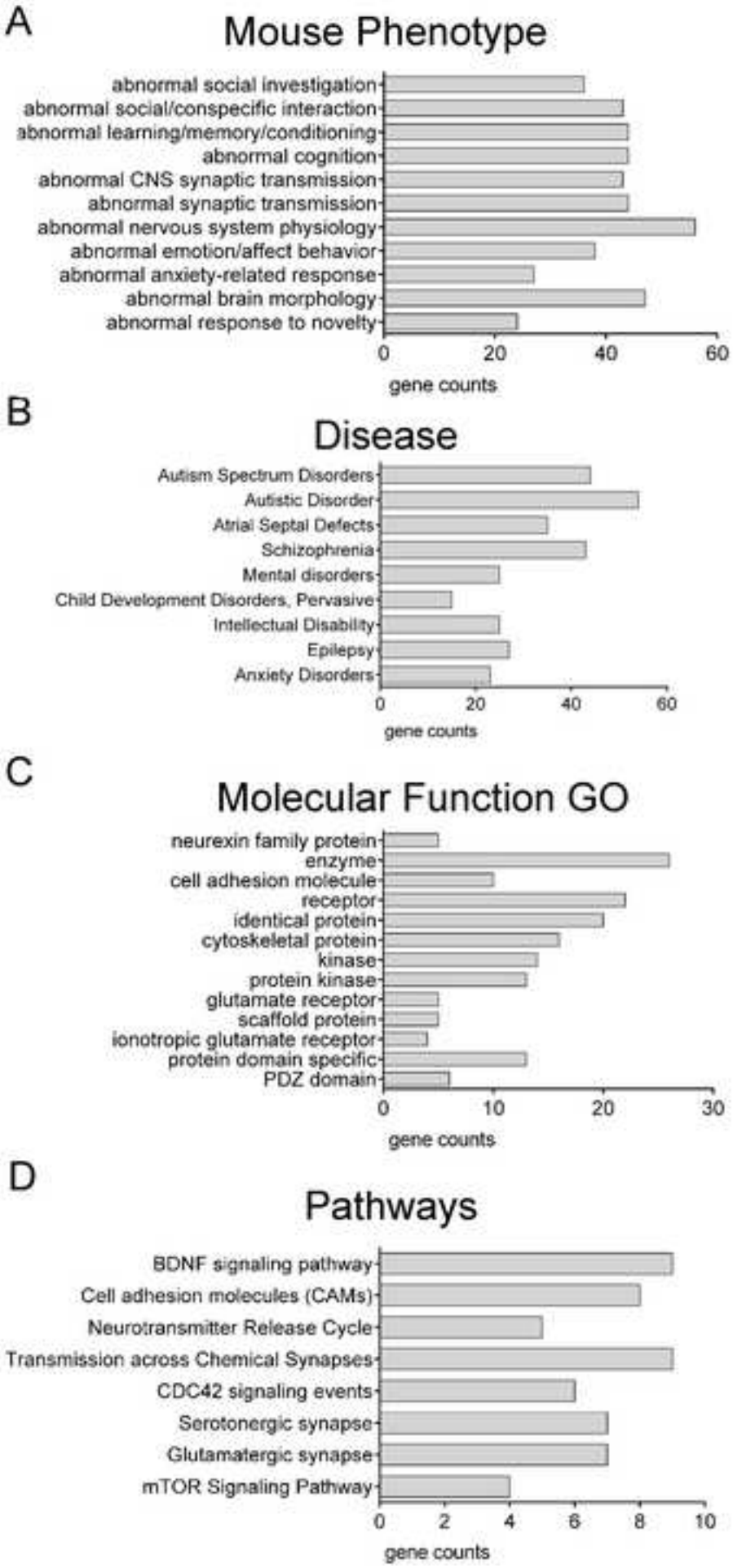

Figure 2
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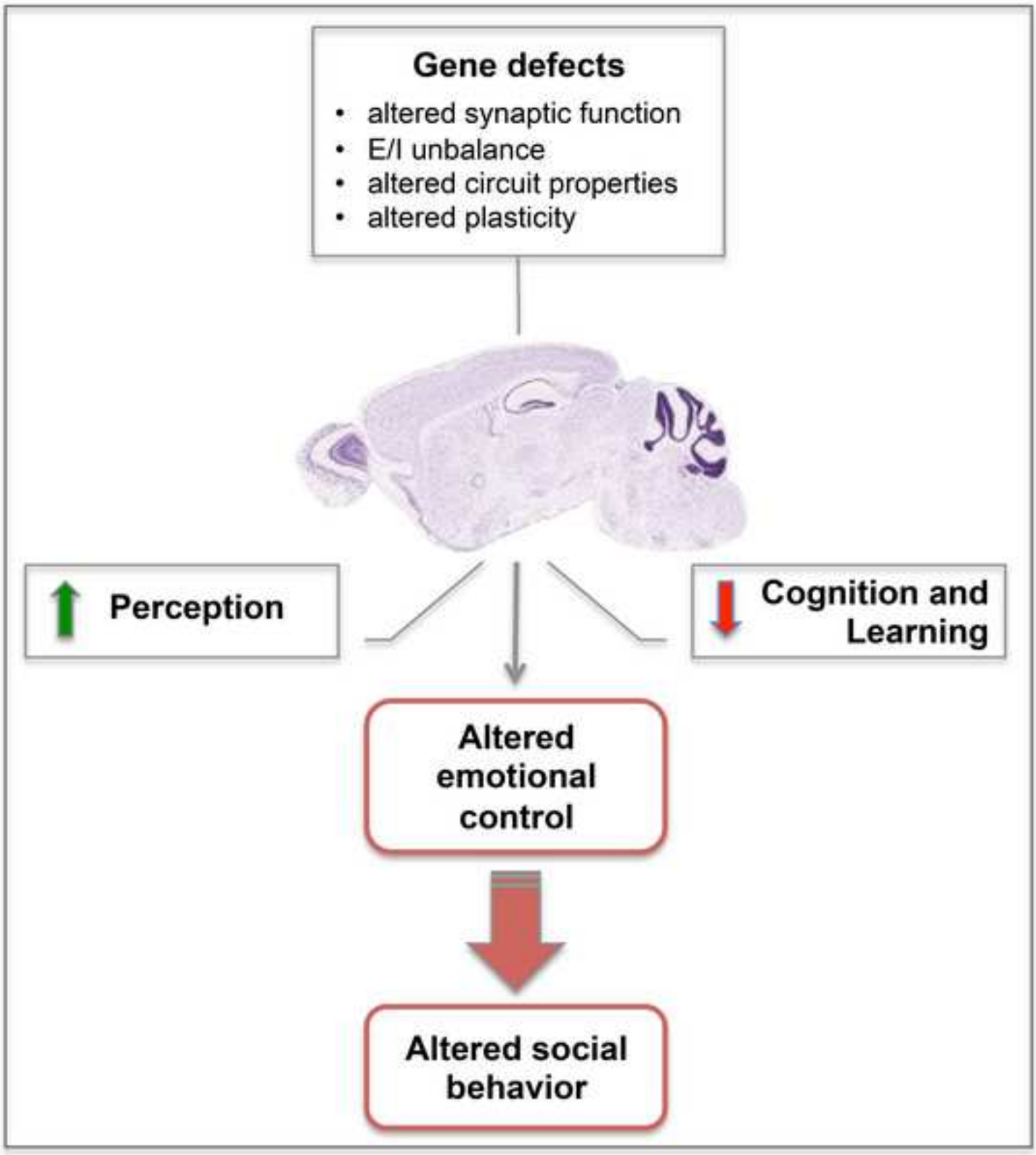

Table 1

Gene Symbol	Function	Model Genotype	Phenotype	Experimental Paradigm	Age at Testing
ADNP	Transcriptional or post-transcriptional regulator	ADNP_1_KO_HT	Decreased	Reciprocal social interaction test	Unreported
			Decreased	Three-chamber social approach test	8 months
		ADNP_2_KO_HT	Decreased	Three-chamber social approach test	7-8 months
ANKRD11	Transcriptional or post-transcriptional	ANKRD11_2_YODA_HT	Decreased	Three-chamber social approach test	6 months
APC	Tumor suppressor	APC_1_cKO_HM	Decreased	Three-chamber social approach test	Unreported
APP	Cell surface receptor	APP_1_Tg_HM	Decreased	Three-chamber social approach test	Unreported
Arhgap33	Rho GTPase activating protein	Arhgap33_1_KO_HM	Decreased	Three-chamber social approach test	Adult
			Decreased	Partition test;only males	Unreported
Atg7	E1 ubiquitin activating enzyme	Atg7_1_cKO_HM	Decreased	Three-chamber social approach test	4 weeks
AVPR1A	Arginine vasopressin receptor	AVPR1A_2_KO_HM	Decreased	Reciprocal social interaction test	7 weeks
BAIAP2	Scaffolding protein	BAIAP2_1_KO_HM	Decreased	Three-chamber social approach test	8-16 weeks
			Decreased	Reciprocal social interaction test	8-16 weeks
CACNA1C	Neurotransmission - calcium channel	CACNA1C_2_KO_HT	Decreased	Three-chamber social approach test	<6 months
CADM1	Cell adhesion molecule	CADM1_1_KO_HM	Decreased	Reciprocal social interaction test	5 months
			Decreased	Social recognition test	11-17 weeks
CADPS2	Calcium binding protein	CADPS2_1_KO_HM	Decreased	##########	4 weeks
		CADPS2_4_KO_HM	Decreased		2 months
			Decreased		2 months
		CADPS2_5_KO_HT	Decreased	Open field test	2 months
CDKL5	Ser/Thr protein kinase	CDKL5_1_KO_HE	Decreased	Three-chamber social approach test	9-12 weeks
CNTNAP2	Cell adhesion molecule	CNTNAP2_5_KO_HM	Decreased	Reciprocal social interaction test	3-6 weeks
			Decreased	Three-chamber social approach test	Unreported
DISC1	Neurite outgrowth and cortical development	DISC1_11_KI_CC_HM	Decreased	Three-chamber social approach test	3-12 months
		DISC1_3_Q31L_HM	Decreased	Three-chamber social approach test	12-16 weeks
DLG4		DLG4_1_KO_HM	Increased	Three-chamber social approach test	Unreported
Dvl1	Scaffolding protein	Dvl1_1_KO_HM	Decreased	Three-chamber social approach test	>6 weeks
		Dvl1_2_KO_HM_Dvl3_DM	Decreased	Three-chamber social approach test	>6 weeks
EIF4EBP2	Transcriptional regulator	EIF4EBP2_1_KO_HM	Decreased	Three-chamber social approach test	2 months
			Decreased	Reciprocal social interaction test	2 months
