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REVIEW ARTICLE

# Strengths and limitations of genetic models of ADHD

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**Abstract** The cause and pathophysiology of attention-deficit hyperactivity disorder (ADHD) are unknown, but compelling evidence suggests an involvement of genetic factors. While dopamine is believed to play a major role in ADHD, the role for norepinephrine and serotonin systems has also been indicated. Mutant mice are valuable tools to dissect the contribution of specific neurotransmitter systems to brain dysfunction and particularly useful to decode complex multi-transmitter interaction that is critical to the pathophysiology of ADHD. Genetically altered mice provided also an opportunity to test experimentally the role of novel candidate genes for this disorder identified in genetic clinical studies. While it is clear that no rodent model would be able to recapitulate fully the complex nature of ADHD, certain endophenotypes could be reasonably well mimicked in these models. Multiple studies have reported associations between polymorphisms in dopamine transporter (DAT) gene and ADHD. Although the functional consequences of these associations are still unclear, it is believed that alterations in DAT-mediated processes might contribute to the pathogenesis of ADHD. Mice lacking the dopamine transporter have elevated dopaminergic tone and represent a genetic animal model in which certain endophenotypes of ADHD can be recapitulated. These mutants as well as other mouse models of DAT dysfunction provided an opportunity to investigate the neuronal circuitry and molecular mechanisms involved in the inhibitory action of psychostimulants on hyperactivity. Several additional knockout and transgenic mouse models have been proposed to model ADHD. Strengths and limitations of currently available genetic mouse models of ADHD are discussed.

**Keywords** ADHD · Dopamine · Serotonin · Dopamine transporter · Animal model · Psychostimulants

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## Introduction

Genetically modified mice have become an indispensable tool to address critical questions in the various fields of medicine. While the exact molecular mechanisms involved in the etiology of attention-deficit hyperactivity disorder (ADHD) are yet to be uncovered, clinical studies clearly indicate that ADHD has a significant genetic component with genetic influence exceeding that in such brain disorders as depression and anxiety disorders (Barkley 1997; Mill 2007; Russell 2007; Swanson et al. 2007). As a highly heritable disorder, ADHD should be relatively easily modeled by targeted genetic manipulations in experimental animals. Obviously, no animal model can recapitulate at full extend the human brain disorder that involves a complex set of cognitive and psychological manifestations. Animals, particularly rodents, do not have such intricate organization of the brain circuitry as humans do. Furthermore, many key endophenotypes of ADHD, such as inattention and impulsivity, can be investigated in rodents, particularly in mice, just partially. At the same time, the opportunity to manipulate with genome of animals and perform direct histological, physiological, or biochemical

measurements in brain tissue provides the most effective approach to investigate the role of specific candidate genes in in vivo physiological settings.

An ideal animal model of ADHD should recapitulate the major endophenotypes of ADHD (face validity), conform to an underlying pathological cause of ADHD (construct validity), and predict pharmacological responses to the clinically effective ADHD therapy (predictive validity) (Sagvolden et al. 2005; Russell 2007; van der Kooij and Glennon 2007). Needless to say that one should be careful with consistency and stability with which the variables of interest are observed in experiments critical for obtaining desired validity criteria and *reliability* of approaches should be considered as an additional criteria (Markou et al. 2009).

Genetic clinical investigations have shown an important role of multiple genes in the development of ADHD. Recent meta-analysis of candidate gene association studies for over the past 15 years have revealed a consistent evidence of significant associations between ADHD with polymorphisms in several candidate genes almost exclusively involved in the regulation of dopaminergic and serotonergic transmission such as *DAT1, DRD4, DRD5, 5HTT, HTR1B* and *SNAP25* (Khan and Faraone 2006; Gizer et al. 2009). Among them, perhaps the most commonly replicated associations were reported for *DAT1* and *DRD4*, but even for these genes a substantial population heterogeneity was observed.

