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Rodent models: Utility for candidate gene studies in human attention-deficit hyperactivity disorder (ADHD)

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Abstract

Attention-deficit hyperactivity disorder (ADHD) is a common neurobehavioral disorder defined by symptoms of developmentally inappropriate inattention, impulsivity and hyperactivity. Behavioral genetic studies provide overwhelming evidence for a significant genetic role in the pathogenesis of the disorder. Rodent models have proven extremely useful in helping understand more about the genetic basis of ADHD in humans. A number of well-characterized rodent models have been proposed, consisting of inbred strains, selected lines, genetic knockouts, and transgenic animals, which have been used to inform candidate gene studies in ADHD. In addition to providing information about the dysregulation of known candidate genes, rodents are excellent tools for the identification of novel ADHD candidate genes. While not yet widely used to identify genes for ADHD-like behaviors in rodents, quantitative trait loci (QTL) mapping approaches using recombinant inbred strains, heterogeneous stock mice, and chemically mutated animals have the potential to revolutionize our understanding of the genetic basis of ADHD.

Keywords: Attention-deficit hyperactivity disorder (ADHD); Genetics; Animal model; Quantitative trait loci (QTL); Rodents; Behavior; Candidate gene

1. Introduction

Attention-deficit hyperactivity disorder (ADHD) is a common neurobehavioral disorder defined by symptoms of developmentally inappropriate inattention, impulsivity and hyperactivity. It is estimated that between 3 and 6% of school age children are diagnosed with ADHD, making it the most prevalent psychiatric disorder of childhood. While the precise etiology of ADHD is yet to be ascertained, family (Barkley and Russell, 1997), twin (Thapar, 2002) and adoption studies (Sprich et al., 2000) provide overwhelming evidence for a significant genetic role in the pathogenesis of the disorder. Heritability estimates from most of these studies suggest additive genetic variance to be in the range of 60–90%, and a figure of 70% seems a reasonable average (Eaves et al., 1997). According to these data, ADHD is more heritable than depression (39% heritability), generalized anxiety disorder (32% heritability), breast cancer (27% heritability) and asthma (39% heritability) (Spencer et al., 2002). It is likely that susceptibility is mediated by the effect of numerous genes of small effect, interacting both epistatically and with the environment. Whilst there has undoubtedly been progress in identifying the loci involved in ADHD, and replicated associations with polymorphisms in several genes now exist (Faraone et al., 2005), we are still a considerable distance from fully understanding the precise genetic causes of the disorder. Furthermore, we need to understand the functional relevance of the associated polymorphisms, and their neurobiological and behavioral consequences, before the goal of using genetics to inform novel diagnostic and therapeutic strategies is fully realized.

Rodent models have proven extremely useful in helping understand more about the genetic basis of ADHD in humans. A number of well-characterised rodent models have been proposed, consisting of inbred strains, selected lines, genetic knockouts, and transgenic animals. It is not the goal of this review to fully document the behavioral and neurobiological characteristics of these rodents—a number of excellent review articles have been published on this subject over the last couple of years (e.g. Davids et al., 2003; Russell et al., 2005; Sagvolden et al., 2005). It is also not the aim of this article to detail the specific behavioral paradigms being applied to rodents to test aspects of the ADHD phenotype; it is worth noting, however, that for

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genetic studies it is important that these tests reliably tap into the same neurobiological circuits postulated to be dysfunctional in the human disorder.

In this article, I will discuss how findings from rodents, in particular mice, are guiding research into the genetic basis of the clinical disorder. The utility of rodents in genetic studies of complex disease has increased dramatically since the publication of the draft mouse genome in 2002 (Waterston et al., 2002) and the rat genome in 2004 (Gibbs et al., 2004). Two general strategies exist to investigate the genetics of behavioral phenotypes in rodents, and both have informed candidate gene studies in humans. First, because their genomes can be manipulated in a way that the human genome cannot, rodents can be used to investigate the *function* of specific *known* risk genes via the generation of mutant inbred lines containing specific DNA sequence alterations (e.g. knockout, knockdown, and transgenic mice). Second, elaborate breeding strategies are possible in rodents meaning that quantitative studies utilizing the naturally occurring genetic variation between and within strains can be employed to systematically hunt for *novel* genes that regulate aspects of ADHD-like behavior, nominating additional candidate loci for investigation in humans. Furthermore, rodent models are ideal for investigating the role of gene–environment interactions via the controlled manipulation of the environment in genetically identical individuals, and can also be used to investigate the gene expression changes associated with ADHD medication. While many of these studies are in their infancy, advances in mutagenic and gene-mapping technologies suggest that rodents will provide a valuable future tool in our efforts to identify specific genetic risk factors for ADHD.

