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Connecting Genotype with Behavioral Phenotype in Mouse Models of Autism Associated with *PTEN* Mutations

Amy E. Clipperton-Allen and Damon T. Page

Department of Neuroscience, The Scripps Research Institute, Jupiter, Florida 33458, USA

Correspondence: paged@scripps.edu

A subset of individuals with autism spectrum disorder (ASD) and macrocephaly carry mutations in the gene *PTEN*. Animal models, particularly mice, have been helpful in establishing a causal role for *Pten* mutations in autism-relevant behavioral deficits. These models are a useful tool for investigating neurobiological mechanisms of these behavioral phenotypes and developing potential therapeutic interventions. Here we provide an overview of various genetic mouse models that have been used to characterize behavioral phenotypes caused by perturbation of *Pten*. We discuss convergent and divergent phenotypes across models with the aim of highlighting a set of behavioral domains that are sensitive to the effects of *Pten* mutation and that may provide useful readouts for translational and basic neuroscience research.

Autism spectrum disorder (ASD) is a neurodevelopmental disorder occurring in 1 in 59 children in the United States (Baio et al. 2018), and is highly heritable (83%; Sandin et al. 2017). The diagnostic criteria for ASD are behavioral—consisting primarily of deficits in social behavior and communication, and restricted, repetitive, and stereotyped patterns of behavior (*DSM-V*, American Psychiatric Association 2013). Additionally, several disorders show frequent comorbidity with ASD, including mood disorders (~60%; Skokauskas and Gallagher 2010), anxiety disorders (~80%; Skokauskas and Gallagher 2010), and intellectual disability (~60%; Matson and Shoemaker 2009).

Although autism is defined and diagnosed based on behavioral criteria, a subset of individuals display alterations in the normal trajectory of head and brain growth (Courchesne et al. 2007). Macrocephaly—consisting of a head circumference more than two standard deviations above normal—is present in ~15%–20% of individuals with ASD (Lainhart et al. 2006; Sacco et al. 2015; Albores-Gallo et al. 2017). Of these, ~10%–25% (Butler et al. 2005; Buxbaum et al. 2007; Varga et al. 2009; McBride et al. 2010; Klein et al. 2013; Hobert et al. 2014; Yeung et al. 2017) also have mutations in *Phosphatase and tensin homolog* (*PTEN*), which is causative of macrocephaly/autism syndrome (MIM #605309).

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In humans, *PTEN* mutations lead to a highly penetrant neurodevelopmental phenotype, resulting in an abnormal brain structure, ASD, and/or intellectual disabilities (Mester et al. 2011; Busch et al. 2013; Hobert et al. 2014). Germline heterozygous *PTEN* mutations are associated with macrocephaly/autism syndrome (MIM #605309), as well as Cowden 1 (MIM #158350; ~80% have *PTEN* mutations; Blumenthal and Dennis 2008), Bannayan–Riley–Ruvalcaba (MIM #153480), and Lhermitte–Duclos syndromes (MIM #158350), collectively known as PTEN hamartoma tumor syndromes (PHTS; see Blumenthal and Dennis 2008 for a review). The primary behavioral phenotype for individuals with *PTEN* mutations, in addition to ASD, include lower verbal and nonverbal IQ (Herman et al. 2007; Conti et al. 2012; Rosti et al. 2014; Frazier et al. 2015; Stessman et al. 2017), decreased processing speed (Frazier et al. 2015), deficits in memory recall, particularly in working memory (Busch et al. 2013; Frazier et al. 2015), and short attention spans (Goffin et al. 2001; Butler et al. 2005; Boccone et al. 2006; Herman et al. 2007), as well as impaired motor and fine motor skills (Goffin et al. 2001; Butler et al. 2005; Herman et al. 2007; Orrico et al. 2009; Conti et al. 2012; Busch et al. 2013; Frazier et al. 2015).

Given the genetic heterogeneity present in the human clinical population, a key question arising from early clinical reports of individuals with *PTEN* mutations and ASD was whether *PTEN* mutations are sufficient to induce ASD-related behavioral symptoms. Animal models proved useful in addressing this question, and it was found that conditional homozygous loss-of-function (Kwon et al. 2006), germline heterozygous loss-of-function (Page et al. 2009), and germline homozygous cytoplasm-predominant knockin (Tilot et al. 2014) mutations in *Pten* all result in ASD-relevant behavioral deficits, along with brain overgrowth, in mice. These and numerous other mouse models of *Pten* mutations have been applied to understanding the neurobiological substrates of behavioral abnormalities caused by *Pten* mutations and to testing potential mechanisms and therapeutic interventions. In this review, we examine similarities and differences in behavioral phenotypes across *Pten* mouse models (see Table 1 for model descriptions), with a focus on those directly related to ASD (social behavior, repetitive behavior) and those modeling ASD comorbidities (mood and anxiety disorders, intellectual disability, sleep and circadian rhythms, motor behavior, and sensory sensitivity; see Table 2). With this scope in mind, we will limit our discussion to studies that help define a "profile" of behavioral phenotypes caused by *Pten* mutations, and we will not discuss here the numerous important studies that are elucidating potential molecular, cellular, neuroanatomical, and circuit-level substrates for these effects.

ASD-RELEVANT BEHAVIORS

Social Behavior

As impaired social behavior and communication is one of the key diagnostic criteria for ASD (American Psychiatric Association 2013), it is not surprising that many studies of mouse models harboring *Pten* mutations have assessed this behavioral domain. However, "social behavior" is a very broad category that encompasses many different motivations, skills, and neural circuits. Thus, we have subdivided this category into social interest, social recognition, and other social behaviors.

Social Interest

Social interest is one of the most widely tested types of behavior across mouse models of ASD, and represents one of the most fundamental aspects of social behavior. Face validity for social impairments in ASD is a common rationale for testing social interest in mouse models; however, it is worth noting that the translational value of mouse sociability as a model for human social deficits in ASD remains to be validated. This same caveat applies to the other ASD-relevant behaviors discussed below.