## Role of dopamine and DAT in ADHD

The plasma membrane dopamine transporter (DAT), the member of a family of the $Na^+/Cl^-$—dependent transporters, plays a critical role in the regulation of extracellular DA concentrations by providing selective mechanism of rapid reuptake of the neurotransmitter into the presynaptic dopamine terminals (Kuhar et al. 1990; Amara and Sonders 1998; Gainetdinov and Caron 2003; Torres et al. 2003). DAT has attracted specific attention in ADHD research due to several reasons. It is well established that psychostimulants exert their major pharmacological actions through the interaction with the plasma membrane monoamine transporters, such as DAT but also with serotonin (SERT) and norepinephrine (NET) transporters. These drugs exert inhibitory and/or specific reversing actions on the function of monoamine transporters thereby disrupting normal reuptake of neurotransmitters from the synaptic cleft and thus causing an elevation in the extracellular monoamine levels. It is commonly believed that the elevation of extracellular dopamine is the primary determinant of psychostimulant action of these drugs (Kuhar et al. 1990). The therapeutic efficacy of psychostimulants in ADHD has been also related to interaction with the DAT. Assumption that mechanisms of action of psychostimulants in ADHD should involve similar neurochemical mechanisms as in normal subjects led to a hypothesis that ADHD represents a hypodopaminergic disorder and that psychostimulants should exert their therapeutic action in ADHD by restoring dopamine levels (Madras et al. 2005; Sagvolden et al. 2005; Russell 2007).

While the role of monoamines, and particularly dopamine, in the pathogenesis of ADHD seems to be generally accepted, there is still ongoing debate whether ADHD may represent hyperdopaminergic or hypodopaminergic disorder (Gainetdinov and Caron 2001; Madras et al. 2005; Russell 2007). Unfortunately, multiple attempts to analyze the status of the dopamine system in ADHD patients in clinical settings has yielded very controversial results, mostly because current approaches used in clinical research, such as analysis of plasma, urine, cerebrospinal fluid, and imaging studies, fail to provide a direct estimations of the most functionally important extracellular level of dopamine in the human brain. At the same time, clinical genetic studies have provided a provocative evidence suggesting that alterations in DAT-mediated processes could significantly contribute to the pathogenesis of ADHD. Most commonly discussed are associations between ADHD and the variable number tandem repeat (VNTR) in the 3′—untranslated region of *DAT1* (Cook et al. 1995). The 10-repeat allele has been associated with ADHD in most of these studies. More recently, additional markers in DAT1 gene have been also associated with ADHD (Mill 2007). It should be noted, however, that the functional consequences of these associations are still remain largely unclear, and it is questionable whether these polymorphisms in the non-coding regions of DAT gene could result in significant alterations in transporter expression or function. To further complicate the situation, brain imaging studies aimed at evaluation of the status of DAT expression were also extremely controversial. Initial observations describing a significant increase in DAT levels in few ADHD patients and normalizing effect of methylphenidate on increased DAT expression have received significant attention (Dougherty et al. 1999; Dresel et al. 2000). However, recent studies have questioned these observations and, in fact, observed a significant decrease in DAT levels in several brain areas of ADHD patients (Volkow et al. 2007, 2009).

It should be emphasized that overwhelming majority of experimental evidence indicates that enhanced dopaminergic transmission in the basal ganglia causes psychomotor stimulation, hyperactivity, euphoria, and reward. Numerous pharmacological studies in the variety experimental models with drugs enhancing or reducing intensity of dopaminergic transmission have shown that hyperactivity is caused by an increased dopaminergic tone

in the subcortical structures (Martin et al. 1998; Carlsson et al. 2001; Gainetdinov et al. 2002). Over the last decade, these pharmacological and neurochemical observations received strong support from the experimental genetic approaches involving mice with targeted genetic alterations in key components of dopaminergic transmission. Particularly important in this regard observations have been gained in several strains of mutant mice with altered function of the DAT (Table 1).

