New mouse genetic model duplicates human 15q11-13 autistic phenotypes, or does it?

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Autism spectrum disorders (ASDs) are a heterogeneous group of neurodevelopmental disorders that manifest deficits in social interaction, and verbal and non-verbal communication, in addition to restrictive interests and repetitive behaviors. Recent reports indicate that ASDs may occur in as many as one in 91 children (Kogan et al., 2009) and encompass several clinically defined conditions, including autism, Asperger syndrome, pervasive developmental disorder – not otherwise specified, and rare syndromic forms, such as fragile X and Rett syndrome (Geschwind and Levitt, 2007). Autism is a highly heritable neuropsychiatric disorder, involving de novo gene mutations, chromosomal abnormalities and common genetic variants. Chromosome abnormalities probably account for 6-7% of autism cases (Abrahams and Geschwind, 2008), of which, maternal duplication of 15q11-13 is the most frequently observed (1-3% of cases). Genes within the 15q11-13 region are typically expressed from a single parental chromosome (imprinted). When these genes are mutated, diverse phenotypes result depending on the parent of origin for the mutation. For instance, maternal deletion of 15q11-13 causes Angelman syndrome; paternal deletion causes Prader-Willi syndrome (Nicholls and Knepper, 2001). Two single genes within the 15q11-13 locus, maternally expressed *UBE3A* and non-imprinted *GABRB3*, are thought to be associated with autism (Nurmi et al., 2001). Consistently, mice lacking *Ube3a* or *Gabrb3* show deficits in neurodevelopment and behavior (DeLorey, 2005; Yashiro et al., 2009).

Most existing mouse models for ASD represent single-gene human disorders that are characterized by autistic symptoms. These candidate genes were identified through human genetic association or linkage studies, or by studying mutations that involve pathways that are potentially altered in autism (Moy and Nadler, 2008; Levitt and Campbell, 2009). However, single-gene mouse models cannot recapitulate complex human genomic disorders that affect multiple contiguous genes. Therefore, a forward genetic approach that is capable of modeling chromosomal rearrangements involving multiple genes, such as the 15q11-13 locus, would help to further delineate autism etiology. Recent advances in chromosome engineering have facilitated the development of mouse models for human genomic disorders involving chromosomal abnormalities (van der Weyden and Bradley, 2006). In issue 137 of *Cell*, Nakatani et al. reported the first targeted chromosomal duplication in a mouse model of the human 15q11-13 duplication that is associated with autism (Nakatani et al., 2009). A key finding of this study is that mice harboring a paternal duplication of the syntenic region for human 15q11-13 display significant behavioral deficits including abnormal social interactions, inflexible behavior and increased anxiety, which are all phenotypes that are observed in individuals with autism. This work presents a mouse model with autistic characteristics that is associated with a genomic change, chromosomal duplication, which is found in human patients.

To generate the mouse model of the human 15q11-13 duplication, Nakatani et al. constructed a 6.4 Mb interstitial duplication of mouse chromosome 7, mirroring the region between common breakpoints in human chromosome 15q11-13 that are seen in autism (see fig. 1 in Nakatani et al.). Gene targeting was used to insert selection cassettes; a clone that was double targeted on homologous chromosomes was then chosen to produce clones with a balanced duplication or deletion following Cre recombination. Germline transmission of the duplication allele was confirmed by comparative genomic hybridization using a mouse bacterial artificial chromosome array (see fig. 2 in Nakatani et al.). Mice with paternal (*patDp/+*) or maternal (*matDp/+*) duplications of the 15q11-13 locus maintain expression levels of several preeminent 15q11-13 genes, which is consistent with their imprinting patterns. This suggests that chromosome duplication does not affect imprinting. Non-imprinted genes in this region and genes adjacent to the duplication region were also expressed at normal levels. Additionally, allele-specific epigenetic regulation by methylation (a major mechanism for imprinting) is conserved in mice with duplicated alleles.

