

The neurobiology of mouse models syntenic to human chromosome 15q

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Abstract Autism is a neurodevelopmental disorder that manifests in childhood as social behavioral abnormalities, such as abnormal social interaction, impaired communication, and restricted interest or behavior. Of the known causes of autism, duplication of human chromosome 15q11–q13 is the most frequently associated cytogenetic abnormality. Chromosome 15q11–q13 is also known to include imprinting genes. In terms of neuroscience, it contains interesting genes such as *Necdin*, *Ube3a*, and a cluster of GABA_A subunits as well as huge clusters of non-coding RNAs (small nucleolar RNAs, snoRNAs). Phenotypic analyses of mice genetically or chromosomally engineered for each gene or their clusters on a region of mouse chromosome seven syntenic to human 15q11–q13 indicate that this region may be involved in social behavior, serotonin metabolism, and weight control. Further studies using these models will provide important clues to the pathophysiology of autism. This review overviews phenotypes of mouse models of genes in 15q11–q13 and their relationships to autism.

Keywords 15q11–q13 · Mouse model · Autism · Chromosome · CNV · Serotonin · Epigenetics

Autism

Autism is a developmental brain disorder whose definition has been extended to autism spectrum disorders (ASDs), including non-pervasive development disorder and Asperger syndrome (Levitt and Campbell 2009). The prevalence of autism is increasing and currently stands at one in every 150 individuals. Its influence on modern society is increasing, especially in developed countries, where birthrates are low and the management of children with these disorders is a serious social issue. However, the cause of autism and its pathophysiology remain unknown and require further research from a number of different approaches. Among the neuropsychiatric diseases, autism is considered as an appropriate disorder that can be approached from biological aspects. This is partly because genetic studies show that the concordance rate of autism in identical twins is over 90%, which is higher than other neuropsychiatric diseases such as schizophrenia and mood disorders (Abrahams and Geschwind 2008).

Most autism cases are sporadic and their causes are still unknown; however, 10–20% have known causes, such as (1) comorbidity with other diseases, such as fragile X syndrome, tuberous sclerosis, and Rett syndrome; (2) genome abnormalities, including copy number variations (CNVs), such as 15q11–q13 and 16q11.2; and (3) single gene mutations such as *NLGN3/4*, *NRXN1*, and *SHANK2/3* (Abrahams and Geschwind 2008; Berkel et al. 2010; Cook and Scherer 2008; Kumar et al. 2008; Marshall et al. 2008; Pinto et al. 2010; Toro et al. 2010; Vörstman et al. 2006; Weiss et al. 2008). Recent genetic screening using cutting-edge technologies has revealed more CNV cases than previously expected (Cook and Scherer 2008). Given the

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rapid progress in current technologies, such as array comparative genomic hybridization (array CGH) and next generation sequencers, and their availability for clinical use, it is likely that more CNV cases will be diagnosed in the near future.

CNVs are important not only in explaining human heterogeneity but also because they are responsible for several diseases, especially neuropsychiatric disorders including autism. Human chromosome 15q11–q13 is the second most frequent region to contain chromosome abnormalities, probably because it contains large repeat sequences. Depending on its copy variant, this region causes different diseases, such as Prader–Willi syndrome (PWS), Angelmann syndrome (AS), and autism, all sharing ASD features. Comparison of mouse models of 15q copy variants with phenotypes of genetically engineered mice of each gene, with or without imprinting, will provide some clues to the pathophysiology of autism.

Mouse chromosome seven is syntenic to human chromosome 15q11–q13

Chromosomal abnormalities were known as causes of autism before the era of current genome technology. Among them, human 15q11–q13 is the most notable chromosome region (Vorstman et al. 2006) (Fig. 1). This region is also known to be an imprinting region (Chamberlain and Lalande 2010a, b; Nicholls and Knepper 2001). Thus, there are three classified regions: paternally, maternally, and biallelically expressed regions. Human chromosome 15q11–q13 is conserved in mouse chromosome seven and it is believed that the imprinting status is conserved between mouse and human (Yang et al. 1998). Knockout (KO) mice for each gene corresponding to a human counterpart have been generated and studied (Table 1).