## DAT mutant mice

Development of mice with genetically altered DAT function provided novel test systems to explore the role of DAT in ADHD (Giros et al. 1996; Gainetdinov and Caron 2003). In particular, these mutants have proven to be extremely valuable in the experimental protocols aimed at understanding the specificity and the mechanism of a psychostimulant drug action in normal conditions and under pathological conditions of dopaminergic dysfunction (Gainetdinov et al. 2002). By using various genetic approaches, several strains of DAT mutant mice with wide variety in the level of DAT expression have been developed and at least partially characterized with regard to ADHD-related behaviors and responses to psychostimulants (Table 1). Most of these investigations have been focused on DAT knockout (DAT-KO) and heterozygous mice (Giros et al. 1996), but important input was gained also in studies involving mice with moderately reduced DAT levels (DAT siRNA-treated mutant mice) (Salahpour et al. 2007), mice with severely reduced DAT levels (DAT knockdown, DAT-KD) (Zhuang et al. 2001), as well as mice with the moderately increased DAT levels (DAT overexpressing mice) (Donovan et al. 1999) and mice with markedly elevated DAT levels (BAC-transgenic DAT overexpressing mice) (Salahpour et al. 2008).

The most investigated DAT mutant strain is the genetically engineered "DAT knockout" mouse that completely lacks expression of the DAT. Disruption of DAT-mediated reuptake process causes remarkable alterations in the both intraneuronal and extracellular DA concentrations (Giros et al. 1996; Gainetdinov and Caron 2003). These mice have about 300-fold increase in the extracellular lifetime of released DA resulting in at least fivefold elevation in the basal extracellular DA levels (Gainetdinov et al. 1998; Jones et al. 1998; Shen et al. 2004). At the same time, a marked depletion of reuptake-dependent intraneuronal dopamine stores (20-fold) and decreased amplitude of the evoked dopamine release (fourfold) was observed in these mutants (Gainetdinov et al. 1998; Jones et al. 1998). Thus, it might be speculated that the steady-state "tonic" extracellular DA levels are increased but "phasic" DA release could be, in fact, diminished due to depleted storage pools. Importantly, lack of DAT-mediated recycling makes remaining dopamine levels in the striatum of these mice totally dependent on the rate of its ongoing synthesis (Jones et al. 1998; Sotnikova et al. 2005).

The mode of dopamine transmission in DAT-KO mice could be described as "volume transmission" which is distinguished by the markedly increased distance that released DA molecules can cover and thereby affect a wide area of postsynaptic receptors and structures (Jones et al. 1998; Zoli et al. 1999). Similar situation could occur also following partial dopaminergic degeneration that causes significant reduction in the number of dopamine terminals and respective dopamine reuptake sites (Zoli et al. 1999). Thus, the fate of extracellular DA in another model of hyperactivity, the neonatal 6-hydroxydopamine (6-OHDA) treated mice, may be to some degree similar to that in

**Table 1** Summary of observations in DAT mutant models with regard to ADHD-related phenotypes

| Mice | DAT expression (%) | Extracellular DA (%) | Locomotor activity | Responses to psychostimulants |
|---|---|---|---|---|
| DAT-KO | 0 | 500 | Extreme hyperactivity | Amphetamine and methylphenidate inhibit hyperactivity |
| DAT knockdown | 10 | 200 | Moderate hyperactivity | Amphetamine inhibits hyperactivity |
| DAT heterozygous | 50 | 200 | Normal | Reduced stimulation after amphetamine |
| DAT siRNA | 60 | N.D. | Normal | Reduced stimulation after amphetamine |
| Wild type | 100 | 100 | Normal | Normal stimulation after amphetamine and methylphenidate |
| Transgenic DAT overexpression | 130 | N.D. | Hypoactivity in a novel environment | N.D. |
| BAC DAT overexpression | 300 | 60 | Normal | Markedly enhanced amphetamine stimulation |

Detailed description of these mutant models and respective references are presented in the text

*N.D.* not determined

DAT-KO mice. In this putative neurotoxic model of ADHD, neonatal treatment with the selective dopaminergic neurotoxin 6-OHDA, that causes partial degeneration of dopamine terminals via DAT-dependent mechanism, induces in a significant portion of developing animals (40–50%) a transient hyperactivity and cognitive deficits that last for about 2 weeks (Kostrzewa et al. 2008; Galinanes et al. 2009). It should be noted, however, that since DAT expression and function are quite low at early neonatal period (Patel et al. 1994; Gordon et al. 1995), only neurons with high expression of DAT should be affected by this toxin, while neurons that have delayed maturation of DAT may be protected and thus operate later quite effectively under conditions of "volume transmission".

