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Genetic factors underlying attention and impulsivity: mouse models of attention-deficit/hyperactivity disorder

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Increasing evidence suggests complex genetic factors for attention-deficit/hyperactivity disorder (ADHD). Animal models with definitive genetic characteristics are indispensable for gaining an understanding of the molecular, cellular, and neural circuit mechanisms underlying ADHD. Toward this aim, mice have several advantages because of their well-controlled genetic backgrounds and the relative ease with which functions of defined neuronal circuits can be manipulated. Dopamine signaling dysfunction was once the major pathogenic focus of interest in ADHD research, but hypotheses have expanded to include functionally distinct molecules. Forward and reverse genetic approaches have produced diverse mouse genetic models for genes involved in monoaminergic signaling, synaptic plasticity, and neuronal circuit formation. Data suggest crucial roles of gene–gene interactions and gene–environment interactions in the pathophysiology of ADHD.

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Introduction

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder defined by inattention and/or hyperactivity-impulsivity that occurs in ~5% of children and ~2.5% of adults worldwide [1]. Attention is the ability to focus on particular (important) sensory information and ignore other (less important) information. Attention can be divided into subdomains comprising alerting, orienting, and executive attention functions; and neuroimaging data in humans suggest the existence of broad attention networks [2•]. Impulse control is required to optimize animal actions, and is divided into subcognitive domains potentially involving distinct neuronal circuits and neurochemistry [3,4]. Imaging studies in ADHD indicate hypofunction and/or volume changes in various brain regions, such as the anterior cingulate, dorsolateral and inferior prefrontal cortices, basal ganglia, thalamus, parietal cortex, and cerebellum [4,5,6]. Cognitive domains for attention and impulsivity may provide foundations of other cognitive/emotional domains and personality [7]. Inattentive and impulsive behaviors are also comorbid with other psychiatric disorders, such as autism spectrum disorders, bipolar disorder, and developmental coordination disorders [1,8,9,10]; and are a risk factor for the development of antisocial and drug-abuse disorders [1].

Human genetics for ADHD

Family, adoption, and twin studies support the heritable etiology of ADHD (for review see: [11]). Psychostimulants, such as methylphenidate and amphetamine, potent dopamine reuptake inhibitors, ameliorate the symptoms of ADHD. The paradoxic effects of these agents, however, led researchers to hypothesize that abnormal dopaminergic signaling causes ADHD and to search for an association between a polymorphism at the dopamine transporter locus (*DAT1*) and ADHD [12]. The findings of hypothesis-driven studies focusing on the genes involved in catecholaminergic systems suggest various genes potentially involved in the pathogenesis of ADHD. Meta-analyses of the hypothesis-driven research support significant associations of several candidate genes, including *DAT1*, *DRD2*, *DRD4*, *DRD5*, *5HTT*, *HTR1B*, and *SNAP25* [13,14]. These studies, however, also revealed modest odds ratios (<1.33) for all of the significant polymorphisms, suggesting that each gene has only a small effect and supporting a multifactorial and polygenic etiology of ADHD.

The polygenic etiology is further supported by hypothesis-free genome-wide scan studies. These studies implicate multiple loci, thus diluting the significance of the classic candidate genes involved in catecholaminergic signaling, and suggest the potential involvement of genes for 'new' neurotransmission and cell-cell communication systems, including *T-cadherin* [15]. A recent genome-wide copy number variation study provided evidence for an association of metabotropic glutamate receptors and their interacting molecules with ADHD [16••]. Taken together, human genetic studies have established a complex etiology of ADHD, similar to that of other psychiatric disorders. Thus, different types of model animals are needed and proposed [17]. This article focuses on the mouse genetic models.

Mouse genetic models of ADHD

Dat1(Slc6a3)-KO/knockdown/cocaine-insensitive mice

DAT is expressed on axon terminals and regulates dopamine (DA) signaling by transporting DA from the synaptic cleft back into the presynaptic terminal. Multiple lines of evidence from genetic, pharmacologic, and imaging studies suggest that *DAT1* is a strong candidate gene involved in the pathogenesis of ADHD. The behavioral phenotypes of mutant mice generated by gene-targeting methods support this notion. *Dat1*-knockout (KO) mice exhibit hyperactivity and deficits in learning and memory [18]. The mice also show attention deficits in an auditory prepulse inhibition (PPI) test [19]. Hyperactivity and PPI deficits in *Dat1*-KO mice are ameliorated by methylphenidate [18,20]. A recent study revealed that *Dat1*-KO mice with a mixed genetic background of C57BL/6J and 129Sv/J were impaired in a cliff avoidance reaction (CAR) test based on their inability to remain on an elevated small round platform without falling, suggesting impulsivity [21]. Methylphenidate or nisoxetine ameliorated the cliff avoidance reaction impairment in the *Dat1*-KO mice [21].