Paternally expressed genes

In the paternally expressed regions of the mouse chromosome, *Mkrn3*, *Magel2*, *Necdin* (*Ndn*), *Snrpn-Snurfl*, and huge clusters of small nucleolar RNAs (snoRNAs) are present. *Magel2* is involved in hypothalamic functions, and

its deficient mice show abnormal circadian rhythm, body weight, and hormonal secretion, such as testosterone (Bischof et al. 2007; Kozlov et al. 2007; Mercer and Wevrick 2009). *Ndn* was first identified as a gene related to neural differentiation (Maruyama et al. 1991). *Ndn* KO mice were generated from multiple laboratories; however, they display different phenotypes, from no abnormality to respiratory distress and lethality, similar to PWS, depending on the strain of mouse (Gerard et al. 1999; Kuwako et al. 2005; Muscatelli et al. 2000; Tsai et al. 1999a). Muscatelli et al. observed a neonatal lethality of variable penetrance in *Ndn* mutants, and they showed a reduction in both oxytocin-producing and luteinizing hormone-releasing hormone (LHRH)-producing neurons in the hypothalamus and emotional and cognitive-related behaviors reminiscent of PWS (Muscatelli et al. 2000). Interestingly, oxytocin is known to be related to social behavior and is expected to be a candidate for therapeutics of ASD (Insel 2010). *Ndn* deficiency induces perinatal serotonergic alterations that affect the maturation and function of the respiratory network, inducing respiratory problems (Zanella et al. 2008). Serotonergic and catecholaminergic projections are reduced in the *Ndn* null embryo (Lee et al. 2005). Provided that serotonin is involved in symptoms of autism, *Ndn* is a candidate for further studies to reveal its involvement in abnormal social behavior. Additionally, from studies using *Ndn* KO mice, it is reported that *Ndn* is involved not only in *TrkA* signaling (Kuwako et al. 2005) but also promotes tangential migration of neocortical interneurons from the basal forebrain (Kuwajima et al. 2010). The fact that the neocortical interneurons are important for development of inhibitory neural circuits also makes *Ndn* a possible candidate for causing an imbalance of the excitatory/inhibitory ratio. Thus, although it is still controversial whether *Ndn* is a cause of PWS, it is an intriguing target gene for further analyses in terms of developmental brain disorders, including ASDs. *Snrpn* is a small nuclear ribonucleoprotein N and the *Snrpn* promoter is located in a maternally methylated CpG island within an imprinting center (IC). Mutations of *Snrpn* are not sufficient to induce PWS in mice, but a larger deletion including the PWS-IC and *Ndn* show phenotypes common to PWS infants (Yang et al. 1998). Paternal transmission of IC deletions results in a PWS-like phenotype in mice (Bielinska et al. 2000). The

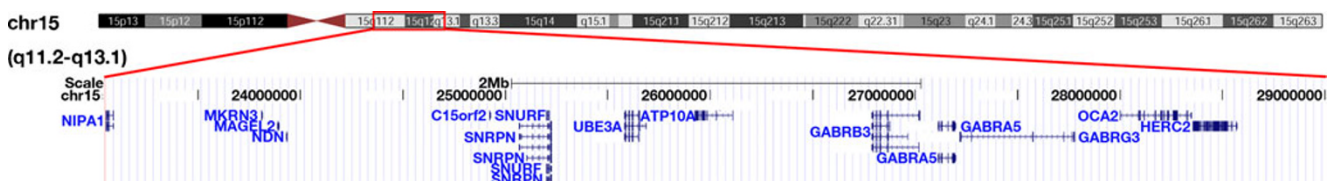


Fig. 1 Genes on human chromosome 15q11–q13. Clusters of non-coding RNA, such as HBII-52 and HBII-85, are omitted. The original data are taken from UCSC Genome Browser (<http://genome.ucsc.edu>)

Table 1 Genes on human chromosome 15q11–q13 and the phenotypes of their genetically engineered mice