In response to the permanently increased DA tone, the D2 class DA autoreceptors are down-regulated and desensitized (Jones et al. 1999) while postsynaptic D1 and D2 DA receptors in the striatum are down-regulated by approximately 50% (Giros et al. 1996). It is also important that hyperdopaminergia causes rearrangement of synaptic structures and regulates molecules involved in neuronal plasticity. Thus, the level of the scaffolding protein postsynaptic density-95 (PSD-95) is significantly reduced in the striatum and nucleus accumbens (Yao et al. 2004), while the levels of brain-derived neurotrophic factor (BDNF) gene expression are reduced in the frontal cortex of DAT-KO mice (Fumagalli et al. 2003). Many of these neurochemical abnormalities observed at a lesser degree in mice heterozygous for DAT deletion that show about twofold elevated extracellular DA levels (Jones et al. 1998, 1999).

It should be noted that the reduced intraneuronal DA storage, the diminished amplitude of evoked DA release, and the down-regulated DA receptor expression might be potentially interpreted as an evidence of "hypodopaminergia" in these mice; however, the most functionally important extracellular levels of DA are markedly elevated that led us to define the neurochemical profile of DAT-KO mice as a state of "functional hyperdopaminergia" (Gainetdinov et al. 1999a; Gainetdinov and Caron 2003).

This "functional hyperdopaminergia" in DAT-KO mice is manifested behaviorally as an extreme spontaneous hyperactivity (Giros et al. 1996; Sora et al. 1998; Gainetdinov et al. 1999b). While this hyperactivity is observed in familiar environment as well, an exposure to a novel environment particularly causes DAT-KO mice to become much more active, indicating that environmental cues can influence this behavior (Gainetdinov et al. 1999b). Behavioral activation caused by a novel environment in normal mice is generally attributed to the environmental stimuli-induced increase in mesolimbic DA release; however, no further rise in the extracellular dopamine accompanies exposure to a novel environment in mutants. Thus, a novel environment-induced potentiation of hyperactivity in DAT-KO mice is likely caused by the stress-related alterations in system(s) other than dopamine (Gainetdinov et al. 1999b). Intriguingly, DAT-KO mice show a perseverative pattern of locomotor hyperactivity (thigmotaxis) and in cognitive tests display deficits related to cognitive inflexibility (Gainetdinov et al. 1999b; Ralph et al. 2001; Morice et al. 2007). Particularly, the deficits were most notable as perseverative errors in the 8-arm maze test, strongly suggesting an impaired behavioral inhibition in these mice (Gainetdinov et al. 1999b). Similarly, DAT-KO mice were deficient in the Morris water maze learning and memory test (Morice et al. 2007). Furthermore, a sleep dysregulation, sensorimotor gating deficits as well as specific alterations in social interaction related to behavioral inflexibility have been also found in mutants (Ralph et al. 2001; Wisor et al. 2001; Rodriguiz et al. 2004). In tests assessing the rewarding values of tastants or food, DAT-KO mice developed a more positive bias toward a hedonically positive tastant (Costa et al. 2007) and showed an enhanced resistance to extinction from food-reinforced operant behavior (Hironaka et al. 2004), thus indicating reward-motivation deficits in these mutants.

DAT-KO mice were particularly instrumental to study the mechanisms of action of psychostimulants in normal conditions and under the conditions of DAT deficiency (Gainetdinov et al. 2002). Since 1937, when Charles Bradley first observed that Benzedrine (amphetamine) exerts therapeutic effect in hyperactive children, the question as to why drugs that induce psychostimulant excitation in normal individuals would have such an effect in ADHD population has remained open. As in ADHD patients, psychostimulants amphetamine and methylphenidate inhibited hyperactivity in DAT-KO mice, while in normal mice these drugs caused normal psychostimulant reaction consisting of hyperactivity and stereotypy (Gainetdinov et al. 1999b). Importantly, normal mice demonstrate the well-known rise in the extracellular DA levels in major DA brain areas, while antihyperkinetic action of psychostimulants in DAT-KO mice was not accompanied by a decrease in elevated DA levels. These findings indicated that in these mutants psychostimulants do not affect the dopamine system directly but exert their antihyperkinetic effects through modulation of other neurotransmitter systems targeted by these drugs such as norepinephrine or serotonin. We hypothesized that the well-known SERT-mediated effects of psychostimulants on serotonin neurotransmission could be particularly important for their inhibitory action on the dopamine-dependent hyperactivity (Gainetdinov et al. 1999b). While detailed pharmacological investigations have indeed revealed a prominent role of serotonin in the modulation of dopamine-dependent hyperactivity, this effect may also involve alterations in norepinephrine transmission by psychostimulants. In fact, in striking contrast to DAT-KO