2. Single gene studies in mutant rodents: investigating the function of ADHD candidate genes

Current aetiological theories postulate that ADHD is a highly complex disorder, caused in part by the action of numerous genes of relatively small effect. Genetic studies of ADHD have predominantly taken a candidate gene approach—investigating the relevance of biologically plausible loci, for which there is *a priori* evidence to support a role in the aetiology of the disorder. In this regard, the major focus to date has been on the role of genetic variation within genes involved in the regulation of catecholaminergic neurotransmission, particularly the dopamine-system. Various lines of evidence support a role for dopamine in the aetiology of ADHD. Stimulant drugs are highly effective in alleviating the symptoms of ADHD in both children (Konrad et al., 2004) and adults (Faraone et al., 2004). The effect of these drugs is mediated principally by the blockade of dopamine reuptake at the dopamine transporter (DAT), with a subsequent increase in synaptic dopamine. Neuroimaging studies further implicate dopamine in the aetiology of the disorder, with brain regions rich in dopaminergic innervations showing structural abnormalities (Castellanos et al., 2003) and altered DAT density (Dougherty et al., 1999). Increasing evidence also alludes to a role for serotonergic, glutaminergic and noradrenergic systems in the development of ADHD (Oades, 2002).

Compared to the progress seen for other psychiatric disorders, the candidate gene approach in ADHD has been relatively successful, finding associations with a number of polymorphisms in various genes encoding neurotransmitter receptors, metabolic enzymes, and the synaptosomal proteins that mediate the vesicular release of neurotransmitters. A recent review and meta-analysis of candidate gene polymorphisms in ADHD by Faraone et al. (2005) identified variants in seven genes (DRD4, DRD5, DAT, DBH, 5-HTT, HTR1B, and SNAP-25) that show the strongest evidence for association with ADHD, with replications of these findings observed in several studies. Knockout, knockdown, and transgenic mutant rodent models have been extremely useful for understanding the biochemical, neuronal and behavioral processes regulated by the specific candidate genes postulated to mediate susceptibility to ADHD (Table 1). The behavioral and neurochemical phenotypes observed in these animals have clearly linked certain neurobiological systems to the aetiology of the disorder. They have also given us insight into the many compensatory responses instigated in response to the severe misregulation of specific neurochemical pathways in order to maintain functional homeostasis in the brain. It is worth noting, however, that whilst these genetically engineered animals are clearly 'models of gene function', they cannot be labeled true 'models of ADHD'. ADHD is a highly heterogeneous disorder caused by the action of numerous interacting genes and environmental factors, synergistically influencing the function of intricate biological systems. Single-gene models provide evidence to link the function of specific genes to *aspects* of the clinical disorder, but for a true genetic model of ADHD we need to investigate strains with more subtle allelic variation resulting in more quantitative changes to gene function.

2.1. The dopamine transporter gene

One of the first dopamine-related genes to be nominated as a candidate for ADHD was the dopamine transporter gene (DAT1), located on chromosome 5p15.3 (Cook et al., 1995). The DAT mediates uptake of dopamine into neurons, and is a major target for various pharmacologically active stimulants such as cocaine and methylphenidate. Initial genetic association studies focused on a variable number tandem repeat (VNTR) polymorphism in the 3′-untranslated region (UTR) of DAT1. The VNTR polymorphism consists of a 40 bp sequence that most frequently occurs as 9 or 10 tandem repeat units, although 3–11 repeats are also observed. The 10-repeat allele of this polymorphism has been associated with ADHD in numerous studies, although several non-replications have also been reported and a meta-analysis reports significant heterogeneity between datasets (Curran et al., 2001). More recent studies have examined additional markers across the DAT1 gene, finding stronger evidence for an association with multi-marker haplotypes containing the 10-repeat VNTR allele (Brookes et al., 2006; Feng et al., 2005b). The 10-repeat allele has been associated with quantitative ADHD-trait scores in general population samples (Cornish et al., 2005; Mill et al., 2005a), suggesting it may act as a quantitative trait locus (QTL) influencing hyperactivity in the normal range above and beyond its role as a risk for clinical ADHD. The mechanism

Table 1
Selected rodent models that have been used to inform candidate gene studies in ADHD