Dat1-knockdown mice also exhibited hyperactivity and risk-taking behavior in a mouse version of the Iowa gambling test [22], reflecting impulsivity. *Dat1*-knockin mice carrying the cocaine-insensitive mutation exhibit reduced DAT activity [23]. Although the *Dat1*-cocaine insensitive mice exhibit hyperactivity, their locomotor activity and responses to amphetamine are dependent on their genetic background [24], suggesting a crucial role of gene–gene interactions for these phenotypes. Other phenotypes relating to attention and impulsivity in these mice have not been documented.

Drd4-KO mice

Although genes encoding DA receptors are classic candidates for ADHD, experimental evidence from *Drd1*, *Drd2*, *Drd3*, *Drd4*, and *Drd5* KO mice for these genes affecting ADHD-relevant endophenotypes is weak [25•]. Interesting results were reported for *Drd4*-heterozygous mice [26]. Young *et al.* applied a 5-choice continuous performance test (5C-CPT), which is a modification of the 5-choice serial reaction time test (5CSRTT) [27] that may more closely correspond to the CPT used in humans [28]. In the 5C-CPT, rodents must continue to respond to signal stimuli (illumination of any 1 of 5 holes), and must also inhibit their response to non-signal stimuli (simultaneous illumination of all 5 holes). Heterozygous but not homozygous *Drd4*-KO mice exhibited attention deficits in the 5C-CPT [26]. High impulsivity was also measured by false alarms but not by premature responses. The mice showed no deficits in PPI or spontaneous exploratory behavior. It is plausible that the complete lack of D4 receptors leads to a robust compensatory system(s) at the molecular and/or neural circuit levels. Interactions of the gene with other genetic or environmental factors require further evaluation.

COMT-KO mice

Recent works for catechol-*O*-methyltransferase (COMT)-KO mice support the notion that gene–environment interactions and gene–gene interactions are involved in attention and impulsivity domains [29••,30••]. COMT methylates and inactivates DA. In the 5CSRTT, male and female $COMT^{+/+,\ +/-}$ and $COMT^{-/-}$ mice equally acquire the task. Interestingly, environmental factors induced genotype-sex interactions in the task. For example, a mild stress (15 min exposure in an empty cage at ~800 lx before test) increased impulsive premature responses in $COMT^{+/-}$ and $^{-/-}$ males, but not in females [29••]. In contrast, females, but not males, exhibited genotype differences in perseverative responses. $COMT^{-/-}$ females showed perseverative responses at a lower rate compared to other genotypes [29••]. Differential effects of various stimuli are consistent with the sex difference in ADHD prevalence [1].

DTNBP1 (dysbindin) is a molecule that has a role in homeostasis of excitatory synapses [31]. C57BL6/J congenic $COMT^{+/-}$ and $^{-/-}$ males and C57BL6/J congenic $Dtnbp1^{+/-}$ and $^{-/-}$ males learn the T-maze working memory task, which demands a high level of attention, faster than wild-type mice. In contrast, double mutants (double heterozygotes and homozygotes) learn slower than wild-types [30••]. Although Papaleo *et al.* [30••] did not directly examine attention and impulsive behaviors, their data clearly demonstrated the significance of gene–gene interactions in behaviors requiring attention. Interestingly, similar interactions between *COMT* and *DTNBP1* are observed in functional magnetic resonance imaging analysis during working memory tasks in healthy humans [30••]. The *COMT* rs4680 Met allele has reduced COMT enzyme activity compared to the Val allele, and the 'Bray haplotype' of *DTNBP1*, carrying three markers rs2619538–rs3213207–rs1047631, has a lower level of mRNA expression. *COMT* M/M carriers show evidence of efficient prefrontal cortical activity during the task, but the effect is canceled by the presence of *DTNBP1* Bray+/+ alleles [30••].

GC-C-KO mice

Guanylyl cyclase-C (GC-C), which is a membrane receptor for the gut peptide hormones guanylin and uroguanylin, is selectively and strongly expressed in dopaminergic neurons in the ventral tegmental area and substantia nigra compacta. GC-C activation by its ligands activates metabotropic glutamate receptors and muscarinic acetylcholine receptors via the activity of guanosine 3′,5′-monophosphate-dependent protein kinase [32]. GC-C-KO mice in the C57BL6 genetic background exhibit hyperactivity in both the home cage and novel open-field. In a Go/No-go test using water as a reward and two distinct auditory stimuli as Go and No-go signals, the GC-C-KO mice showed impulsivity and attention deficits [32]. The hyperactivity observed in the open field was ameliorated by