EN2	Transcriptional regulator	EN2_1_HD_KO_HM	Decreased	General observations	4-6 weeks
			Decreased	Three-chamber social approach test	8–10 weeks
Eps8		Eps8_1_KO_HM	Decreased	Three-chamber social approach test	Unreported
FMR1	Post-transcriptional regulator	FMR1_1_KO_HM	Decreased	##########	2.5 - 6 months
			Decreased		2-3 months
		FMR1_8_KO_HM	Decreased	Three-chamber social approach test	Unreported
			Increased	Open field test; males only	Unreported
FOLH1	Glutamate metabolism	FOLH1_1_KO_HT	Abnormal	Reciprocal social interaction test	7 weeks - 6 months 3-4 months
			Abnormal	Three-chamber social approach test	
FOXP1	Transcriptional or post-transcriptional regulator	FOXP1_1_CKO_HM	Decreased	Reciprocal social interaction test	Adult
GABRB3	Neurotransmission - GABA receptor	GABRB3_1_KO_HM	Abnormal	Open field test	24-52 weeks
			Abnormal	Three-chamber social approach test	16-52 weeks
Gad1	GABA synthesis	Gad1_2_KO_HT	Decreased	Three-chamber social approach test	15-16 weeks
GAP43	Kinase A binding activity	GAP43_1_KO_HT	Abnormal	Three-chamber social approach test	3-9 months
			Abnormal	Reciprocal social interaction test	3-9 months
GRID1	Glutamate receptor	GRID1_1_KO_HM	Abnormal	Three-chamber social approach test	Unreported
GRIN1	Glutamate receptor	GRIN1_1_KO_HM	Decreased	Three-chamber social approach test	7 months
		GRIN1_2_CN_KO_HM	Decreased	Three-chamber social approach test	10-18 weeks
Grm5	Glutamate receptor	Grm5_6_cKO_HM_PVN	Decreased	Three-chamber social approach test; social memory	8-12 weeks
			Increased	Three-chamber social approach test; self grooming	8-12 weeks
GRPR	G-protein coupled receptor	GRPR_1_KO	Increased	Reciprocal social interaction test	4 months
GSK3B	Serine-threonine kinase	GSK3B_1_KI_HM	Abnormal	Three-chamber social approach test	3 months
GSTM1	Glutathione transferase activity	GSTM1_1_KO_HM	Abnormal	Reciprocal social interaction test	Unreported
GTF2I	Transcriptional or post-transcriptional regulator	GTF2I_2_KO_HT	Decreased	Three-chamber social approach test	Unreported
HMGN1	Transcriptional regulator	HMGN1_1_OE_HM	Decreased	Three-chamber social approach test	6-7 months
		HMGN1_2_KO_HM	Decreased	Three-chamber social approach test	6-7 months
IL1RAPL1	Regulation of calcium channel activity	IL1RAPL1_2_KO_HM	Increased	Three-chamber social approach test	11-14 weeks
ITGB3	Cell adhesion molecule	ITGB3_1_KO_HM	Decreased	Three-chamber social approach test	Unreported
JAKMIP1	GABAergic system	JAKMIP1_1_KO_HM	Decreased	Three-chamber social approach test	Unreported
			Decreased	Open field test	Unreported
MAOA		MAOA_3_KO_HM	Decreased	Reciprocal social interaction test	3-4 months