mice, both SERT-KO and NET-KO mice demonstrate the spontaneous hypoactivity (Xu et al. 2000; Kalueff et al. 2007) and thus the well-established action of psychostimulants on these systems may dampen hyperactivity caused by enhanced dopamine tone. Notably, serotonin and norepinephrine systems can intimately interact at multiple levels and very often changes in one system cause indirect alterations in another (Bortolozzi and Artigas 2003).

With regard to an involvement of serotonin system, it is well established that serotonin plays a critical role in impulse regulation and inhibitory control on the external stimuli-induced behavioral activation (Lucki 1998; Winstanley et al. 2005). It should be mentioned that at least 14 subtypes of serotonin receptors are found in mammals and serotonin can have an extremely complex set of actions on behavior involving both the "inhibitory" and "stimulatory" actions (Martin et al. 1998; Gainetdinov et al. 1999b; Rocha et al. 2002; Barr et al. 2004). It is believed that the likely candidates for "stimulatory" action are 5-HT1B and 5-HT2A receptors, while 5-HT1A and 5HT2C receptors can serve as "inhibitory" receptors (Martin et al. 1998). It has been hypothesized that a balance between the action of serotonin on these "stimulatory" and "inhibitory" receptors could be important for determining the state of behavioral activation (Martin et al. 1998) and thus, dopamine-dependent hyperactivity could be inhibited either by agonists of "inhibitory" receptors or by antagonists of "stimulatory" serotonin receptors. In fact, hyperactivity of DAT-KO mice can be suppressed either by direct and indirect serotonin agonists (Gainetdinov et al. 1999b; Spielewoy et al. 2001; Morice et al. 2004; Powell et al. 2004; Beaulieu et al. 2006) or by an antagonist of "stimulatory" 5-HT2A receptors (Barr et al. 2004). Further studies are necessary to determine which subtype(s) of serotonin receptors are primarily involved in the antihyperkinetic effects of psychostimulants. It is also important that the antihyperkinetic action of psychostimulants and serotonin drugs requires an intact fronto-striatal glutamatergic transmission suggesting that the site of antihyperkinetic action of serotonin is likely localized within the frontal cortex. In particular, these inhibitory actions could be completely disrupted by the pretreatment with the glutamate NMDA receptor antagonist (+)-MK-801. In contrast, drugs that enhance efficacy of glutamatergic transmission via positive modulation of AMPA glutamate receptors, such as AMPAkines, can effectively suppress hyperactivity in DAT-KO mice (Gainetdinov et al. 2001).

Recently, DAT-KO mice were used to understand the intracellular signaling mechanisms that could be involved in the paradoxical inhibitory effects of psychostimulants (Beaulieu et al. 2006). Accumulating evidence suggests that the regulation of locomotor activity by dopamine involves three major striatal signaling pathways, namely protein kinase A (PKA)-mediated, Akt/glycogen synthase kinase 3 (GSK-3)-mediated, and extracellular signal-regulated kinase (ERK)-mediated. Analysis of these pathways following treatment with psychostimulants and various serotonin agonists in DAT-KO mice revealed that the inhibition of ERK signaling is a common determinant for the ability of these drugs to antagonize DA-dependent hyperactivity. In striking contrast, psychostimulant treatment of normal animals resulted in increased phosphorylation of ERK. Importantly, direct inhibition of the ERK-mediated signaling cascade by intracerebroventricular infusion of the mitogen-activated protein kinase kinase (MEK) inhibitor SL327 potently inhibited hyperactivity of DAT-KO mice and similarly blocked the hyperlocomotor effect of amphetamine in normal mice. Thus, the paradoxical inhibitory action of psychostimulants in the hyperdopaminergic mice seems to be mediated via intracellular signaling mechanism involving regulation of striatal ERK that likely serves as a coincidence detector of dopamine and glutamate activity at the level of medium-spiny GABA neurons (Beaulieu et al. 2006).