Locus	Line	Phenotypes	Reference
Mkrm3	KO	No obvious phenotypes	Jong et al. 1999
Magel2	Targeted reporter (LacZ insertion)	Neonatal growth retardation, excessive weight gain, and increased adiposity after weaning	Bischof et al. 2007
	Targeted reporter (LacZ insertion)	Altered circadian rhythms, hypophagia, reduced orexin neurons in the lateral hypothalamus	Kozlov et al. 2007
	Targeted reporter (LacZ insertion)	Extended and irregular estrous cycle, decreased testosterone, reduced olfactory preference for female odors	Mercer and Wevrick 2009
Ndn	Conventional KO	Neonatal lethality due to impaired suckling	Schaller et al. 2010
	Conventional KO	Early postnatal lethality due to respiratory defect	Gerard et al. 1999
	Targeted reporter (LacZ insertion)	No obvious phenotypes	Tsai et al. 1999a
	Conventional KO	Postnatal lethality, reduced oxytocin producing and luteinizing hormone releasing hormone producing neuron, skin scraping, increased spatial learning	Muscattelli et al. 2000
	Conventional KO	Reduced substance P containing neuron, high tolerance to thermal pain	Kuwako et al. 2005
	Conventional KO	Central respiratory deficits, altered serotonergic metabolism, enlargement of 5-HT vesicles	Zanella et al. 2008
	Conventional KO	Impaired tangential migration of cortical interneuron	Kuwajima et al. 2010
PWS-IC	Conventional KO	Died shortly after birth, small	Yang et al. 1998
	Conventional KO	Imprinting defect	Bielinska et al. 2000
	Gene targeting (SNRPN human exon 1 switch)	Paternal transmission postnatal lethality (not fully penetrant), growth deficiency. Loss of Ndn, Magel2, and Mkrm3 expression	Johnstone et al. 2006
Snurf	Conventional KO	No obvious phenotype	Tsai et al. 1999b
Snrpn	Conventional KO	No obvious phenotype	Yang et al. 1998
	Conventional KO (Ex1=0.9 kb)	No obvious phenotype	Bressler et al. 2001
	Conventional KO (Ex1=4.8 kb) PWS-IC ^{+/-}	40–50% lethality (strain dependency)	Bressler et al. 2001
MBII-85/ Snord116	Chromosome engineering (Cre lox deletion)	Early onset postnatal growth deficiency, defect of motor learning, increased anxiety, hyperphagia	Ding et al. 2008
	Chromosome engineering (Cre lox deletion)	Postnatal lethality (strain dependency), growth retardation	Skryabin et al. 2007
MBII-52/ Snord115	Radiation induced mutant (large deletion) p ^{30pU^b}	Paternal transmission: loss of MbII-52/Snord115 and Ube3a expression. No obvious phenotype	Ding et al. 2005
	Conventional KO (Ex1=4.8 kb) PWS-IC ^{+/-}	Increased in editing of 5htr2c, impulsive responding, less locomotor activity, reactivity to palatable foodstuffs	Doe et al. 2009
Snurf-Snrpn to Ube3a deletion	Chromosome engineering (Cre-loxP deletion)	Partial postnatal lethality, growth deficiency	Tsai et al. 1999b
	Conventional KO (maternal transmission)	Maternal transmission: reduced brain weight, impaired motor function, inducible seizures, deficit in context-dependent fear conditioning, abnormal hippocampal electroencephalographic recordings and severely impaired long-term potentiation	Jiang et al. 1998
	Targeted reporter (maternal transmission LacZ insertion)	Maternal transmission: reduced brain weight, impaired motor function, impaired spatial learning, deficit in context-dependent fear conditioning, abnormal hippocampal electroencephalographic recordings	Miura et al. 2002
	Targeted reporter (maternal transmission LacZ insertion)	Sleep disturbance	Colas et al. 2005

Table 1 (continued)

Locus	Line	Phenotypes	Reference
	Conventional KO (maternal transmission)/ CamKII-T305V/T306A double mutant	Rescued various abnormalities in Ube3a ^{-/+} mutant; reduced seizure, improved motor coordination and spatial learning	van Woerden et al. 2007
	YFP fused to Ube3a	Visualized ube3a protein, Ube3a is imprinted in all regions of the brain	Dindot et al. 2008
	Conventional KO (maternal transmission)	Altered licking behavior, defects in rope climbing, grip strength, gait, and a raised beam task	Heck et al. 2008
	Conventional KO (maternal transmission)	Impaired experience dependent maturation of excitatory cortical circuits, deficits in ocular dominance plasticity	Yashiro et al. 2009
	Conventional KO (maternal transmission)	Lack of rapid and mature ocular dominance plasticity	Sato and Stryker 2010
Atp10a	Radiation induced chromosomal deletion p ^{30PUb} (Ube3a to Gabrb3 deletion)	Maternal deletion showed greater body weight, adiposity index, plasma insulin, leptin, triglyceride concentration	Dhar et al. 2004
Ube3a-Gabrb3	1.6 Mb deletion (Ube3a to Gabrb3) chromosome engineering	Similar to the phenotypes of Ube3a and Gabrb3 KO mice, increased USVs	Jiang et al. 2010
Gabrb3	Conventional KO mouse	90% KO mice display neonate (–24 h) lethal, 60% cleft palate, some mice are viable that showed hyperactive, higher sensitivity to sensory stimuli, motor difficulties, myoclonus, epileptic seizures	Homanics et al. 1997
	Conventional KO mouse	Electroencephalographic abnormality, seizures, deficits in learning and memory, poor motor skills	DeLorey et al. 1998
	Conventional KO mouse	44% decreased norepinephrine in spinal cord	Ugarte et al. 2001
	Conventional KO mouse	Lower REM sleep time, greater EEG delta power	Wisor et al. 2002
	Point mutation KI mouse (N265M; second transmembrane region)	Abolished suppression of noxious-evoked movements in response to the intravenous anesthetics	Jurd et al. 2003
	Conventional KO mouse	Enlarged pericoerulear dendritic zone of the locus coeruleus, hypotonia, less marble burying behavior	Hashemi et al. 2007
	Conventional KO mouse	Deficits in activities related to social behavior, altered exploratory behavior, hypoplasia of the cerebellar vermis	DeLorey et al. 2008
	Conditional KO (CamKII or Synapsin I Cre)	Synapsin I=>60% died in neonate, CamKII=>30% died in 15–25 days of age, hyperactive, sensitive for etomidate	Ferguson et al. 2007
Gabra5	Conventional KO mouse	Enhanced spatial learning, decreased amplitude of IPSC, and enhanced amplitude of paired-pulse facilitation of fEPSP in CA1 of hippocampus	Collinson et al. 2002
	Conventional KO mouse	Normal behavioral phenotype for fear-associated contextual learning	Cheng et al. 2006
	Conventional KO mouse	Reduced threshold for the induction of LTP	Martin et al. 2010
	Point mutation KI mouse (H105R; diazepam insensitive)	Impaired the activity of diazepam, reduction of Gabra5 protein level, facilitated trace fear conditioning	Crestani et al. 2002
	Point mutation KI mouse (H105R)	Attenuated prepulse inhibition, increased spontaneous locomotor activity	Hauser et al. 2005
Gabrg3	No	No	No
Oca2	Spontaneous, ENU induced, radiation induced mutant mouse	Hypopigmentation, melanosomal defect	Gardner et al. 1992 Johnson et al. 1995
Herc2	Spontaneous, ENU induced, radiation induced mutant mouse (many mutants)	Sterility, defects in growth, coordination, fertility, maternal behavior. Jerky gait	Lehman et al. 1998 Walkowicz et al. 1999