MAPK1	Serine/threonine kinase (MAPK pathway)	MAPK1_1_CN_KO_HM	Abnormal	Open field test	Unreported
			Abnormal	Three-chamber social approach test	Unreported
			Decreased	Social recognition test	Unreported
MAPKBIP2	C-Jun-amino-terminal kinase-interacting protein	MAPKBIP2_1_CN_KO_HM	Decreased	Reciprocal social interaction test	5-6.5 weeks
MECP2	Transcriptional regulator	MECP2_17_CN_KO_HE	Increased	Partition test	12-13 weeks
		MECP2_18_CN_KO_HE	Increased	Partition test	12-13 weeks
		MECP2_23_308T_HT	Decreased	Partition test	Unreported
		MECP2_24_308T_HE	Decreased	Three-chamber social approach test	5-6 weeks
			Decreased	Partition test	Unreported
		MECP2_27_KO_HT	Decreased	Three-chamber social approach test	5 months
		MECP2_28_KO_HT	Decreased	Three-chamber social approach test	3-5.5 months
		MECP2_29_KO_HT	Decreased	Three-chamber social approach test	5.5 months
		MECP2_32_CKO_HE_PVN	Abnormal	Partition test	3.5 months
NBEA	Protein kinase A binding activity	NBEA_2_GH240B_HT	Decreased	Three-chamber social approach test	Unreported
NF1	RAS GTPase-activating protein	NF1_4_N31_HT	Decreased	Reciprocal social interaction test	Adult
NLGN1	Cell adhesion molecule	NLGN1_2_Tg_HM	Abnormal	Reciprocal social interaction test	2.5-6 months
NLGN2	Cell adhesion molecule	NLGN2_1_KO_HM	Decreased	Open field test	7-8 weeks
NLGN3	Cell adhesion molecule	NLGN3_1_KI_R451C	Decreased	Reciprocal social interaction test: confined stimulus	Unreported
			Decreased	mouse Reciprocal social interaction test: juvenile play	2-4 months
			Decreased	Three-chamber social approach test	Unreported
		NLGN3_4_KO	Decreased	Three-chamber social approach test	12-15 weeks
NLGN4X	Cell adhesion molecule	NLGN4X_1_KO_HM	Abnormal	Three-chamber social approach test	3 months
			Decreased	Reciprocal social interaction test	3 months
NRCAM	Cell adhesion molecules	NRCAM_1_KO_HM	Decreased	Three-chamber social approach test	Unreported
NRG1	Membrane glycoprotein	NRG1_1_KO_HT	Decreased	Three-chamber social approach test	Unreported
Nrxn2	Cell adhesion molecule	Nrxn2_1_KO_HM	Decreased	Three-chamber social approach test	10 weeks
		Nrxn2_1_KO_HM	Decreased	Reciprocal social interaction test	10 weeks
OXTR	Oxytocin receptor	OXTR_1_KO_HM	Abnormal	##########	Unreported
		OXTR_1_KO_HM	Decreased		12-16 weeks
		OXTR_2_KO_HM	Decreased	Social recognition test: two-trial	10 weeks
		OXTR_5_KO_HM	Decreased	Three-chamber social approach test	10-14 weeks
		OXTR_5_KO_HM	Decreased	Reciprocal social interaction test	10-14 weeks
P2RX4	Purinergic receptor	P2RX4_1_KO_HM	Decreased	Reciprocal social interaction test	3-5 months
