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Review article

The BTBR mouse model of idiopathic autism – Current view on mechanisms☆

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ABSTRACT

Autism spectrum disorder (ASD) is the most commonly diagnosed neurodevelopmental disorder, with current estimates of more than 1% of affected children across nations. The patients form a highly heterogeneous group with only the behavioral phenotype in common. The genetic heterogeneity is reflected in a plethora of animal models representing multiple mutations found in families of affected children. Despite many years of scientific effort, for the majority of cases the genetic cause remains elusive. It is therefore crucial to include well-validated models of idiopathic autism in studies searching for potential therapeutic agents. One of these models is the BTBR $T^{+}Itpr3^{tf}$/J mouse. The current review summarizes data gathered in recent research on potential molecular mechanisms responsible for the autism-like behavioral phenotype of this strain.

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1. Introduction

Despite decades of research, autism spectrum disorder (ASD) remains the most commonly diagnosed neurodevelopmental disorder. Current epidemiology studies place its prevalence at as high as 1 in 45 children (Zablotsky et al., 2015) or, using a more conservative questionnaire, 1 in 68 children (Christensen et al., 2016) in the US. National statistics for ASD prevalence vary across countries but in most of them the numbers oscillate around 1% of the population (for review see, Elsabbagh et al., 2012).

The etiology of the disorder remains unclear, with genetic, epigenetic and environmental factors interacting to produce a very heterogeneous group of patients sharing a similar behavioral

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profile. Recent studies point to over a hundred affected genes, with the majority being *de novo* mutations and copy number variants (Huguet et al., 2013; Jeste and Geschwind, 2014; Iossifov et al., 2014). Nevertheless, only a small percentage of cases is associated with known mutations, leaving the majority of cases to be described as idiopathic. In a recent work Iossifov et al. (2014), screened over 2500 simplex families (families in which only one child was diagnosed with ASD) to find that de novo missense mutations and de novo likely gene-disrupting (LGD) mutations contribute to 12% and 9%, respectively, of ASD diagnoses. Upon inclusion of copy number variants into these numbers, de novo mutations were found responsible for 30% of simplex cases and 45% of female cases of ASD.

Preclinical animal studies rely on well-validated rodent models to facilitate an understanding of clinical conditions. In the absence of a clear genetic, molecular, physiological or structural mechanism, animal models of ASD are typically validated in terms of two main behavioral clusters: 1. social behavior and communication impairments and 2. excess of repetitive behaviors (DSM-V, APA). The BTBR $T^{+}Itpr3^{tf}$/J mouse (BTBR), originally bred for studies on insulin-resistance, diabetes-induced nephropathy and phenyloketonuria, was identified only a decade ago as displaying strong and consistent autism-relevant behaviors (Bolivar et al., 2007; Moy et al., 2007; Nadler et al., 2006). Here we will summarize the data from recent genetic and proteomic studies identifying several clusters of genes and proteins differently expressed in the BTBR mice, as compared with C57BL6/J (B6) mice (unless otherwise noted). The latter strain is commonly used as a highly social "control" for autism-related studies employing BTBR mice. We will also discuss recent developments in the search for the neuroanatomical correlates of autism-like behaviors of the BTBR mouse, as well as possible molecular mechanism responsible for this phenotype.

2. Altered gene and protein expression in the BTBR mouse

Recent research has provided several gene expression and proteomic studies emphasizing the unique phenotype of the BTBR mouse strain. The inositol triphosphate receptor 3 gene (*Itpr3*), was identified as responsible for the mouse tufted (tf) locus (Ellis et al., 2013), which resulted with a change of the strain name from BTBR T^{+}tf/J to BTBR $T^{+}Itpr3^{tf}$/J. More importantly, the deletion within the *Itpr3* gene was found to cause indifference of BTBR mice to sweet, Polycose, umami, bitter, and calcium tastes (Tordoff and Ellis, 2013), which in turn affects their food intake (preferential fat consumption, as compared to carbohydrate-rich diet, Tordoff et al., 2014). In the light of these results, the reports of impaired social communication of food-preference in this strain (McFarlane et al., 2008), as well as the use of BTBR mice to validate food reward based tasks (Martin et al., 2014) need to be reconsidered.