The diagnosis of autism is based on behavioral criteria. The authors reason that a valid mouse model should display autism-related behavioral phenotypes. Therefore, they performed 16 behavioral tests to compare the social behavior, anxiety-related behaviors, learning, reversal of learning and motor function in *patDp/+* and *matDp/+* mice with their respective controls. *patDp/+* mice display the most striking phenotype, showing a lack of preference for a stranger mouse relative to an inanimate object in the three-chamber social interaction test (see fig. 4 in Nakatani et al.). Further, *patDp/+* pups emit greater ultrasonic vocalizations when separated from their mothers, which is an indication of increased anxiety and impaired development of communicative behavior (see fig. 4F in Nakatani et al.). Adult *patDp/+* mice also display a generalized fear response with higher freezing scores in an altered contextual environment during a paired noise-foot shock conditioning task.

Finally, the Morris water maze and Barnes maze tests measured the spatial learning, memory acquisition and behavioral flexibility in this mouse model (see fig. 5 in Nakatani et al.). *patDp/+* mice are capable of locating an escape platform in the target quadrant equally as well as the wild-type mice in the Morris water maze test, indicating normal spatial learning. However, after moving the target platform to the opposite quadrant in a subsequent probe trial, these mice did not learn the new platform location, whereas the wild-type

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mice quickly adapted to the new location. Similar results are obtained for *patDp/+* mice in the Barnes maze. Together, these behavioral findings reveal social communication deficits, an enhanced anxiety/fear response to stress, and behavioral inflexibility/stereotypy in *patDp/+* mice, all of which are behavioral hallmarks of autism.

To identify a potential mechanism for the abnormal behaviors observed in *patDp/+* mice, Nakatani et al. examined the expression of a candidate gene in the duplication region, *MBII52* (also known as *Snord115*), which encodes a mouse brain-specific small nucleolar RNA. *MBII52* is maternally imprinted and is a post-transcriptional regulator of the G-protein-coupled serotonin 2c receptor (5-HT2cR). Editing of 5-HT2cR pre-mRNA by adenosine deamination (A-to-I editing) leads to amino acid substitutions that affect receptor physiology and serotonin-guided behavior (Seeburg, 2002). RNA blot hybridization reveals a twofold increase in *MBII52* transcription in *patDp/+* brains compared with wild-type and *matDp/+* brains, consistent with the significantly higher editing ratio of 5-HT2cR RNA in *patDp/+* mice (see figs 6A and S13 in Nakatani et al.). The authors reasoned that altered *MBII52* transcripts might alter signaling through the 5-HT2cR and thus facilitate intracellular calcium increase. Primary neurons isolated from *patDp/+* mice were then cultured and treated with a 5-HT2cR-specific agonist. Consistent with increased 5-HT2cR signaling, these neurons show a significantly higher intracellular calcium increase compared with wild types (see fig. 6B in Nakatani et al.). Collectively, these experiments indicate that increased expression of *MBII52* enhances activation of 5-HT2cR, which in turn may contribute to the abnormal behavior observed in *patDp/+* mice.

It remains unclear why only paternal duplication of 15q11-13 genes in mice produces autism-like phenotypes, whereas such a duplication is usually inherited maternally in human autism (Cook et al., 1997). A similar result is observed in mouse models of Rett syndrome: male *Mecp2* mutant mice recapitulate phenotypes associated with the female-dominated human condition (Chahrour and Zoghbi, 2007). These mouse models may eventually provide clues to the reason for parental gender bias that is observed in some neurodevelopmental disorders, including autism.

There are interesting differences between these models and human disease. It is curious that the *patDp/+* model recapitulates the majority of the autism-related behavioral phenotypes, whereas 15q11-13 duplication occurs only in a small percentage of all autism cases. Although this new mouse model phenocopies relevant autism symptoms, it remains to be determined whether this chromosome duplication involves additional molecular mechanisms that affect synapse formation, balancing of neural networks, and the formation of behaviorally-relevant neurocircuitries. These are fundamental processes in autism etiology and have been validated previously in single-gene autism mouse models (Moy and Nadler, 2008). Considering the existence of multiple 15q11-13 autism risk genes that affect synapse formation (e.g. *UBE3A*) (Yashiro et al., 2009) and excitatory-inhibitory networks (e.g. GABA$_A$ receptor clusters), it is likely that multiple gene dosage effects converge with other signaling pathways (Levitt and Campbell, 2009) to produce a number of stable, recognizable, abnormal behavioral states that are commonly seen here in *patDp/+* mice and human autistic patients.

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