		P2RX4_2_KO_HT	Decreased	Reciprocal social interaction test	3-5 months
PAFAH1B1	Platelet-activating factor acetylhydrolase	PAFAH1B1_1_KO_HT	Decreased	Three-chamber social approach test	3-4 weeks
		PAFAH1B1_2_CN_KO_HM	Decreased	Three-chamber social approach test	3-4 weeks
POU3F2	Transcriptional regulator	POU3F2_2_KO_HT	Decreased	Three-chamber social approach test	8-10 weeks
PRICKLE2	Unknown	PRICKLE2_2_KO_HM	Decreased	Three-chamber social approach test	8-12 weeks
PTEN	Signal transduction	PTEN_1_CKO_HM	Decreased	Three-chamber social approach test	Unreported
		PTEN_17_CKO_HM	Decreased	Reciprocal social interaction test	Unreported
			Decreased	Three-chamber social approach test	Unreported
		PTEN_2_CKO_HT	Decreased	Reciprocal social interaction test	3-4months
		PTEN_6_KO_HT	Decreased	Three-chamber social approach test	5-7 months
			Decreased	Three-chamber social approach test	7-20 weeks
			Decreased	Reciprocal social interaction test	7-20 weeks
		PTEN_7_CKO_HM	Decreased	Three-chamber social approach test	7-15 weeks
		PTEN_9_CKO_HM	Decreased	Reciprocal social interaction test	1 month
PVALB	Calcium-binding albumin protein	PVALB_1_KO_HM	Decreased	Reciprocal social interaction test	3-4 weeks
		PVALB_2_KO_HT	Decreased	Reciprocal social interaction test	3 weeks
RELN	Extracellular matrix protein	RELN_4_KO_HT_CPO	Increased	Three-chamber social approach test	4 weeks
RPS6KB1	Serine/threonine kinase (mTOR pathway)	RPS6KB1_1_KO_HM	Increased	Three-chamber social approach test	Unreported
SCN1A	Neurotransmission - sodium channel	SCN1A_11_cKO_HT-PVN_SSTN	Decreased	Three-chamber social approach test	Unreported
		SCN1A_6_KO_HT	Decreased	Reciprocal social interaction test	6-8 months
			Decreased	Three-chamber social approach test	6-8 months
			Decreased	Reciprocal social interaction test	6-8 months
		SCN1A_8_CN_KO_HT	Decreased	Reciprocal social interaction test	6-8 months
			Decreased	Three-chamber social approach test	3-5 months
		SCN1A_9_cKO_HT-PVN	Decreased	Three-chamber social approach test	3-5 months
SHANK2	Scaffolding protein	SHANK2_1_KO_HM	Decreased	Three-chamber social approach test	Unreported
		SHANK2_3_KO_HM	Decreased	Three-chamber social approach test	4-5 weeks
			Decreased	Reciprocal social interaction test	2-5 months
		SHANK2_5_KO_HM	Decreased	Three-chamber social approach test	2-4 months
SHANK3	Scaffolding protein	SHANK3_13_cKO_HM	Decreased	Reciprocal social interaction test	3-5 months
		SHANK3_15_KI_HM	Decreased	Reciprocal social interaction test	3 weeks
			Decreased	Three-chamber social approach test	Adult
		SHANK3_17_KI_HM	Decreased	Three-chamber social approach test	Adult