human PWS-IC could acquire a methylation imprint in mouse oocytes but the imprint is lost in somatic cells, suggesting that the human imprinting center demonstrates conserved acquisition, but diverged maintenance, of imprinting in a mouse model for AS imprinting defects (Johnstone et al. 2006). The main promoter of *Snrpn* is not required for the function of the PWS-IC, and the 4.8-kb deletion induces a severe phenotypic effect and a partial imprinting defect; however, this defect is less severe than that with the 42-kb deletion of the PWS-IC (Bressler et al. 2001; Yang et al. 1998). Paternal deletion from *Snrpn* to *Ube3a* in the mouse causes hypotonia, growth retardation, and partial lethality (Tsai et al. 1999b). The non-coding RNA region includes *HBII-52* and *HBII-85*. Knockout mice of *MBII-85*, a mouse homolog of *HBII-85*, show growth retardation, increased anxiety, and hyperphagia (Ding et al. 2008; Skryabin et al. 2007). Recently, *HBII-85* was reported as a cause of PWS (Duker et al. 2010; Sahoo et al. 2008). On the other hand, knockout mice of *MBII-52*, a mouse homolog of *HBII-52*, display increased RNA editing of the serotonin 2c receptor (5-HT_{2c}R) and less locomotor activity (Doe et al. 2009). In terms of the relationship between serotonin and autism, *MBII52* is an interesting candidate gene.

Maternally expressed genes

Maternally expressed genes in chromosome 15q11–q13 include *Ube3a* and *Atp10a*. *Ube3a* is an E3 ubiquitin ligase and is known to be a causative gene for AS (Kishino et al. 1997; Matsuura et al. 1997). *Ube3a* knockout mice show impaired motor function, inducible seizures, learning deficits (Jiang et al. 1998; Miura et al. 2002), and sleep disturbance (Colas et al. 2005). The ataxic and long potentiation phenotypes are rescued by reduction of α CaMKII inhibitory phosphorylation (van Woerden et al. 2007). Knock-in (KI) *Ube3a*-YFP fusion reporter mice show that *Ube3a* is imprinted in all regions of the brain, but is biallelically expressed in glial cells (Dindot et al. 2008). Maternally *Ube3a*-deficient mice show altered linking behavior, which has been associated with cerebellar control (Heck et al. 2008). Recently, *Ube3a* was extensively characterized in neurons and found to be involved in synaptic plasticity (Greer et al. 2010; Margolis et al. 2010; Sato and Stryker 2010; Yashiro et al. 2009). A genetic study also reported the involvement of *Ube3a* in ASD (Glessner et al. 2009). *Ube3a* is the primary candidate gene for causing the phenotypic features in duplication 15q autism. In contrast, *Atp10a*, an aminophospholipid translocase, has been less comprehensively studied than *Ube3a*, and its imprinting status remains unclear (DuBose et al. 2010; Kayashima et al. 2003). Mice with a radiation-induced

maternal deletion, including *Ube3a* and *Gabrb3*, are a model of obesity and type two diabetes (Dhar et al. 2004). Mice with a large maternal deletion from *Ube3a* to *Gabrb3*, produced by chromosome engineering, show altered ultrasonic vocalization, impaired learning and memory, and display parent of origin effects (Jiang et al. 2010).