There are two main ways in which social interest is analyzed in animal models. The first is by simply measuring the amount of time spent investigating a novel conspecific (adult or juve-

Table 1. List of mouse models with *Pten* mutations included in this review

Type	*Pten* allele	*Pten* perturbation	Site of *Pten* perturbation	Common name	References	MGI link
Germline	*Pten*$^{+/-}$ [*Pten*^{tm1Rps}]	Loss-of-function (haploinsufficiency)	Ubiquitous	*Pten*$^{+/-}$	Page et al. 2009; Clipperton-Allen and Page 2014, 2015; Séjourné et al. 2015; Huang et al. 2016	www.informatics.jax.org/allele/MGI:2151804
Germline	*Pten*^{m3m4} [*Pten*^{tm1Engc}]	↓ Nuclear: cytoplasm ratio	Ubiquitous	*Pten*^{m3m4}	Mester et al. 2011; Tilot et al. 2014	www.informatics.jax.org/allele/reference/J:210487
Germline	*Pten*α^{mu}	Deletion of PTENα isoform	Ubiquitous	*Pten*α^{mu}	Wang et al. 2017	n/a
Germline	*Pten*^{Tg} [*Tg(Pten)1Srn*]	Overexpression	Ubiquitous	*Pten*^{Tg}	Sanchez-Puelles et al. 2019	www.informatics.jax.org/allele/MGI:5645732
Germline	*Pten*$^{\Delta PDZ}$ [*Pten*$^{tm1.1Jaes}$]	Deletion of PDZ-binding domain	Ubiquitous	*Pten*$^{\Delta PDZ}$	Sanchez-Puelles et al. 2019	www.informatics.jax.org/allele/key/868490
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak}	Loss-of-function	CB and DG granule cells, some CTX neurons [*Tg(Gfap-cre)1Sbk*]	*NS-Cre*; *Pten*^{loxP}	Backman et al. 2001; Kwon et al. 2001, 2003; Ljungberg et al. 2009; Lugo et al. 2014, 2017; Nguyen et al. 2015; Smith et al. 2016; Binder and Lugo 2017; Hodges et al. 2018	*Tg(Gfap-cre)1Sbk*: www.informatics.jax.org/allele/MGI:2448664 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak} *Pten*^{tm1Hwu}	Loss-of-function	Mature CTX and HIPP neurons [*Tg(Eno2-cre)39Jme*, *Tg(Eno2-cre)2Lfp*]	*Nse-Cre*; *Pten*^{loxP}	Kwon et al. 2006; Ogawa et al. 2007; Zhou et al. 2009; Napoli et al. 2012; Nolan et al. 2019	*Tg(Eno2-cre)39Jme*: www.informatics.jax.org/allele/MGI:2177175 *Tg(Eno2-cre)2Lfp*: www.informatics.jax.org/allele/MGI:3621475 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak}	Loss-of-function	Inducible; postnatal NSCs in SGZ, SVZ [*Tg(Nes-cre/ERT2)73Lfp*]	*Nestin-Cre*; *Pten*^{loxP}	Amiri et al. 2012	*Tg(Nes-cre/ERT2)73Lfp*: www.informatics.jax.org/allele/MGI:3840184 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	NSCs [*Tg(Gfap-cre)77.6Mvs*]	*NSC-Cre*; *Pten*^{loxP}	Gregorian et al. 2009	*Tg(Gfap-cre)77.6Mvs*: www.informatics.jax.org/allele/MGI:3838840 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
Conditional (*Pten*^{loxP})	*Pten*$^{tm1.1Mwst}$	Loss-of-function	Mesenchymal cells and WM astrocytes [*Tg(S100a4-cre)1Gle*]	*Fsp1-Cre*; *Pten*^{loxP}	Borniger et al. 2016	*Tg(S100a4-cre)1Gle*: www.informatics.jax.org/allele/MGI:3775823 *Pten*$^{tm1.1Mwst}$: www.informatics.jax.org/allele/MGI:4366755
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	NeoCTX and HIPP neurons, subset of glia [*Emx1*$^{tm1(cre)Krj}$]	*Emx1-Cre*; *Pten*^{loxP}	Huang et al. 2016	*Emx1*$^{tm1(cre)Krj}$: www.informatics.jax.org/allele/MGI:2684610 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086

Continued

Table 1. *Continued*

Type	*Pten* allele	*Pten* perturbation	Site of *Pten* perturbation	Common name	References	MGI link
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	Oxytocinergic neurons [*Oxt*$^{tm1.1(cre)Dolsn}$]	*Oxt-Cre; Pten*^{loxP}	Clipperton-Allen et al. 2016	*Oxt*$^{tm1.1(cre)Dolsn}$: www.informatics.jax.org/allele/MGI:5523143 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	Purkinje cells [*Tg(Pcp2-cre)2Mpin*]	*L7-Cre; Pten*^{loxP}	Cupolillo et al. 2016	*Tg(Pcp2-cre)2Mpin*: www.informatics.jax.org/allele/MGI:2137515 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	Dopaminergic neurons [*Slc6a3*$^{tm1.1(cre)Bkmn}$]	*DAT-Cre; Pten*^{loxP}	Diaz-Ruiz et al. 2009; Clipperton-Allen and Page 2014	*Slc6a3*$^{tm1.1(cre)Bkmn}$: www.informatics.jax.org/allele/MGI:3689434 *Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak}	Loss-of-function	Subsets of DG, HAN, AMYG neurons [*Tg(Pomc-cre)1Lowl*]	*Pomc-Cre; Pten*^{loxP}	Matsushita et al. 2016	*Tg(Pomc-cre)1Lowl*: www.informatics.jax.org/allele/MGI:4362028 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak}	Loss-of-function	Excitatory neurons [*Tg(Camk2a-cre)T29-1Stl*]	*CaMKIIa-Cre; Pten*^{loxP}	Sperow et al. 2012	*Tg(Camk2a-cre)T29-1Stl*: www.informatics.jax.org/allele/MGI:2177650 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005
Conditional (*Pten*^{loxP})	*Pten*^{tm2Mak}	Loss-of-function	MGE and MPOA progenitors [*Tg(Nkx2-1-cre)2Sand*]	*Nkx2.1-Cre; Pten*^{loxP}	Vogt et al. 2015	*Tg(Nkx2-1-cre)2Sand*: www.informatics.jax.org/allele/MGI:3773076 *Pten*^{tm2Mak}: www.informatics.jax.org/allele/MGI:2182005
Conditional (*Pten*^{loxP})	*Pten*^{tm1Hwu}	Loss-of-function	AAV-Cre in somatosensory CTX at P1	n/a	Gutilla et al. 2016	*Pten*^{tm1Hwu}: www.informatics.jax.org/allele/MGI:2156086
shRNA targeting *Pten*	n/a	shRNA knockdown	BLA, LA	n/a	Haws et al. 2014	n/a

(CB) cerebellum, (DG) dentate gyrus, (CTX) cortex, (HIPP) hippocampus, (NSC) neural stem cells, (SGZ) subgranular zone, (SVZ) subventricular zone, (WM) white matter, (HAN) hypothalamic arcuate nucleus, (AMYG) amygdala, (MGE) medial ganglionic eminence, (MPOA) medial preoptic area, (shRNA) short hairpin RNA, (BLA) basolateral amygdala, (LA) lateral amygdala.