		SHANK3_18_KI_HT	Decreased	Three-chamber social approach test	Adult
		SHANK3_2_CN_KO_HT	Decreased	Reciprocal social interaction test	2.5-4 months
		SHANK3_4_KO_HM	Abnormal	Three-chamber social approach test	5-6 weeks
			Decreased	Open field test	5-6 weeks
		SHANK3_5_KO_HM	Abnormal	Three-chamber social approach test	Unreported
		SHANK3_6_KO_HM	Abnormal	Three-chamber social approach test	3-6 months
			Decreased	Reciprocal social interaction test	3-6 months
		SHANK3_8_KO_HM	Decreased	Reciprocal social interaction test	3-6 months
		SHANK3_9_KO_HT	Decreased	Three-chamber social approach test	1.5-6 months
			Decreased	Three-chamber social approach test	6-8 weeks
SLC6A4	Serotonin transporter	SLC6A4_4_SERTAla56_HM	Decreased	Three-chamber social approach test	Unreported
SYN1	Synaptic vesicle transport	SYN1_1_KO_HM	Decreased	Social transmission of food preference	2, 6 months
SYN2	Synaptic vesicle transport	SYN2_1_KO_HM	Decreased	Three-chamber social approach test, Social recognition test	2, 6 months 2, 6 months
SYN3	Synaptic vesicle transport	SYN3_1_KO_HM	Decreased	Three-chamber social approach test	2 months
SYNGAP1	Scaffolding protein	SYNGAP1_4_KO_HT	Decreased	Three-chamber social approach test	Unreported
Tbr1	Transcriptional regulator	Tbr1_2_KO_HT	Decreased	Three-chamber social approach test	2-4 months
		Tbr1_3_KO_HT	Decreased	Three-chamber social approach test	2-4 months
TBX1	Transcriptional regulator	TBX1_1_KO_HT	Decreased	Reciprocal social interaction test	2 months
TPH2	Serotonin synthesis	TPH2_1_KO_HM	Decreased	Reciprocal social interaction test, Three-chamber social approach test	Adult Adult
TSC1	Signal transduction	TSC1_5_CN_KO_HM	Decreased	Three-chamber social approach test	Unreported
		TSC1_6_CN_KO_HT	Decreased	Three-chamber social approach test	Unreported
		TSC1_7_KO_HT	Decreased	Reciprocal social interaction test	3-7 months
TSC2	Signal transduction	TSC2_10_KO_ATG7_DM	Decreased	Three-chamber social approach test	Unreported
		TSC2_4_DN_HM	Decreased	Open field test	3-6 months
		TSC2_5_CN_KO_HM	Decreased	Three-chamber social approach test	2 months
		TSC2_7_CN_KO_HM	Decreased	Three-chamber social approach test	2 months
		TSC2_8_KO_HT	Decreased	Three-chamber social approach test	4-5 weeks
			Decreased	Reciprocal social interaction test	1-7 months
TYR	Amine oxidase enzymes	TYR_1_Tg_HM	Decreased	Three-chamber social approach test	Unreported
UBA6	Protein ubiquitination	UBA6_1_CN_KO_HM	Decreased	Three-chamber social approach test	3 months
UBE3A	Protein ubiquitination	UBE3A_5_Tg_HM	Abnormal	Three-chamber social approach test	Unreported