Rodent	Type	Selected references	Behavioral/neurobiological summary	Genes implicated
Spontaneously hypertensive rat (SHR)	Selected line	Sagvolden (2000); Russell et al. (2005); Mill et al. (2005b)	Motor hyperactivity, increased impulsiveness and deficient sustained attention. Sensitive to immediate behavioral reinforcement. Responsive to stimulant medication. Altered dopaminergic and noradrenergic neurotransmission.	*Dopaminergic and noradrenergic genes*: altered dopaminergic and noradrenergic neurotransmission relative to WKY control animals suggests that hyperactive phenotype may be influenced by genes in these systems. *DAT1*: sequencing of dopaminergic loci identified polymorphisms in the dopamine transporter gene.
DAT-KO mouse	Gene knockout	Giros et al. (1996); Gainetdinov et al. (1999)	Increases in spontaneous behavioral activity. Prolonged dopamine persistence in synapse. Hyperactivity alleviated by stimulant medication. Severe developmental problems.	*DAT1*: total ablation of dopamine transporter gene expression causes extreme hyperactivity. *Serotonergic and noradrenergic genes*: paradoxical response to stimulants implicates serotonergic and noradrenergic system genes.
DAT-KD mouse	Gene knockdown	Zhuang et al. (2001)	Dopamine transporter expression reduced to 10% of wild-type levels. Normal home cage activity but hyperactivity and impaired response habituation in novel environments. No developmental problems.	*DAT1*: reduced dopamine transporter gene expression leads to less severe behavioral phenotype than observed in DAT-KO mice, suggesting DAT1 expression is linked to hyperactivity.
Coloboma mouse	Hemizygous deletion	Hess et al. (1996); Jones and Hess (2003); Bruno and Hess (2006)	Severe spontaneous hyperactivity that is reduced after administration of certain stimulants.	*SNAP-25*: hemizygous for a deletion spanning the *Snap25* gene—reintroduction of SNAP-25 via a transgene abolished hyperactive phenotype. *ADRA2C*: noradrenalin levels in *coloboma* mice also linked to hyperactivity, with the alpha(2C)-adrenergic receptor (ADRA2C) particularly important.
D4-KO mouse	Gene knockout	Rubinstein et al. (1997); Avale et al. (2004)	Less active than wild type controls in open-field activity tests. Exhibit reduced exploration of novel stimuli. Supersensitive to ethanol, cocaine, and methamphetamine. Elevated dopamine synthesis. Unlike wild-type mice, DRD4-KO mice lesioned with 6-OHDA do not develop hyperactivity.	*DRD4*: implicates DRD4 in the modulation of normal, coordinated, and drug-stimulated motor behaviors.
6-Hydroxydopamine (6-OHDA) lesioned neonatal rat/mouse	Chemically lesioned animal	Shaywitz et al. (1976); Avale et al. (2004)	6-OHDA selectively damages catecholaminergic neurons and produces hyperactivity that can be alleviated with stimulant medication. In DRD4-KO mice 6-OHDA lesioning does not produce hyperactivity.	*DRD4*: suggests DRD4 is essential for the expression of juvenile hyperactivity and impaired behavioral inhibition in 6-OHDA lesioned animals.
Alpha4beta2 nicotinic receptor KO mouse	Gene knockout	Granon and Changeux (2006)	Demonstrate ADHD-like symptoms, which are alleviated by nicotinic agonists.	*Nicotinic acetylcholine receptor subunit genes*: lack of functional receptor causes hyperactivity phenotype.
TRβ mutant	Transgenic mutant mouse	Siesser et al. (2006)	Show no thyroid abnormalities, but exhibit numerous ADHD-like symptoms including inattention, hyperactivity, and impulsivity.	*TRβ*: could explain comorbidity between ADHD and resistance to thyroid hormone syndrome.

behind this association is not yet understood, although several lines of evidence implicate variation in gene expression (Mill et al., 2002a; VanNess et al., 2005).

The postulated role of DAT1 in ADHD is also supported by brain imaging studies. Several *in vivo* analyses using single photon emission computed tomography (SPECT) demonstrate increased DAT density in ADHD probands compared to controls (Dougherty et al., 1999; Dresel et al., 2000), although such findings are not ubiquitous (van Dyck et al., 2002). Interestingly, methylphenidate has been shown to normalize levels of DAT in the brain in adults with ADHD (Dresel et al., 2000). In addition, several studies suggest there may be an association between DAT1 genotype and DAT density (e.g. Heinz et al., 2000; Jacobsen et al., 2000). Finally, the most thorough investigation of methylphenidate response in relation to DAT1 genotype suggests that the 10-repeat allele is associated with a positive response to the drug (Kirley et al., 2003), as would be expected if the density of the DAT to which methylphenidate binds is increased in individuals with the 10-repeat allele. Again, this finding is not ubiquitously replicated (Roman et al., 2002; Winsberg and Comings, 1999) suggesting that the role of the DAT1 gene is complex, and likely to be mediated by other polymorphisms in addition to the VNTR.