Non-imprinting genes

In the conventional biallelic region, there is a cluster of GABA_A receptor subunits (*Gabrb3*, *Gabra5*, *Gabrg3*), *Oca2*, and *Herc2*. The GABA_A receptor is one of the major neurotransmitter receptors and forms the most notable target in this region because an imbalance between excitatory and inhibitory neurons during development is a pathophysiological hypothesis of developmental brain disorders, including ASD (Rubenstein and Merzenich 2003). Of the GABA_A receptor subunits, the GABA_A β 3 subunit (encoded by the *Gabrb3* gene) is well studied. Its deficient mice show epileptic seizures, deficits in learning and memory, poor motor skills (DeLorey et al. 1998; Homanics et al. 1997), as well as sleep disturbance, such as lower REM sleep time and greater EEG delta power than the wild-type (WT) (Wisor et al. 2002). The level of norepinephrine in the spinal cord of *Gabrb3* KO mice was less than that of WT mice, suggesting that β 3 subunit-containing GABA_A receptors mediate the trophic effects on a subpopulation of spinally projecting noradrenergic neurons (Ugarte et al. 2001). Furthermore, *Gabrb3* N265M KI mice display altered general anesthetic actions (Jurd et al. 2003). *Gabrb3* KO mice show expansion of the plexus of locus coeruleus dendrites, with a concurrent reduction in muscle tone and marble burying behavior (Hashemi et al. 2007). They also exhibit hypoplasia of cerebellar vermal lobules and impaired social and exploratory behaviors in addition to deficits in non-selective attention (DeLorey et al. 2008). Survivors of pan-neuronal *Gabrb3* knockouts are overtly normal, whereas those of forebrain selective knockouts have reduced fertility, are hyperactive, and some become obese (Ferguson et al. 2007). An association between a specific GABA_A β 3 receptor defect and autism has been reported recently (Delahanty et al. 2010). Thus, *Gabrb3* is also an intriguing candidate gene in the region with respect to autism. *Gabra5* KO mice display enhanced spatial learning, decreased amplitude of inhibitory post-synaptic current (IPSC), and enhanced amplitude of paired-pulse facilitation of excitatory postsynaptic potential (EPSP) (Collinson et al. 2002). While *Gabra5* KO mice exhibit a normal behavioral phenotype for fear-associated contextual learning (Cheng et al. 2006) and no differences in long-term potentiation (LTP) in hippocampal slices (Collinson et al. 2002), they show reduced threshold for the induction of LTP via mechanisms that are indepen-

dent of synaptic inhibition (Martin et al. 2010). On the other hand, $\alpha 5$ (H105R) KI mice, in which a partial reduction of $\alpha 5$ GABA_A receptor restricted to CA1-3 subfield of the hippocampus was achieved, exhibit facilitated trace fear conditioning (Crestani et al. 2002). Moreover, H105R mutant mice show attenuated prepulse inhibition (PPI) and increased spontaneous locomotor activity (Hauser et al. 2005). *Herc2* contains a COOH-terminal HECT domain and functions as an assembly factor for the complex of an E3 ubiquitin ligase (Bekker-Jensen et al. 2010). Mutant mice of *Herc2* show defects in growth (Lehman et al. 1998), but the physiological functions of *Herc2* in the brain remain unknown.

Genes between BP1 and BP2

In addition to genes between breakpoints BP2 and BP3, there are four highly homologous genes (*Nipa1*, *Nipa2*, *Cyfp1*, and *Tubgcp5*) in between BP1 and BP2 (Chai et al. 2003), which appear to result in developmental behavioral problems when disturbed (Doornbos et al. 2009; Tam et al. 2010; van der Zwaag et al. 2010; von der Lippe et al. 2011). *Cyfp1* (cytoplasmic FMRP interacting protein, also known as *Sra-1*) is an intriguing candidate among them because it binds with the Fragile X protein (Schenck et al. 2001). Microarray analysis using lymphoblastoid cells from males with autism due to a 15q11–q13 duplication reveal that *Cyfp1* is upregulated (Nishimura et al. 2007). Although there is no mouse model of *Cyfp1* at this moment, it would be interesting to make both *Cyfp1* deletion and transgene animals since individuals with 15q duplications and AS deletion patients have a larger deletion that includes *Cyfp1* (Sahoo et al. 2006).