Table 2. Summary of behavioral phenotypes in mouse models of *Pten* mutations (red, increase in behavior; gray, no change in behavior; blue, decrease in behavior)

Mouse model	Type:	Germline	Germline	Germline	Germline	Germline	Conditional	Conditional	Conditional	Conditional
	Line:	*Pten*$^{+/-}$	*Pten*^{m3m4}	*Ptenα*^{mu}	*Pten*^{Tg}	*Pten-ΔPDZ*	*NS-Cre*	*Nse-Cre*	*Nestin-Cre*	*NSC-Cre*
Behavior category	Perturbation / Behavior	Germline heterozygous	↓ Nuclear: cytoplasm ratio	PTENα isoform	*Pten* over-expression	PDZ-binding domain	CB and DG granule cells, some CTX neurons	Mature CTX and HIPP neurons	Inducible; postnatal NSCs in SGZ, SVZ	NSCs
Social behaviors	Social interest	—[1] ↓[1-4]	↑[6] —[6]		↑[9]	↓[9]	—[10] ↓[11]	↓[20-22]	↓[25]	
	Social recognition	—[2] ↓[1,2]	—[6]				↓[11]	↓[20-22]	↓[25]	
	Aggression	↓[5]								
	Other				↓[9]	—[9]	—[11] ↓[12]	↓[20]	—[25]	
Repetitive behaviors	Marble burying	↑[2] —[2]					—[10] ↓[11]			
	Self-grooming	—[5]			—[9]		—[10] ↓[11]	↑[22]		
	Stereotypies	—[5]					↑[13]			
Mood and anxiety	Anxiety	—[2] ↓[2]	—[6]	—[8]	↓[9]	—[9]	—[10] ↓[11]	↑[20,21] —[20]		
	Depression	↑[2] —[2]								
Intellectual disability	Fear conditioning	—[2] ↓[2]		↓[8]	↓[9]	—[9]	—[10]	—[20]		
	Aversive spatial L&M			↓[8]	↓[9]		—[10]	↓[20]		
	Nonaversive spatial L&M	—[4]			—[9]		↓[14]			
	Novelty recognition		—[6]				↓[14]			
Circadian	Activity	—[2] ↓[2]						↓[23]		
	Tau	—[2]						↑[23]		
Sensorimotor	Startle							↑[20]		
	PPI	↓[1]						↓[20]		

Continued

Table 2. *Continued*

Mouse model	Type:	Germline	Germline	Germline	Germline	Germline	Conditional	Conditional	Conditional	Conditional
	Line:	*Pten*$^{+/-}$	*Pten*^{m3m4}	*Ptenα*^{mu}	*Pten*^{Tg}	*Pten-ΔPDZ*	*NS-Cre*	*Nse-Cre*	*Nestin-Cre*	*NSC-Cre*
Behavior category	Perturbation / Behavior	Germline heterozygous	↓ Nuclear: cytoplasm ratio	PTENα isoform	*Pten* over-expression	PDZ-binding domain	CB and DG granule cells, some CTX neurons	Mature CTX and HIPP neurons	Inducible; postnatal NSCs in SGZ, SVZ	NSCs
Basic sensory/motor	Auditory orientation									
	Contact placing									
	Visual placement									
	Olfaction	—[1]					—[11]	—[20]		↑[26]
	Pain perception	—[2]	—[6]							
	Endurance							—[20]		
	Grip strength							—[20]		
Motor behaviors	Locomotion	—[2]	—[6]	—[8]	—[9]		↑[11,13] —[13]	↑[20,21] —[20,21]	—[25]	
	Balance	—[2]	↓[6]					—[20] ↓[24]		
	Motor learning	—[2]	—[6]					↑[20]		
	Fine motor skills							↓[24]		
Spontaneous observations	Seizures						↑[15-19]	↑[20-21,23]	↑[25]	
	Ataxia						↑[15,18]			
	Limb clasping									
	Premature death		↑[7]				↑[15-19]			

Continued

Table 2. *Continued*

Mouse model	Type:	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	shRNA
	Line:	*Fsp1-Cre*	*Emx1-Cre*	*Oxt-Cre*	*L7-Cre*	*DAT-Cre*	*Pomc-Cre*	*CaMKIIα-Cre*	*Nkx2.1-Cre*	AAV-Cre	BLA complex
Behavior category	Perturbation / Behavior	Mesenchymal cells and WM astrocytes	NeoCTX and HIPP neurons, subset of glia	Oxytocinergic cells	Purkinje cells	Dopaminergic cells	Subsets of DG, HAN, AMYG neurons	Excitatory neurons	MGE and MPOA progenitors	AAV-Cre to somatosensory CTX at P1	shRNA to BLA, LA
Social behaviors	Social interest	—[27]	↓[4]	—[28]	↓[29]	—[2] ↓[2]			↓[33]		—[35]
	Social recognition			—[28] ↓[28]		—[2] ↓[2]					—[35]
	Aggression	↓[27]		—[28]							
	Other										
Repetitive behaviors	Marble burying			—[28] ↓[28]							
	Self-grooming				↓[29]						
	Stereotypies				↓[29]						
Mood and anxiety	Anxiety	—[27]		—[28] ↓[28]					—[33]	—[34]	—[35]
	Depression			—[28]							
Intellectual disability	Fear conditioning										—[35]
	Aversive spatial L&M				—[29]			↓[32]			
	Nonaversive spatial L&M	—[27]									
	Novelty recognition	—[27]							↓[33]		
Circadian	Activity										
	Tau										
Sensorimotor	Startle										—[35]
	PPI										