Several rodent models have been used to examine the role of the DAT1 gene, and explore its putative relationship with the ADHD phenotype. The dopamine transporter knockout (DAT-KO) mouse shows dramatic increases in spontaneous behavioral activity compared to wild-type mice (Giros et al., 1996). In homozygous DAT-KO mice, dopamine persists for ~100 times longer in the synaptic space, demonstrating the importance of a properly functioning DAT in controlling dopamine flux. Interestingly, a number of compensatory responses are observed in these animals including decreases in dopamine release and the down-regulation of dopamine receptors, giving us insight into how dopaminergic homeostasis is maintained in the brain. A potential limitation of DAT-KO mice is that they totally lack the dopaminergic target for the psychostimulant drugs widely used as medication for ADHD, and thus, cannot be used to investigate potential dopaminergic mechanisms in the treatment of ADHD. Despite expressing no functional dopamine transporter, it appears counter-intuitive that behavioral hyperactivity in DAT-KO mice is still alleviated by the administration of psychostimulants such as amphetamine, methylphenidate, and cocaine (Gainetdinov et al., 1999). These experiments suggest a role for other systems, particularly the serotoninergic system, in the pharmacological effects of these drugs. Noradrenaline may also be important, given that the behavioral response to amphetamine and cocaine is mimicked by selective inhibitors of the noradrenaline transporter (NET), but not of the DAT (Carboni et al., 2001). In this regard, perhaps the greatest insight for genetic studies gained from the study of DAT-KO mice is confirmation that other neurotransmitter systems, in addition to dopamine, are likely to be important mediators of the hyperactive phenotype.

Completely knocking out the function of a gene is obviously an extreme situation, unlikely to mimic effects seen in common human behavioral disorders like ADHD. It is worth noting, therefore, that DAT-KO mice demonstrate a number of behaviors generally not seen in children with ADHD including growth retardation, changes in reproductive behavior, and premature death (Bosse et al., 1997). In order to better model the variation in gene function likely to occur in normal populations, Zhuang et al. (2001) created a dopamine transporter knockdown (DAT-KD) mouse strain in which expression of the dopamine transporter is reduced to 10% of that observed in wild-type animals. Like DAT-KO mice, these animals appear to clear dopamine from the synapse at a much slower rate than observed in wild-type animals, resulting in a significantly higher extracellular dopamine concentration. Unlike DAT-KO mice, however, they show no apparent developmental defects. Behaviorally, DAT-KD animals have normal home-cage activity but display-marked hyperactivity and impaired response habituation in novel environments. Whilst both the DAT-KO and DAT-KD mice are highly informative about the function of the dopamine transporter gene, neither may be a realistic model of the changes in dopamine transporter expression observed in ADHD. As discussed above, several brain imaging studies suggest an increase in DAT density in the brains of human subjects with ADHD, and the 'risk' allele of the most-studied polymorphism in the DAT1 gene has been widely linked to increased DAT expression (Madras et al., 2005).

2.2. The SNAP-25 gene

SNAP-25 (synaptosomal-associated protein of 25 kDa) is a presynaptic plasma membrane protein with an integral role in synaptic transmission. It forms a complex with syntaxin and the synaptic vesicle proteins (synaptobrevin and synaptotagmin) that mediates the Ca^{2+}-mediated exocytosis of neurotransmitter from the synaptic vesicle into the synaptic cleft. Expression studies suggest that SNAP-25 is differentially expressed throughout the brain, and present primarily in the neocortex, hippocampus, anterior thalamic nuclei, substantia nigra, and cerebellar granule cells (Oyler et al., 1989). During development, SNAP-25 appears to be involved in synaptic plasticity and axonal growth (Osen-Sand et al., 1993), but in the mature nervous system expression is generally only seen in presynaptic terminals (Oyler et al., 1989). Several lines of evidence suggest a role for SNAP-25 in the genetic etiology of ADHD. Most notable is the coloboma mouse mutant, which displays spontaneous hyperactivity and is hemizygous for a deletion spanning this gene. Given its function, it is postulated that variation in the SNAP-25 gene, located at chromosome 20p13, may mediate susceptibility to ADHD by altering the release of dopamine and other neurotransmitters at the synapse. Several groups have reported an association between polymorphisms in SNAP-25 and clinical ADHD (Feng et al., 2005a; Mill et al., 2004), although to date the precise causal variant has yet to be ascertained.