		UBE3A_6_Tg_HM	Decreased	Three-chamber social approach test	Unreported

Table 2

Model Gene Symbol	Model Genotype	Treatment	Phenotype	Experimental Paradigm	Age at Testing
BAIAP2	BAIAP2_1_KO_HM	Memantine i.p. injection (10 mg/Kg) 30 minutes before testing	Restored	Three-chamber social approach test	8-16 weeks
		2-methyl-6-(phenylethynyl)pyridine (MPEP) i.p. injection (30 mg/Kg) 30 minutes before testing	Restored	Three-chamber social approach test	8-16 weeks
CNTNAP2	CNTNAP2_5_KO_HM	Arginine vasopressin i.p. injection (10 mg/kg) 20-30 min before social behavioral test	Restored	Three-chamber social approach test	6-8 weeks
		Arginine vasopressi intranasal administration (200ug/kg) 20-30 min before social	Restored	Reciprocal social interaction test:	4-6 weeks
		Arginine vasopressin receptor (AVP-V1a receptor) antagonist (reclovaptan) administered intranasally (300ug/kg), followed by AVP at ~ 200ug/kg or saline intranasally 20-30 min before testing in social behavioral tests	Restored	Reciprocal social interaction test: juvenile	4-6 weeks
		Oxytocin receptor antagonist (L371,257) intranasally at a dose of 300ug/kg, followed by AVP	Restored	Reciprocal social interaction test: juvenile	4-6 weeks
		Oxytocin i.p. injection (10 mg/kg) in juvenile (4-6 wks old) mice 20-30 minutes before testing in social behavioral paradigm(s)	Restored	Three-chamber social approach test	6-8 weeks
		Oxytocin i.p. injection (10 mg/kg) in juvenile (4-6 wks old) mice 20-30 minutes before testing in social behavioral paradigm(s)	Restored	Reciprocal social interaction test: juvenile	4-6 weeks
		Oxytocin intranasal administration (200ug/kg) 20-30 min before testing in social behavioral tests.	Restored	Reciprocal social interaction test: juvenile	4-6 weeks
		Oxytocin intranasal administration (200ug/kg) on pups between P7- P21	Restored	Three-chamber social approach test	4 weeks
		Oxytocin intranasal administration (200ug/kg) on pups between P7- P21	Restored	Reciprocal social interaction test	4 weeks
		Genetic rescue: DREADD-Oxytocin neurons	Restored	Three-chamber social approach test	adult
Dvl1	Dvl1_1_KO_HM	CHIR99021 (Tocris) i.p. injection (4 mg/kg) on Dvl1 null pregnant dams daily from E9.5 to E14.5	Restored	Three-chamber social approach test	8-10 weeks
EIF4EBP2	EIF4EBP2_1_KO_HM	4EGI	Restored	Three-chamber social approach test	2 months
		shRNAs (TRCN0000032022 and TRCN0000032020)	Restored	Three-chamber social approach test	2 months
Fmr1	FMR1_8_KO_HM_S6K1_DM-hm	Genetic rescue: inactivation of S6K1 gene	Restored	Three-chamber social approach test	adult
FOLH1	FOLH1_1_KO_HT_FD	Low folate	Restored	Three-chamber social approach test	3.5 months