Continued

Table 2. *Continued*

Mouse model	Type:	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	Conditional	shRNA
	Line:	*Fsp1-Cre*	*Emx1-Cre*	*Oxt-Cre*	*L7-Cre*	*DAT-Cre*	*Pomc-Cre*	*CaMKIIα-Cre*	*Nkx2.1-Cre*	AAV-Cre	BLA complex
Behavior category	Perturbation / Behavior	Mesenchymal cells and WM astrocytes	NeoCTX and HIPP neurons, subset of glia	Oxytocinergic cells	Purkinje cells	Dopaminergic cells	Subsets of DG, HAN, AMYG neurons	Excitatory neurons	MGE and MPOA progenitors	AAV-Cre to somatosensory CTX at P1	shRNA to BLA, LA
Basic sensory/motor	Auditory orientation	↓[27]									
	Contact placing	—[27]									
	Visual placement	—[27]									
	Olfaction	—[27]									
	Pain perception										—[35]
	Endurance										
	Grip strength	—[27]	↓[27]								
Motor behaviors	Locomotion			—[28]	—[29]	—[30]	↓[2]			—[34]	—[35]
	Balance	↓[27]		—[28]	↓[29]					—[34]	
	Motor learning	—[27]		—[28]	↓[29]					—[34]	
	Fine motor skills										
Spontaneous observations	Seizures						↑[31]				
	Ataxia										
	Limb clasping										
	Premature death						↑[31]				

(L&M) learning and memory, (PPI) Prepulse inhibition, (CB) cerebellum, (DG) dentate gyrus, (CTX) cortex, (HIPP) hippocampus, (NSC) neural stem cells, (SGZ) subgranular zone, (SVZ) subventricular zone, (WM) white matter, (HAN) hypothalamic arcuate nucleus, (AMYG) amygdala, (MGE) medial ganglionic eminence, (MPOA) medial preoptic area, (shRNA) short hairpin RNA, (BLA) basolateral amygdala, (LA) lateral amygdala.

References: [1]Page et al. 2009; [2]Clipperton-Allen and Page 2014; [3]Séjourné et al. 2015; [4]Huang et al. 2016; [5]Clipperton-Allen and Page 2015; [6]Tilot et al. 2014; [7]Mester et al. 2011; [8]Wang et al. 2017; [9]Sanchez-Puelles et al. 2019; [10]Smith et al. 2016; [11]Lugo et al. 2014; [12]Binder and Lugo 2017; [13]Lugo et al. 2017; [14]Hodges et al. 2018; [15]Backman et al. 2001; [16]Kwon et al. 2001; [17]Kwon et al. 2003; [18]Ljungberg et al. 2009; [19]Nguyen et al. 2015; [20]Kwon et al. 2006; [21]Zhou et al. 2009; [22]Napoli et al. 2012; [23]Ogawa et al. 2007; [24]Nolan et al. 2019; [25]Amiri et al. 2012; [26]Gregorian et al. 2009; [27]Borniger et al. 2016; [28]Clipperton-Allen et al. 2016; [29]Cupolillo et al. 2016; [30]Diaz-Ruiz et al. 2009; [31]Matsushita et al. 2016; [32]Sperow et al. 2012; [33]Vogt et al. 2015; [34]Gutilla et al. 2016; [35]Haws et al. 2014.

nile), either freely moving or contained within a tube (thus requiring the experimental animal to initial social contact). The second uses the three-chamber social approach test. In this assay, the experimental animal is habituated to an arena containing three chambers of equal size. A novel conspecific in a tube or cage is placed in one chamber, and a tube or cage (empty or containing a novel object) is placed in another, with the center chamber left empty. The amount of time spent either in the chambers, in the area around the tubes, or actively investigating the tubes is then calculated, and a significant difference between the social and nonsocial investigation indicates social interest or a social preference.

The majority of *Pten* mutant models tested have shown a decrease in or lack of social preference (see Table 2; Kwon et al. 2006; Page et al. 2009; Zhou et al. 2009; Amiri et al. 2012; Napoli et al. 2012; Clipperton-Allen and Page 2014; Lugo et al. 2014; Séjourné et al. 2015; Vogt et al. 2015; Cupolillo et al. 2016; Huang et al. 2016; Sanchez-Puelles et al. 2019). Only two models showed increased social interest: *Pten* overexpression mice ($Pten^{Tg}$; Sanchez-Puelles et al. 2019) and cytoplasm-predominant Pten mice ($Pten^{m3m4}$; Tilot et al. 2014). However, a few models showed normal social approach behavior (see Table 2; Haws et al. 2014; Borniger et al. 2016; Clipperton-Allen et al. 2016). Whereas most studies used only one sex, combined sexes, or did not state the sex of the animals tested, those that did analyze males and females separately found that the phenotype displayed sexual dimorphism and was generally only present in one sex (see Table 3; Page et al. 2009; Clipperton-Allen and Page 2014; Tilot et al. 2014; Smith et al. 2016). The exception to this pattern was a line in which *Pten* was conditionally deleted in oxytocinergic neurons (*Oxt-Cre*; $Pten^{loxP}$), which displayed no deficits in either sex (Clipperton-Allen et al. 2016). This highlights the importance of including both sexes and analyzing them separately, particularly in models of a disorder as highly sexually dimorphic as ASD. Interestingly, of the models that did identify sex differences, the deficits were in females ($Pten^{+/-}$ [Page et al. 2009], *DAT-Cre*; $Pten^{loxP}$ [Clipperton-Allen and Page 2014], *NS-Cre*; $Pten^{loxP}$ [Lugo et al. 2014; Smith et al. 2016]), whereas the increased social interest observed in the $Pten^{m3m4}$ mice was only present in males (Tilot et al. 2014). This raises the intriguing possibility that gonadal hormones may interact with *Pten* mutations to influence sociability phenotypes.

Social Recognition

Social recognition, in its simplest form, is the ability to distinguish between a novel conspecific and one that has been encountered before. There is some evidence that this process may be disrupted in ASD (Weigelt et al. 2012; Ewbank et al. 2017). Social recognition can be tested in several ways using mouse models. Three-chamber social novelty follows from the three-chamber social approach test, with a novel social stimulus being placed into the tube that previously was empty or held an object; the test animal should show a preference for the novel social chamber, tube, or stimulus. Alternatively, social recognition can be assessed using habituation (with or without dishabituation). This takes advantage of the fact that mice will spend decreasing amounts of time investigating a conspecific with repeated exposures (habituation). In some cases, this is followed by a dishabituation trial in which a novel stimulus is introduced, and the test animal should show an increase in investigation, indicating that it has habituated to the individual stimulus and not the testing situation.