Genomic diseases in human chromosome 15q11–q13

In humans, a paternal deletion of 15q11–q13 causes PWS in which the patient shows hypotonia, obesity, cognitive deficit, and behavioral problems, including obsessive-compulsive disorder (Cassidy and Driscoll 2009; Chamberlain and Lalonde 2010b). Maternal deletion of this region causes AS, which includes motor dysfunction, frequent seizures, learning disability, absent speech, and characteristic happy demeanor (Van Buggenhout and Fryns 2009). Both PWS and AS share ASD features (Schanen 2006; Sutcliffe and Nurmi 2003). On the other hand, duplication of this region is known to be the most frequent cytogenetic abnormality in ASDs (Hogart et al. 2010; Vorstman et al. 2006). Autism with 15q duplication syndrome was first reported as partial trisomy of chromosome 15 (Gillberg et al. 1991). It is widely believed that maternally duplicated cases cause autism, but paternally duplicated ones do not (Bolton et al. 2001; Browne et al.

1997; Cook et al. 1997; Mao et al. 2000; Repetto et al. 1998; Roberts et al. 2002). On the other hand, cases with developmental delay are documented with paternal duplications (Bolton et al. 2004; Mao et al. 2000; Mohandas et al. 1999; Roberts et al. 2002; Veltman et al. 2005). A recent screening using multiplex ligation-dependent probe amplification (MLPA) did not detect maternal duplication, suggesting that ASD cases in maternal duplication are not as frequent as previously assumed (Depienne et al. 2009). Recent advances in genetic analyses, such as array CGH, and their clinical application are expected to detect larger numbers of clinical cases for 15q duplication.

A mouse model for 15q duplication

Using a chromosome-engineering technique (Mills and Bradley 2001; van der Weyden and Bradley 2006; Yu and Bradley 2001), a model for human chromosome 15q duplication has been generated (Nakatani et al. 2009). The duplication was confirmed in ES cells by Southern blotting and in mice by fluorescence in situ hybridization (FISH) and CGH arrays. Southern blot analysis using methylation sensitive and insensitive restriction enzymes, and a probe near the IC, indicated that allele-specific methylation is conserved in mice with the duplication. As the region is imprinted, the duplication is classified into two types: patDp/+ mice, in which the duplicated region is derived from the paternal allele; and matDp/+ mice, in which the duplication is derived from the maternal allele.

Both patDp/+ and matDp/+ mice breed normally and are fertile (Nakatani et al. 2009). The patDp/+ male mice begin to show an increase in body weight after 15 weeks and the body weight of patDp/+ mice is significantly greater than that of the WT after 20 weeks. The fact that both decreased (PWS) and increased (15q duplication) gene expressions of PWS regions lead to obesity is contradictory, but rather intriguing. As described above, *Atp10a* seems to be involved in metabolism and body weight (Dhar et al. 2004). Further, some of forebrain specific KO mice of *Gabrb3* also become obese (Ferguson et al. 2007). The PWS region is involved in aspects of metabolism and above a certain critical threshold, the amounts of the genes and their products, regardless of any increase or decrease, may result in an abnormal pathway of metabolism.

Histological analyses, either at the macroscopic level or at the microscopic level, using simple staining such as hematoxylin–eosin or Bodian staining, detect no significant difference between mice with the duplication and WT mice (Nakatani et al. 2009). Recent studies reveal that synaptic adhesion molecules, and their associated proteins, are known to be rare mutation cases in autism (Betancur et al. 2009; Bourgeron 2009; Toro et al. 2010). For example,

Shank3 mutant mice display abnormality in spines (Bozdagi et al. 2010; Peca et al. 2011). Thus, it will be quite interesting as to whether spine or synaptic abnormality is observed in 15q duplication mice or other models.

Autistic-like behavior of 15q duplication mice

Behavioral analyses are essential for animal models of autism because major phenotypes of ASDs are behavioral abnormalities and the diagnosis is made by three major phenotypes: impairment in social interaction, impaired social communication, restricted interest and behavior, or resistance to change. Mouse behavioral tests corresponding to human autistic phenotypes have been reported (Moy and Nadler 2008; Silverman et al. 2010). The systematic behavioral analyses were performed for 15q duplication mice (Nakatani et al. 2009). First, in the three-chamber test for social interaction, WT mice display more time spent near a novel mouse than an empty cage, while no difference of time spent around the novel mouse and the empty cage is observed in *patDp/+* mice. In comparison with time between an inanimate object and an empty cage, both *patDp/+* and WT mice spend more time near the object than empty cage, indicating that *patDp/+* mice are able to see the object. Interestingly, WT mice spend more time around a novel mouse compared with an inanimate object, whereas there was no difference in time spent between the novel mouse and the inanimate object in *patDp/+* mice. On the other hand, there was no significant difference between *matDp/+* and WT mice in the three-chamber test. These results indicate that *patDp/+* mice exhibit impaired social interaction. Similarly, *Gabrb3*^{-/-} mice spend less time in the proximity of unfamiliar mice than do their control counterparts in a similar social behavior test, although some aspects of social curiosity appear to remain intact (DeLorey et al. 2008).