In summary, DAT-KO mice display several key endophenotypes of ADHD, including spontaneous hyperactivity, impaired behavioral inhibition, cognitive deficits, deficient extinction of previously reinforced behavior and inhibitory responses to psychostimulants, thereby conforming to certain face and predictive validity (Gainetdinov et al. 1999b; Gainetdinov and Caron 2000, 2001). As long as *DAT1* remains a major candidate gene for ADHD, all DAT mutant mice have at least partial construct validity, although more definitive understanding of the mechanism of DAT involvement in the pathogenesis of ADHD is necessary. Nevertheless, as with any model, DAT-KO mice have certain limitation. Obviously, the level of DAT dysregulation and dopamine dysfunction is too extreme and DAT-KO mice have some other phenotypes (dwarfism (Bosse et al. 1997), hormonal dysregulation (Bosse et al. 1997), remarkable neurochemical alterations (Jones et al. 1998)) that may not be relevant for classical ADHD. Second, more studies are necessary to explore effect of the NET inhibitors in these mice to further support the predictive validity of this model. Third, robust approaches have to be developed to assess the effects of psychostimulants and other clinically effective compounds in the cognitive tasks relevant for ADHD in mice, but the recent progress in the development of *reliable* tests to analyze attention and impulsivity in mice (Helms et al. 2008; Young et al. 2009) give a hope that these shortcomings should be soon alleviated. At the same time, the extreme level of hyperactivity in DAT-KO mice gives certain advantages to this model as a very simple and sensitive test system to reveal the effects of novel pharmacological treatments to affect DA-dependent

hyperactivity. For example, a more subtle model of DAT dysfunction, DAT heterozygous mice that have about 50% reduction in DAT expression and twofold increase in extracellular DA levels, do not display such hyperactivity. In these mice, however, a decrease in stimulatory action of amphetamine was observed with only one (relatively low) dose of amphetamine demonstrating the inhibitory action (Spielewoy et al. 2001). Similar observations have been made in another model of mild DAT deficiency developed by local injections of small interfering RNA (siRNA) against DAT into the ventral tegmental/substantia nigra of adult mice (Salahpour et al. 2007). This approach achieved about 40% decrease in DAT levels but no changes in spontaneous activity levels were observed. At the same time, hyperlocomotor responses to amphetamine were reduced in DAT siRNA-treated mice.

Additional model of severe DAT dysfunction, DAT knockdown (DAT-KD) mice, has been also developed. These mutants have about 90% reduction in DAT levels and display similar but less pronounced phenotype in comparison to DAT-KO mice (Zhuang et al. 2001). DAT-KD mice have about twofold increased extracellular DA levels and show a relatively milder level of spontaneous hyperactivity in a novel environment. DAT-KD mice demonstrate normal weight and development and display an impaired response habituation and paradoxical hypolocomotor response to amphetamine (Zhuang et al. 2001), but not to cocaine (Tilley et al. 2007). These mutants have also demonstrated an enhanced motivation, but not learning, to rewarding stimuli (Pecina et al. 2003; Cagniard et al. 2006) and altered regulation of the corticostriatal glutamatergic neurotransmission (Zhuang et al. 2001) that could contribute to the abnormal striatal information processing critical for the behavioral deficits in these mutants. As a model of ADHD, these mice certainly have some face, construct, and partially predictive validity, but as with other mouse strains a more detailed characterization of the cognitive abnormalities should be performed. Furthermore, the responses to other than amphetamine drugs used in ADHD (methylphenidate, atomoxetine) should be tested to further validate the use of these mice in ADHD research.