Although fewer models have been tested for social recognition than social interest, most lines do show deficits (see Table 2; Kwon et al. 2006; Zhou et al. 2009; Amiri et al. 2012; Napoli et al. 2012; Clipperton-Allen and Page 2014; Lugo et al. 2014; Clipperton-Allen et al. 2016), although others show no alterations in social recognition (Haws et al. 2014; Tilot et al. 2014). As with social interest, the majority of studies did not look for sexual dimorphism, but of those that did, with the exception of the $Pten^{m3m4}$ line, the deficit was restricted to the male subjects (see Table 3; Page et al. 2009; Clipperton-Allen and Page 2014; Clipperton-Allen et al. 2016).

Table 3. Behavioral phenotyping in studies of mouse models that compared sexes (black, males; white, females; red, increase in behavior in males; gray, no change in behavior; blue, decrease in behavior in males [dark] or females [light])

Mouse model	Type:	Germline	Germline	Conditional (*Pten*^{loxP})	Conditional (*Pten*^{loxP})	Conditional (*Pten*^{loxP})
	Line:	*Pten*$^{+/-}$	*Pten*^{m3m4}	*NS-Cre*	*Oxt-Cre*	*DAT-Cre*
Behavior category	Perturbation	Germline heterozygous	↓ Nuclear: cytoplasm ratio	CB and DG granule cells, some CTX neurons	Oxytocinergic cells	Dopaminergic cells
	Sex / Behavior	♂ ♀	♂ ♀	♂ ♀	♂ ♀	♂ ♀
Social behaviors	Social interest	—[1] ↓[2]	↓[1,2,4,5]	↑[6] —[6]		—[8] —[8]	—[2] ↓[2]
	Social recognition	↓[1,2]	—[2]	—[6] —[6]		↓[8] —[8]	↓[2] —[2]
	USVs				↓[7] ↓[7]		
Repetitive behaviors	Marble burying	↑[2]	—[2]			—[8]	↓[8]
	Self-grooming	—[3]	—[3]				
	Stereotypies	—[3]	—[3]				
Mood and anxiety	Anxiety	↓[2]	—[2]	—[6] —[6]		—[8] ↓[8]	—[8]
	Depression	↑[2]	—[2]			—[8]	—[8]
Intellectual disability	Fear conditioning	—[2]	↓[2]				
	Nonaversive spatial L&M	—[4]	—[4]				
	Novelty recognition			—[6] —[6]			
Circadian	Activity	—[2]	↓[2]				
	Tau	—[2]	—[2]				
Sensorimotor	PPI	↓[1]	↓[1]				
Basic sensory/motor	Olfaction	—[1]	—[1]				
	Pain perception	—[2]	—[2]	—[6] —[6]			
Motor behaviors	Locomotion	—[2]	—[2]	—[6] —[6]		—[8]	—[8]
	Balance	—[2]	—[2]	↓[6] ↓[6]		—[8]	—[8]
	Motor learning	—[2]	—[2]	—[6] —[6]		—[8]	—[8]

(CB) cerebellum, (DG) dentate gyrus, (CTX) cortex, (L&M) learning and memory, (PPI) prepulse inhibition, (USVs) ultrasonic vocalizations.

References: [1]Page et al. 2009; [2]Clipperton-Allen and Page 2014; [3]Clipperton-Allen and Page 2015; [4]Huang et al. 2016; [5]Séjourné et al. 2015; [6]Tilot et al. 2014; [7]Binder and Lugo 2017; [8]Clipperton-Allen et al. 2016.

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Other Social Behaviors

Increased aggression has been reported in up to 70% of individuals with ASD (McClintock et al. 2003; Holden and Gitlesen 2006; Hartley et al. 2008; Kanne and Mazurek 2011; Maskey et al. 2013). When a novel male is introduced to the cage of a male mouse, they will often engage in agonistic behavior to establish a dominance hierarchy. The effect of *Pten* mutations on these interactions has only been assessed in males the germline heterozygous $Pten^{+/-}$ line and the conditional mutants *Fsp1-Cre*; $Pten^{loxP}$ and *Oxt-Cre*; $Pten^{loxP}$, with the latter showed no major differences (Clipperton-Allen et al. 2016). Interestingly, agonistic dominance behavior, but not overt attacks, were decreased in both $Pten^{+/-}$ and *Fsp1-Cre*; $Pten^{loxP}$ males (Clipperton-Allen and Page 2015; Borniger et al. 2016). Although this is the opposite of what the ASD phenotype would predict, it suggests that the circuitry controlling aggression may be abnormal when *Pten* is perturbed.

A few other social behavior assays have also been tested in *Pten* mutant models. Nest building was decreased in *Nse-Cre*; $Pten^{loxP}$ mice (Kwon et al. 2006), and *L7-Cre*; $Pten^{loxP}$ males showed decreased social interaction, including decreased sexual contact, with a novel female in a free social interaction (Cupolillo et al. 2016). Social olfaction was found to be normal in *Nestin-Cre*; $Pten^{loxP}$ males (Amiri et al. 2012).

Although it is challenging to assess communication deficits in mice, measurements of ultrasonic vocalizations (USVs), typically assessed during brief maternal separation in mice <2 weeks of age, has proven to be a useful proxy. In the few lines tested for USV abnormalities, no differences were found in the *Pten-ΔPDZ* line (Sanchez-Puelles et al. 2019), but deficits were observed in $Pten^{Tg}$ overexpression mice (Sanchez-Puelles et al. 2019). Interestingly, one study found USV deficits in both male and female *NS-Cre*; $Pten^{loxP}$ juveniles (Binder and Lugo 2017), but another study in mice of the same line found no differences in juveniles of unidentified sex(es) (Lugo et al. 2014).