GRIN1	GRIN1_1_KO_HM	Baclofen i.p. injection 15-30 mins before tests	Restored	Three-chamber social approach test	7 months
MECP2	MECP2_25_308T_HT	BDE-47 oral administration (0.03 mg /kg/day for 10 weeks from preconception to lactation)	Restored	Three-chamber social approach test	5.5-10 weeks
		BDE-47 oral administration (0.03 mg /kg/day for 10 weeks from preconception to lactation)	Restored	Partition test	16-18 weeks
		BDE-47 oral administration (0.03 mg /kg/day for 10 weeks from preconception to lactation)	Restored	Three-chamber social approach test	10 weeks
NF1	NF1_4_N31_HT	IPA-3	Restored	Reciprocal social interaction test	adult
	NF1_8_N31_HT_PAK1_KO	Genetic rescue:targeted disruption of exon 31 of the Nf1 gene	Restored	Reciprocal social interaction test	adult
OXTR	OXTR_1_KO_HM	Arginine vasopressin	Restored	Three-chamber social approach test	12-16 weeks
		Oxytocin	Restored	Three-chamber social approach test	12-16 weeks
SCN1A	SCN1A_6_KO_HT	Clonazepam (CLZ) i.p. injection (0.0625 mg/kg)	Restored	Reciprocal social interaction test	6-8 months
		Clonazepam (CLZ) i.p. injection (0.0625 mg/kg)	Restored	Three-chamber social approach test	6-8 months
SHANK2	SHANK2_3_KO_HM	3-cyano-N-(1,3-diphenyl-1H-pyrazol-5-yl)benzamide (CDPPB) treatment (10mg/kg)	Restored	Three-chamber social approach test	2-5 months
SHANK3	SHANK3_9_KO_HT	Tat-p-cofilin injection (15 picomol/g) before behavioral testing in juvenile male mice	Restored	Three-chamber social approach test	6-8 weeks
		Cofilin inhibitory peptide bilateral stereotaxic injections into the prefrontal cortex prior to social behavior testing	Restored	Three-chamber social approach test	6-8 weeks
Tbr1	Tbr1_2_KO_HT	D-cycloserine administration (20mg/kg) 30 min before behavioral experiments: two 25 gauge guide tubes implanted bilaterally into the amygdala	Restored	Three-chamber social approach test	2-4 months
		D-cycloserine intraperitoneal administration (20mg/kg) 30 min before behavioral experiments	Restored	Reciprocal social interaction test	2-4 months
	Tbr1_3_KO_HT	Clioquinone(CQ)	Restored	Three-chamber social approach test	2-4 months
TSC1	TSC1_5_CN_KO_HM	Rapamycin	Restored	Three-chamber social approach test	7-9 weeks
	TSC1_7_KO_HT	Rapamycin intraperitoneal administration (5 mg/kg) once daily for 2 consecutive days	Restored	Reciprocal social interaction test	3-7 months
TSC2	TSC2_8_KO_HT	Rapamycin intraperitoneal administration (5 mg/kg) once daily for 2 consecutive days	Restored	Reciprocal social interaction test	3-7 months
		Rapamycin treatment (3mg/kg) from P21 to P28	Restored	Three-chamber social approach test	4 weeks