Second, *patDp/+*, but not *matDp/+*, mice display abnormal ultrasonic vocalizations (USVs) (Nakatani et al. 2009). When the pups are separated from their dams, they produce USVs. In WT pups, the numbers of USVs reach a peak around postnatal day five (P5) and then gradually decrease until P14, when their eyes are open and they can communicate through the visual pathway. In contrast, the numbers of USVs remain high at P7 or even at P14 in *patDp/+* mice, suggesting that the developmental course of USVs in *patDp/+* mice is delayed compared with WT. These results demonstrate that *patDp/+* mice may be developmentally abnormal in comparison with WT, although the larger number of USVs in *patDp/+* pups may reflect higher anxiety and fear in response to isolation stress. No difference of USVs between *matDp/+* and WT mice was observed. The increased USVs were also

observed in mice with a maternal deletion from *Ube3a* to *Gabrb3* (Jiang et al. 2010).

Third, ritualistic behavioral inflexibility seen in autistic patients is examined by reversal tasks using learning mazes. In the Barnes maze, WT, *patDp/+*, and *matDp/+* mice exhibit intact spatial learning and memory. In a reversal test, in which the target is altered to the opposite position, WT and *matDp/+* mice learn the new target position, whereas *patDp/+* mice return to the original position more often than WT and *matDp/+* mice, indicating that *patDp/+* mice display resistance to change or perseveration (Nakatani et al. 2009). *patDp/+* mice also show similar behavior in the Morris water maze. These results suggest that *patDp/+* mice exhibit behavioral inflexibility.

In addition to these phenotypes, *patDp/+* mice display novelty-induced anxiety and decreased exploratory activity (Tamada et al. 2010). Interestingly, anxiety is also observed in paternal duplication of 15q11–q13 in humans (Mao et al. 2000; Veltman et al. 2005). Mice with maternal deficiency for just the *Ube3a* gene also show decreased exploratory behavior (Allensworth et al. 2011). In several tests involving food deprivation, such as the novelty suppressed test, radial maze, and Y-maze, *patDp/+* mice display peculiar behavior (Tamada et al. 2010). Even though *patDp/+* mice must be hungry after food deprivation, they do not rush to feed and take more time to proceed to feed than WT mice, and finally perform some tasks that are equivalent to the WT. This behavior is likely to reflect a lack of desire. *patDp/+* mice also show less marble burying behavior (Tamada et al. 2010), a method commonly used to assess obsessive-compulsive behavior, which is also seen in *Gabrb3* KO mice (Hashemi et al. 2007).

Abnormal serotonin in 15q duplication mice

The mouse model for 15q duplication can be considered an artificial founder mouse to unravel which gene and/or region in 15q11–q13 is critical for autistic phenotypes. A series of bacterial artificial chromosome (BAC), yeast artificial chromosome (YAC), or other engineered mice with longer insertions that cover the duplicated region will provide useful materials for further investigation. Alternatively, genetic rescue experiments crossed with a series of knockout or deficient mice will be required to identify the gene.

The primary benefit of the mouse model is that it allows us to study abnormality at the molecular and cellular levels. Major behavioral phenotypes appear in *patDp/+* mice but not in *matDp/+* mice (Nakatani et al. 2009); therefore, genes in the maternally imprinted region can be regarded as candidates for abnormal behaviors observed in *patDp/+* mice. Among them, *MBII-52*, a mouse brain specific

snoRNA 52 in a huge cluster of non-coding RNAs, would be an interesting candidate for the following reasons. First, *MBII-52* is a paternally expressed gene. Second, *HBII-52*, a human homolog of *MBII-52*, is reported to be involved in posttranscriptional modification of the serotonin 2c receptor (5-HT_{2c}R) (Kishore and Stamm 2006). Third, serotonin (5-HT) is well known to be related to the pathophysiology of autistic phenotypes (Whitaker-Azmitia 2005). Fourth, 5-HT_{2c}R is the only G-protein coupled receptor that undergoes A to I RNA editing, resulting in an amino acid substitution in the second loop of the cytoplasmic domains (Seeburg 2002), which may affect G-protein coupling efficiency. *MBII-52* RNA displays an approximately two-fold increase in expression in the patDp/+ mice brain compared with matDp/+ and WT mice (Nakatani et al. 2009). Pyrosequencing analyses revealed that the RNA editing ratio in potential editing sites of 5-HT_{2c}R in patDp/+ mice was higher than that in matDp/+ or WT mice. This suggests that increased snoRNA in the patDp/+ brains may affect the RNA editing of 5-HT_{2c}R. Furthermore, a specific agonist for 5-HT_{2c}R induces higher intracellular Ca²⁺ concentration in primary cultured neurons derived from patDp/+ mice compared with matDp/+ and WT neurons, indicating that serotonergic signals in patDp/+ neurons are different from matDp/+ and WT neurons. This result suggests that alterations in RNA editing of 5-HT_{2c}R may affect serotonergic signals in neurons and that this alteration in serotonergic signals in patDp/+ neurons may recapitulate abnormal behavior in patDp/+ mice.