Taken together, these data clearly indicate that *Pten* mutations can disrupt social behavior in mouse models, although the nature of the disruption may depend on the type and site of *Pten* perturbation. Further research on the less common social behaviors across models, especially social recognition, aggression, and USVs, may help to elucidate the circuitry by which social behavior is altered. Additionally, the sexual dimorphism observed in the few studies that compared males and females highlights the importance of analyzing both sexes separately, particularly in light of the sex disparity in humans with ASD.

Repetitive Behaviors

In addition to abnormal social behavior and communication, restricted, repetitive behaviors and interests is the other core symptom of ASDs (American Psychiatric Association 2013). This has been assessed in mice in three main ways. The marble burying test assesses repetitive digging by placing a mouse in a cage with clean bedding and a number of marbles; more marbles buried indicates increased repetitive behavior (Hoeffer et al. 2008; Thomas et al. 2009; Silverman et al. 2010). The observation of spontaneous stereotypies, including flips, spins, circling the cage, etc., is another indicator of repetitive behavior. Finally, self-grooming is often considered a repetitive behavior, although as this is also a normal cleaning behavior, it is preferable to only use either excessive self-grooming resulting in hair loss, or abnormal or interrupted sequences of grooming as an indication of repetitive behaviors.

Altered repetitive behavior is seen in several models of *Pten* mutations, often with sexually dimorphic phenotypes in those studies that assessed sex differences (see Tables 2 and 3). Marble burying was increased in male, but not female $Pten^{+/-}$ mice (Clipperton-Allen and Page 2014). Female, but not male *Oxt-Cre*; $Pten^{loxP}$ mice (Clipperton-Allen et al. 2016), and likely female, but not male *NS-Cre*; $Pten^{loxP}$ mice, showed decreased marble burying (Lugo et al. 2014; Smith et al. 2016). *NS-Cre*; $Pten^{loxP}$ mice showed variable results in terms of other repetitive behaviors: in unspecified sex(es), repetitive behavior on the hole-board test, another assay of

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repetitive behavior, and self-grooming in the open field were decreased in one study (Lugo et al. 2014), although increased stereotypy was also shown in 6-week-old males (Lugo et al. 2017), and normal self-grooming was also observed (Smith et al. 2016). No differences in this behavior were found in *Pten*^{Tg} mice (Sanchez-Puelles et al. 2019); decreased self-grooming and stereotypes were also found in *L7-Cre*; *Pten*^{loxP} males (Cupolillo et al. 2016).

Because of the paucity of repetitive behavior testing in *Pten* mutant mice, it is difficult to draw meaningful conclusions; thus, more testing of models of both sexes is needed. However, the data do suggest that repetitive behavior may be increased in males with decreased *Pten*, and possibly decreased in females; *Pten* overexpression does not appear to affect this behavior.

COMORBID AND OTHER BEHAVIORS

Mood and Anxiety

Anxiety

ASD is frequently comorbid with anxiety disorders (Skokauskas and Gallagher 2010). Anxiety-like behavior is commonly measured using three assays: the open field test ([OFT]; an empty box lit with white light), the elevated plus maze test ([EPM]; a plus-shaped maze with two open and two enclosed arms), and the dark–light assay (a box with one dark, covered chamber and one light, brightly lit chamber). These paradigms all take advantage of the tendency of mice to avoid bright open spaces, which make them more vulnerable to predation, especially from above; as such, less time spent in the center of the open field, the open arms of the EPM, or the light chamber of the dark–light assay all indicate more anxiety-like behavior (Mozhui et al. 2010).

Although the majority of models tested for anxiety-like behavior show no phenotypes (see Table 2; Haws et al. 2014; Tilot et al. 2014; Vogt et al. 2015; Borniger et al. 2016; Gutilla et al. 2016; Wang et al. 2017; Sanchez-Puelles et al. 2019), increased anxiety-like behavior was observed in *Nse-Cre*; *Pten*^{loxP} mice on the OFT and dark–light tests, but not the EPM (Kwon et al. 2006; Zhou et al. 2009). Interestingly, all other models showing phenotypes showed decreased anxiety-like behavior (see Table 2; Clipperton-Allen and Page 2014; Lugo et al. 2014; Clipperton-Allen et al. 2016; Sanchez-Puelles et al. 2019). As in other assays, sexual dimorphism was observed in those studies comparing males and females (see Table 3): decreased anxiety was observed in *Pten*$^{+/-}$ and *Oxt-Cre*; *Pten*^{loxP} males but not females (Clipperton-Allen and Page 2014; Clipperton-Allen et al. 2016), and as well as being shown by *NS-Cre*; *Pten*^{loxP} mice of indeterminate sex (Lugo et al. 2014) but not by males (Smith et al. 2016), suggesting that the observed phenotype could be present in females.

Depression

Depression and other mood disorders are frequently comorbid with ASD (Skokauskas and Gallagher 2010). Depressive-like behavior in mice is tested by measuring immobility in the forced swim test ([FST]; the mouse is put into a small pool of water and the time floating (immobile) and swimming or climbing is measured) or the tail suspension test ([TST]; the mouse is suspended by the tail and the time spent immobile or struggling is measured). Both the FST and the TST are responsive to antidepressants.

Only two *Pten* mutant models were tested for depressive-like behavior (see Table 2; Clipperton-Allen and Page 2014; Clipperton-Allen et al. 2016), and of these, only male *Pten*$^{+/-}$ mice showed any phenotype, showing increased depressive-like behavior. These data suggest that *Pten* mutations may leave mood and anxiety phenotypes largely unaffected, although further testing is warranted. Notably, anxiety or depression have not been commonly reported in patients with ASD and *PTEN* mutations to date.

Intellectual Disability

Intellectual disability is another common comorbidity with ASD (Matson and Shoemaker 2009). No *Pten* mutants showed substantially improved learning and memory when tested on aversive or appetitive tasks, both spatial and non-

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spatial. However, approximately half of the lines tested on each task showed deficits, whereas the remainder showed no impairment (see Table 2).

Aversive Learning and Memory Tasks

When tested in fear conditioning, which involves learning to associate a context or cue with a foot shock and thus is aversively motivated, *NS-Cre*; *Pten*^{loxP} mice, as well as *Nse-Cre*; *Pten*^{loxP}, *Pten-ΔPDZ*, and mice with shRNA in the lateral amygdala, showed no impairments (see Table 2; Kwon et al. 2006; Haws et al. 2014; Smith et al. 2016; Sanchez-Puelles et al. 2019). However, female, but not male, *Pten*$^{+/-}$ mice did show deficits, as did *Pten*α^{mu} males and *Pten*^{Tg} mice of combined sexes (Clipperton-Allen and Page 2014; Wang et al. 2017; Sanchez-Puelles et al. 2019).