In striking contrast to DAT-deficient models, mice with an increased DAT function show no behaviors relevant to ADHD. For example, transgenic mice that have modestly increased DAT expression in tyrosine hydroxylase–expressing neurons (approximately 30% up-regulation) show hypoactivity in a new environment, that is likely reflects the decreased extracellular level of DA in these mutants (Donovan et al. 1999). Recently, a novel model of robust overexpression of DAT has been developed by using the bacterial artificial chromosome (BAC) transgenic approach. These mice have a threefold increased density of DAT in DA neurons that results in about 40% decrease in extracellular DA levels and 30–60% up-regulation in the expression and function of postsynaptic DA receptors (Salahpour et al. 2008; Ghisi et al. 2009). Behaviorally, DAT overexpressing animals display similar locomotor stimulation when treated with the DAT blockers such as GBR12909, methylphenidate, and cocaine. However, amphetamine induced in these mice a markedly increased locomotor stimulation and caused a threefold greater increase in the amount of extracellular DA compared with control animals (Salahpour et al. 2008). While one might argue for the construct validity of DAT overexpressing mice as a model of ADHD, there is little or none evidence that these mice have face or predictive validity. Thus, experimental evidence with targeted mutagenesis approach does not support postulated increase in DAT levels in ADHD patients (Dougherty et al. 1999; Dresel et al. 2000; Madras et al. 2005), rather strongly supporting clinical observations reporting a decrease in the transporter expression (Volkow et al. 2007, 2009). It should be noted, that, at least in rodents, the DAT function is relatively low at birth and there is an extended period of DAT maturation during postnatal development (Patel et al. 1994; Gordon et al. 1995). If similar developmental maturation occurs with the DAT in humans, pathological mechanisms that could disrupt this process could be suspected to contribute to the development of ADHD.

## Other genetic models of ADHD

Among various genetic animal models that have been proposed to model ADHD, several have unknown genetic origin. These models, such as the hyperactive wheel-running mouse, the acallosal I/LnJ mouse, and spontaneously hyperactive rat (SHR), have been described in several recent excellent reviews (Sagvolden et al. 2005; Mill 2007; Russell 2007; van der Kooij and Glennon 2007) and will not be a subject of the current essay that focuses on the well-defined genetic backgrounds for the model development. With regard to other most defined candidate genes for ADHD (Khan and Faraone 2006; Gizer et al. 2009), it should be noted that only 5HT1B receptor knockout mice display hyperactivity (Brunner et al. 1999). Knockouts of all the other genes from this short list do not show phenotypes relevant for ADHD. Observations in mice lacking one the most reported gene of interest for ADHD, D4 dopamine receptor, are particularly disappointing. The mice do not show hyperactivity while the hyperlocomotor effects of psychostimulants are significantly increased (Rubinstein et al. 1997). Attempts to investigate impulsivity in these mice have shown that D4 receptor deficiency in mice has only limited effects on impulsivity and novelty seeking (Helms et al. 2008). Thus, despite certain construct

validity, D4 knockout mice have no face or predictive validity. With regard to SERT-KO mice, their hypoactivity (Kalueff et al. 2007) suggests that SERT is unlikely to be involved in the pathophysiological mechanisms of ADHD development per se, but may be important determinant of efficacy of ADHD treatment. In fact, the recent report indicated that SERT gene was among the most important candidate genes in moderating treatment effects of methylphenidate (McGough et al. 2009).

Another gene of interest is *SNAP-25* (Hess et al. 1996). While only limited data with SNAP-25 knockout mice are available, the most attention has attracted Coloboma mutant mouse that has been developed as a result of neutron irradiation in mice. The mutations in the Coloboma mutant are located on chromosome 2 and include about 20 genes disrupted along with *SNAP-25* (Hess et al. 1996). Coloboma mice have a very complex phenotype including dysmorphology of the eye, delayed attachment of the lens vesicle and microphthalmia. These mice are suggested as a model for ADHD because they demonstrate spontaneous hyperactivity that mostly manifested as circling with characteristic head bobbing. It has been reported that amphetamine attenuates hyperactivity in these mutants, but methylphenidate increases locomotor activity similarly both in Coloboma and in controls (Hess et al. 1996). While these mice have some construct validity with regard to ADHD model, they certainly have only limited face or predictive validity (Sagvolden et al. 2005; Mill 2007; Russell 2007; van der Kooij and Glennon 2007). Further studies are necessary to investigate the role of SNAP-25 in ADHD, but with regard to animal models it would be preferable to have a model of selective SNAP-25 deficiency such as SNAP-25 heterozygous mice. It should be noted, however, that recent study involving SNAP-25 heterozygous mice failed to detect significant hyperactivity or any other behavioral abnormalities in SNAP-25 heterozygous mice and thus the phenotype of Coloboma mice is likely caused by the deficiency in other genes disrupted in this strain (Oliver and Davies 2009).