Neurochemical analyses revealed abnormal levels of 5-HT and its metabolites in the adult cerebellum and midbrain of the duplication mouse. Interestingly, 5-HT in patDp/+ mice was also observed to decrease in all brain regions tested during postnatal weeks 1–3 (Tamada et al. 2010). These results suggest that abnormal 5-HT levels during developmental stages affect development of neurons and neural circuits, and may recapitulate the abnormal behavior seen in patDp/+ mice. Interestingly, the mean 5-HT concentrations are larger in *Ndn* KO mice than in WT, and the morphologies of 5-HT fibers are altered (Zanella et al. 2008). Combined with these findings, *Ndn* in the region may be involved in the metabolism and development of 5-HT. *Ndn* facilitates *TrkA* signaling for sensory neuron survival (Kuwako et al. 2005). The molecular mechanisms by which *Ndn* might affect 5-HT metabolism and development remain unknown.

Phenotypes (in)dependent on copy variants

Mice with a 15q duplication and other genetically engineered mice for genes in 15q11–q13 share some phenotypes, such as obesity, social behavior, and 5-HT concentration. Serotonin

concentration seems to correlate with gene dosage of *Ndn* (Tamada et al. 2010; Zanella et al. 2008); however, complex phenotypes such as metabolism, weight control, or social behavior are unlikely to be dependent on simple gene dosage or copy number variants, but above a certain critical threshold, increases or decrease of gene expression may produce the abnormal pathway of these phenotypes.

The idea that phenotypes are dependent on copy variants may not be correct and their underlying mechanisms are unlikely to be simple in humans. In contrast to the interstitial duplication of human chromosome 15q, termed idiopathic (15) syndrome individuals, the presence of a supernumerary marker chromosome formed by the inverted duplication of proximal chromosome 15, resulting in partial tetrasomy 15q, often display increased, yet inappropriate, social behaviors (Battaglia et al. 2010).

A gap between the mouse model and the human patient

The mouse model for 15q duplication as an animal model for human neuropsychiatric disease fulfills construct validity, in which it possesses the same chromosomal abnormality as human patients. Thus, the model can be regarded as a humanoid mouse model for autism in one aspect (Takumi 2010). In terms of humanized models, Tabuchi et al. report a KI mouse for an *NLGN3* R541C mutant (Tabuchi et al. 2007). However, the same KI mouse independently generated by Dr. Heintz's group displays no significant or mild social abnormality (Chadman et al. 2008). Further humanized model mice with rare mutations of other molecules, such as serotonin transporter G56A KI mice (Veenstra-Vanderweele et al. 2009), will be expected to display the phenotypes seen in humans.

The allele-specific phenotypes that appeared in 15q model mice will imply some biological significance that can provide a general concept. However, it is surprising that patDp/+ mice, but not matDp/+ mice, display abnormal social behavior (Nakatani et al. 2009) because maternally duplicated cases in humans are likely to result in an ASD phenotype, while paternal duplications do not (Cook et al. 1997). Although allele-specific methylation seems to be conserved, at least around the IC, in mice with a duplication, the possibility that the imprinting status in other regions is different between human and mouse is not excluded. The timing and mechanism of the expression of the downstream non-coding *Snurf-Snrpn* exons seem to be different between human and mouse (Chamberlain et al. 2010). Recent systematic analyses of the mouse epigenome reveal that *Ube3a* and a GABA receptor subunit are differentially imprinted in different brain areas (Gregg et al. 2010). Alternatively, epigenetic status other than DNA methylation may be different in mice from that in humans. Quantitative

analysis of *GABRB3* transcripts in PWS and AS brain samples show evidence for a paternal expression bias when a biparental 15q11–q13 contribution is lacking (Hogart et al. 2007). The *Ube3a*-ATS is thought to regulate expression by turning off *Ube3a* on the paternal allele. This may cause difference between maternal and paternal duplication animals as well and should be investigated. At the very least, there is a significant variability and diversity of clinical phenotypes and gene expression in human chromosome 15q11–q13 duplication syndrome (Hogart et al. 2009; Piard et al. 2010). Further detailed epigenetic analyses both in humans and mice will provide an insight into the imprinting mechanisms.

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