Several lines were also tested on a variety of aversively motivated spatial learning and memory tasks. The most common aversive spatial learning task is the Morris water maze (MWM), which involves a mouse learning the location of a hidden platform in a pool of water; shorter trials or distances traveled indicate improved learning. Additionally, probe trials (with no platform present) measure the amount of time or number of crossings of the area where the platform should be. A similar task is the Barnes maze, which relies on bright light and sound to motivate mice to escape a platform by learning which hole in the round maze leads to a "safe" cage. Thus, these aversively-motivated tasks test spatial learning (acquiring the location of the safe area, shown by shorter times and distances); the probe trials in the MWM also assess memory for where the escape platform should be located. Spatial learning was normal in *NS-Cre*; *Pten*^{loxP}, *L7-Cre*; *Pten*^{loxP}, and *CaMKIIα-Cre*; *Pten*^{loxP} mice (see Table 2; Sperow et al. 2012; Cupolillo et al. 2016; Smith et al. 2016), but impaired in *Pten*α^{mu}, *Nse-Cre*; *Pten*^{loxP}, and *Pten*^{Tg} mutants (Kwon et al. 2006; Wang et al. 2017; Sanchez-Puelles et al. 2019). Spatial memory was also impaired in *Pten*α^{mu} and *Nse-Cre*; *Pten*^{loxP} mice, as well as in *CaMKIIα-Cre*; *Pten*^{loxP} mutants (Kwon et al. 2006; Sperow et al. 2012; Wang et al. 2017).

Nonaversive Learning and Memory Tasks

In novel object recognition or investigation (mice should show increased investigation of the novel object, indicating that they recognize the familiar object), *NS-Cre*; *Pten*^{loxP} and *Nkx2.1-Cre*; *Pten*^{loxP} mice showed impairments, whereas *Fsp1-Cre*; *Pten*^{loxP} and *Pten*^{m3m4} mice did not (see Table 2; Tilot et al. 2014; Vogt et al. 2015; Borniger et al. 2016; Hodges et al. 2018).

Normal spatial ability was seen in the three models that used the spontaneous alternation and novel object location assays (see Table 2), which both use the tendency of mice to prefer to explore a novel location or an object that has been moved to a new location (Borniger et al. 2016; Huang et al. 2016; Sanchez-Puelles et al. 2019). The only appetitive assay to show a deficit was the Lashley maze, in which mice are required to learn a path from the start area to the goal box; *NS-Cre*; *Pten*^{loxP} mice made more errors and took longer to complete this task (Hodges et al. 2018).

Sleep and Circadian Rhythms

Parental reports estimate 50%–80% of children with ASD have difficulty with sleep, compared with 9%–50% of age-matched control children (Polimeni et al. 2005; Allik et al. 2006; Doo and Wing 2006; Giannotti et al. 2008; Richdale and Schreck 2009). These difficulties include problems with sleep initiation and maintenance, less time in slow-wave, REM, and overall sleep, as well as unstable sleep and irregular sleep–wake patterns (Schenck et al. 1987; Miano et al. 2007; Malow and McGrew 2008; Stores 2008; Maes et al. 2011; Vriend et al. 2011; Kotagal and Broomall 2012).

Sleep disturbances and circadian rhythm have been lightly studied in mouse models of *Pten* mutations. *Nse-Cre*; *Pten*^{loxP} males show a longer free-running circadian rhythm (tau) when in constant dark (Ogawa et al. 2007), but neither male nor female *Pten*$^{+/-}$ mice showed an abnormal tau (Clipperton-Allen and Page 2014). However, both lines showed abnormal activity (as measured by wheel running) during a normal 12:12 h light/dark cycle, with *Pten*$^{+/-}$

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females and *Nse-Cre*; *Pten*^{loxP} males showing decreased activity during the dark phase (Ogawa et al. 2007; Clipperton-Allen and Page 2014). More research is needed to form conclusions about the effect of *Pten* mutations on sleep and circadian rhythms.

Sensorimotor and Basic Sensory and Motor Assessments

Acoustic Startle and Prepulse Inhibition

A few models of *Pten* mutations were assessed for their startle response to an acoustic stimulus, and/or their ability to inhibit this response following a lower decibel tone preceding the startle stimulus (prepulse inhibition [PPI]). *Nse-Cre*; *Pten*^{loxP} mice showed an increased response to the 120 dB white noise stimulus during initial trials, but this response normalized with repeated presentations (Kwon et al. 2006). Additionally, both *Nse-Cre*; *Pten*^{loxP} and both sexes of *Pten*$^{+/-}$ mice showed impaired PPI (Kwon et al. 2006; Page et al. 2009).

Basic Sensory and Motor Abilities

No deficits were found in any *Pten* mutant models for olfaction or pain perception, with deletion of *Pten* in neural stem cells actually improving olfactory abilities (see Table 2; Kwon et al. 2006; Gregorian et al. 2009; Page et al. 2009; Clipperton-Allen and Page 2014; Haws et al. 2014; Lugo et al. 2014; Tilot et al. 2014; Borniger et al. 2016). The only sensory deficit observed was in auditory orientation in the *Fsp1-Cre*; *Pten*^{loxP} model (Borniger et al. 2016). Thus, *Pten* mutations appear to leave basic sensory skills largely intact. Similarly, although few studies have investigated basic motor abilities, *Nse-Cre*; *Pten*^{loxP} mice show normal endurance and grip strength (Kwon et al. 2006), although the grip strength of *Fsp1-Cre*; *Pten*^{loxP} males, but not females, was impaired (Borniger et al. 2016).

Given the growing evidence of sensory abnormalities, more investigation, particularly of tactile and auditory sensory perception, is necessary.

Motor Behaviors

One of the most common phenotypes reported in humans with *PTEN* mutations, outside of the core ASD domains, is motor behavior impairments.