Among the other genetic models that may have some preliminary level of validation, the most noticeable are mice mutant in thyroid receptor $\beta$ (Siesser et al. 2005, 2006). There is some evidence that thyroid hormone dysfunction could be related to ADHD in some specific populations and, for example, a relatively high proportion of patients with resistance to thyroid hormone syndrome, resulting from mutations in the thyroid receptor $\beta$ (Thr$\beta$) gene, have comorbid ADHD. Transgenic mice that express a human mutant $\beta$1 thyroid receptor have thyroid resistance demonstrate a very minor hyperactivity after repeated application to a novel environment as well as abnormalities in some indirect measures of impulsivity and attention (Siesser et al. 2005, 2006). This certainly may contribute to some face validity of this specific type of ADHD model, but the normal stimulatory response to methylphenidate challenges the validity of these transgenics with regard to predictive value. Thus, the limited construct validity and the ambiguous face and predictive validity of these mutants significantly question the use of these mice as a putative ADHD model. Recent preliminary investigations have suggested that the mice deficient in NK1 receptor (Yan et al. 2010) and steroid sulfatase (Davies et al. 2009) might be also relevant for ADHD, but a very limited information available at present prevents from the unambiguous categorization of these mice as putative models of ADHD.

## Conclusions

Establishing the construct, face, and predictive validity of genetic animal models of ADHD is limited by the paucity of rigorous experimental data on the pathogenesis of this disorder gained from the clinical studies. The major source of validation remains in the ability to model in animals the major endophenotypes of ADHD and demonstrate the efficacy of psychostimulants and other clinically effective treatments. While there may be no single perfect animal model of ADHD, it is clear that each model has certain strengths and limitations that need to be recognized in order to use the model effectively in the investigation of the pathogenesis of this disorder and development of new therapies. As with other psychiatric disorders, multiple animal models are likely needed for ADHD to allow investigation of the various endophenotypes of the disease and to provide convergent validation of the research findings from each model. At the same time, the process of developing and validating animal models is critically dependent on the progress in identification of reliable measures of the human pathophysiology and uncovering novel pathogenetic mechanisms and genetic factors (Markou et al. 2009). One example of such fruitful approach in translational medicine is the development and validation of DAT mutant mice as a genetic model of ADHD. Mice with genetically altered DAT function provided a powerful approach to model in animals various pathological conditions of DAT dysfunction that may occur in ADHD and investigate the in vivo effects of pharmacological compounds in conditions of severe dopaminergic dysfunction. In fact, hyperdopaminergic DAT-KO mice display several endophenotypes of ADHD, including hyperactivity, cognitive and reward-motivation deficits, impaired behavioral inhibition and paradoxical inhibitory responses to psychostimulants. In contrast, mice with persistent hypodopaminergia induced by the increased expression of DAT show no phenotypes relevant to ADHD. It should be noted that these studies

involved mice generated with a constitutive mutagenesis approach, thus a possibility exist that developmental compensations could contribute to the overall phenotypes of these mice. Recent progress in the development of conditional mutant mouse strains that have a desired genetic manipulation with the specific spatiotemporal characteristics, particularly in the adult animals, could significantly further application of these models to ADHD research.

In general, while multiple genetic defects could contribute to ADHD, the consequences of dopaminergic dysregulation that are observed in DAT-deficient mice provide a strong evidence for the role of mutations affecting the function of dopamine system as the most likely cause of development of ADHD. At the same time, mutations in other monoaminergic systems may provide important contributing factors particularly for the therapy effectiveness. Future identification of novel genetic factors and pathophysiological mechanisms in ADHD patients combined with the development of appropriate mutant models in mice bearing these abnormalities should significantly further our knowledge regarding this disorder and pave the way for more effective and safe therapies.

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