The first genetic comparisons between BTBR and B6 mice were done almost a decade ago (see McFarlane et al., 2008) and yielded several single nucleotide polymorphisms (SNPs) in the BTBR genetic background. The most interesting finding was a nonsynonymous coding region polymorphism in the *Kmo* gene encoding kynurenine 3-hydroxylase, an enzyme regulating the metabolism of kynurenic acid (a glutamate antagonist). Further studies showed that deficiency in cholinergic transmission and increased levels of kynurenic acid in the prefrontal cortex of BTBR mice may be responsible for their inaccurate performance in the 5-choice serial reaction time task (McTighe et al., 2013).

Quantitative Trait Loci analysis using F2 intercross between the BTBR and B6 strains identified loci for autism-relevant traits and commissural morphology on chromosomes 1, 3, 9, 10, 12, and X (Jones-Davis et al., 2013). Additionally, four novel QTL for commissural morphology were found on chromosomes 4, 6, and 12. Detailed analysis yielded several candidate genes in the domains of developmental proteins (including genes regulating cell cycle, cell adhesion, axon growth/guidance and actin binding), synaptic proteins, kinases and immune and heat shock proteins.

Recently, Daimon and collaborators (Daimon et al., 2015) collected transcriptomic and proteomic data indicating differential expression of several genes and proteins in the hippocampus and cortex of BTBR and B6 mice. Among others, brain derived neurotrophic factor [*Bdnf*], p21-activated kinase type1 [*Pak1*] and cortistatin [*Cort*] were downregulated in BTBR hippocampus and cortex. While solute carrier family 25 [mitochondrial carrier; phosphate carrier], member 3 [*Slc25a3*] was downregulated in cortical samples, Serpin peptidase inhibitor, clade A [*Serpina*] was found decreased in the hippocampi of BTBR mice. Signaling pathway analysis (Ingenuity Pathway Analysis and Kyoto Encyclopedia of Genes and Genome) identified numerous down and up-regulated pathways. While transcripts related to gap junction, long-term depression and potentiation, Parkinson's disease, and adherence junction were upregulated, pathways related to metabolic stress response were downregulated. Others, such as members of MAPK signaling pathway, were both up and downregulated. These data are in line with previous reports showing BDNF deficiency (in adult BTBR mice, Scattoni et al., 2013; Stephenson et al., 2011) and MAPK signaling disruption (Faridar et al., 2014; Seese et al., 2014, reporting increased *p*-ERK levels, but fewer double labeled *p*-ERK/PSD95 puncta). The high level of *p*-ERK in the prefrontal cortex, but not in the cerebellum or total level, was associated with impaired juvenile sociability (Faridar et al., 2014) and adult memory formation in the Object Location Memory task (Seese et al., 2014).

Levels of other ASD-relevant mRNAs were also altered. These included: Caskin 1, which binds to Neurexin 1, and Homer31, which binds to Shank 1 and 3. The use of *Textrous!* natural language processing-based informatics analysis allowed for extraction of functional groups of altered genes. These included: *axon guidance*, *neurogenesis* and *regulation of actin cytoskeleton* (Daimon et al., 2015).

A recent comparison of transcriptomic data from BTBR and Engrailed ($En2^{-/-}$) hippocampi showed a total of 153 genes similarly deregulated in both ASD models (Provenzano et al., 2016). Pathway analysis revealed that these were involved in abnormal behavioral response, chemokine/MAP kinase signaling, as well as in dysfunction of the immune system and abnormal synaptic transmission/seizures.

Another proteomic study of BTBR cortex showed that apart from aberrant regulation of actin cytoskeleton BTBR mice have down-regulated levels of the stable tubule only polypeptide protein (STOP) and myelin-related proteins (e.g. myelin basic protein, MBP and myelin associated glycoprotein, MAG). They also displayed reduced levels of staining with ferric alum, indicating myelin disruption, in comparison to B6 controls (Wei et al., 2016a). These results are in line with histopathological examination of BTBR brain tissue (Stephenson et al., 2011), which showed reduction of myelin markers such as 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase) and MBP, as well as an increase in the oligodendrocyte precursor NG2. MBP and CNPase were expressed in small ectopic white matter bundles within the cingulate cortex. Contrary to the findings of Heo et al. (2011), Stephenson and colleagues found no evidence of gliosis, but described the orientations of glial fibers as altered in specific white-matter areas.

Analysis of fetal brain proteins showed a decreased level of glial fibrillary acidic protein, as well as increased BDNF and MBP levels in BTBR compared to FVB/NJ mice. No significant difference was obtained for NGF (nerve growth factor) between the two strains (Hwang et al., 2015). The upregulation of BDNF expression at an early developmental stage (in stark contrast to the BDNF signaling deficiency in later life of BTBR mice) is in line with clinical data