Locomotion

Very few *Pten* mutant mice showed abnormal locomotion, which is typically measured by the distance traveled in an open field, or during the three-chamber assay, both as an assessment of basic activity and as a control for assays requiring locomotion (e.g., social approach, social novelty, light–dark, etc.). Only a small subset of conditional knockout models showed altered locomotor behavior, and even in these the results were inconsistent. Both the *NS-Cre*; *Pten*^{loxP} and *Nse-Cre*; *Pten*^{loxP} lines showed increases in distance traveled, although only when older than 6 weeks of age, and in the case of *Nse-Cre*; *Pten*^{loxP} mice, only under stressful conditions (Kwon et al. 2006; Zhou et al. 2009; Lugo et al. 2014, 2017). *DAT-Cre*; *Pten*^{loxP} mice of unknown sex(es) showed normal open field activity, but males traveled a shorter distance than controls in the three-chamber test (Diaz-Ruiz et al. 2009; Clipperton-Allen and Page 2014). Given these results, and the normal locomotion observed in the vast majority of models tested (see Table 2; Amiri et al. 2012; Clipperton-Allen and Page 2014; Haws et al. 2014; Tilot et al. 2014; Vogt et al. 2015; Clipperton-Allen et al. 2016; Cupolillo et al. 2016; Gutilla et al. 2016; Wang et al. 2017; Sanchez-Puelles et al. 2019), it is highly likely that effects of a *Pten* mutation on locomotor activity are minimal at most.

Balance, Motor Learning, and Fine Motor Skills

Rotarod testing is a standard assay used to assess balance and motor learning. This simple test involves placing the animal on a rotating bar that typically accelerates from 4 to 40 rpm over a period of 2–3 min. Balance is measured by the latency to fall, whereas motor learning is assessed by looking at increases in fall latency

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across several trials or days. Fall latency was increased in half of the *Pten* mutant models tested, specifically the *Pten*^{m3m4}, *Fsp1-Cre*; *Pten*^{loxP}, and 5- to 6-month-old *L7-Cre*; *Pten*^{loxP} mutants (see Table 2; Clipperton-Allen and Page 2014; Tilot et al. 2014; Borniger et al. 2016; Clipperton-Allen et al. 2016; Cupolillo et al. 2016; Gutilla et al. 2016). Additionally, *Nse-Cre*; *Pten*^{loxP} mice were impaired in one of the studies that tested them on this assay (Nolan et al. 2019) but not the other (Kwon et al. 2006). Interestingly, only the *L7-Cre*; *Pten*^{loxP} mice showed less improvement across trials than controls (Cupolillo et al. 2016), whereas acquisition of the task was actually improved in *Nse-Cre*; *Pten*^{loxP} mutants (Kwon et al. 2006). No other tested line showed alterations on rotarod motor learning (Clipperton-Allen and Page 2014; Tilot et al. 2014; Borniger et al. 2016; Clipperton-Allen et al. 2016; Gutilla et al. 2016).

Despite the increasingly well-established impairment in fine motor skills in humans with *PTEN* mutations and ASD, only one study assessed this behavior in mice. This study used the sticker removal task, where a small adhesive sticker is placed on the nose of the animal, and the time to attempt and/or completely remove the sticker is measured. Although normal on the first trial, *Nse-Cre*; *Pten*^{loxP} mice showed impairment on the second and third trials (Nolan et al. 2019). Clearly, more investigation of fine motor skills in mouse models of *Pten* mutations is a crucial need.

Spontaneous Observations

Spontaneous seizures, ataxia, and/or premature death were observed in a subset of the conditional knockout models (see Table 2). Seizures were noted in *NS-Cre*; *Pten*^{loxP}, *Nse-Cre*; *Pten*^{loxP}, *Nestin-Cre*; *Pten*^{loxP}, and *Pomc-Cre*; *Pten*^{loxP} mutants (Backman et al. 2001; Kwon et al. 2001, 2003, 2006; Ogawa et al. 2007; Ljungberg et al. 2009; Zhou et al. 2009; Amiri et al. 2012; Nguyen et al. 2015; Matsushita et al. 2016), with the *NS-Cre*; *Pten*^{loxP} line also showing ataxia and premature death (Backman et al. 2001; Kwon et al. 2001, 2003; Ljungberg et al. 2009; Nguyen et al. 2015). No such phenotypes were noted in germline or conditional heterozygous *Pten* mutant lines, with the only exception being premature death in *Pten*^{m3m4} mice and in *Pten*$^{+/-}$ mice also lacking the serotonin receptor 2C (Page et al. 2009; Mester et al. 2011; Napoli et al. 2012; Clipperton-Allen and Page 2014, 2015; Tilot et al. 2014; Séjourné et al. 2015; Huang et al. 2016; Wang et al. 2017; Sanchez-Puelles et al. 2019). Thus, although conditional knockout *Pten* models are highly valuable for understanding the neurobiology of *Pten*, it seems that more disease-relevant heterozygous mutations have a much more subtle effect on seizure susceptibility in mice.

CONCLUDING THOUGHTS AND FUTURE DIRECTIONS

The aim of this review has been to define a "profile" of behaviors that are sensitive to the effects of *Pten* mutations based on mouse model studies to date. Although we have highlighted some areas in which deeper phenotyping is needed, some of the most reproduced behavioral deficits across models thus far are in the domains of social interest, social recognition, and repetitive behavior. Considering the broad expression of *Pten* in the developing brain and the substantial changes in brain and neuronal growth, structural connectivity, intrinsic properties, and synaptic connectivity/plasticity caused by loss of *Pten* function, the relatively selective effects on behavior are striking (see Table 2). Identifying brain areas and circuits that are vulnerable to reduced *Pten* function will be a critical area of future research and will be informative for developing targeted therapeutic strategies to offset the consequences of *PTEN* haploinsufficiency. Conditional genetic approaches to manipulating *Pten* have already shown the importance of forebrain glutamatergic and GABAergic neurons, midbrain dopaminergic neurons, and cerebellar Purkinje cells. Next steps will involve narrowing down these broad categories of cell types into specific circuits that may underlie abnormal social information processing and behavior in a *Pten* mutant background. Likewise, it is clear that, across *Pten* models in which both males and females have been tested, sexual dimor-

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phism in behavioral phenotypes appears to be more the rule than the exception (see Table 3). Thus, research into neurobiological mechanisms by which gonadal hormones may interact with *Pten* mutations to influence ASD-related behaviors is an important area for the future.

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