The *coloboma* mouse mutant was produced by neutron irradiation and is only viable in the heterozygous form. It demonstrates a number of behavioral traits that resemble some of the deficits seen in ADHD, including severe spontaneous hyperactivity (Hess et al., 1996). In addition, hyperactivity in coloboma mice is alleviated by low doses of amphetamine, although

methylphenidate does not have this 'therapeutic' effect. The extreme hyperactivity exhibited by these mice results from a 2-cM deletion on mouse chromosome 2, in a region containing the mouse SNAP-25 gene (Hess et al., 1992). The introduction of a transgene containing a functional *Snap-25* gene to counteract this deletion returned the mice to normal levels of locomotor activity. As with DAT-KO mice, neurobiological research on *coloboma* mice suggests that the functional effects of the deleted gene may be mediated by additional neurochemical pathways. In particular, it appears that while brain dopamine utilization is reduced in *coloboma* mice, calcium-dependent norepinephrine release is significantly increased (Jones and Hess, 2003). Furthermore, artificial depletion of noradrenalin in these animals significantly reduces their locomotor hyperactivity. Recently, it has also been demonstrated that the alpha (2C)-adrenergic receptor (ADRA2C) plays a role in mediating hyperactivity in *coloboma* mice (Bruno and Hess, 2006), suggesting that this could be another good candidate gene for ADHD, although as yet there is little evidence to support an association (De Luca et al., 2004).

2.3. The dopamine D4 receptor gene (DRD4)

Perhaps the most consistent association finding in ADHD genetics has been with the dopamine D4 receptor (DRD4) gene located on chromosome 11p15.5. DRD4 was first cloned by Van Tol et al. (1991) and found to have high homology with DRD2 and DRD3. Its high affinity for the antipsychotic drug clozapine means it has been one of the most widely studied genes in neuropsychiatric genetics. DRD4 was first reported to be associated with novelty seeking and impulsivity-two personality traits that are known to be correlated with ADHD (Ebstein et al., 1996; Benjamin et al., 1996). Most studies have investigated a 48 base-pair VNTR polymorphism encoding a portion of the third intracellular loop region of the transcribed protein that spans the nerve cell membrane and mediates interaction with secondary signaling proteins (Van Tol et al., 1992). The number of repeats ranges from 2 to 11, with a meta-analysis demonstrating a strong association between the 7-repeat allele and ADHD (Faraone et al., 2005). Although the functional significance of this polymorphism is yet to be fully ascertained, evidence suggests that different D4 receptor variants may display different pharmacological properties (Asghari et al., 1995). There is some evidence that the 7-repeat allele acts to dull the response of dopaminergic cells to dopamine, although these findings are not ubiquitously replicated (Kazmi et al., 2000; Watts et al., 1999). Other polymorphisms in DRD4 have also been associated with ADHD, although the evidence for these findings is less conclusive (Faraone et al., 2005).

DRD4-knockout (D4-KO) mice have been used to examine the function of the gene, although they are rarely cited as models of the ADHD phenotype. Behaviorally, these animals appear less active than wild type controls in open-field activity tests (Rubinstein et al., 1997). The mice are supersensitive to ethanol, cocaine, and methamphetamine, and appear to show elevated dopamine synthesis. These observations suggest that DRD4 modulates normal, coordinated, and drug-stimulated motor behaviors, as well as the activity of nigrostriatal dopamine neurons. Given that there is a clear link between ADHD and novelty-seeking, and there is evidence to suggest an association of the 7-repeat DRD4 VNTR allele with both phenotypes, another interesting observation is that DRD4-KO mice exhibit reduced exploration of novel stimuli (Dulawa et al., 1999).

One of the first rodent models of ADHD was the 6-hydroxydopamine (6-OHDA) lesioned neonatal rat. 6-OHDA selectively damages catecholaminergic neurons and produces hyperactivity that can be alleviated with stimulant medication. Investigations of the neurobiology of these rats first established a role for dopamine and the nucleus accumbens in the expression of hyperactivity (Shaywitz et al., 1976). 6-OHDA lesioning is now being used in conjunction with gene knockout technology to investigate the role of targeted gene mutations in mediating the development ADHD-like phenotypes in mice (Avale et al., 2004). As is observed in rats, 6-OHDA lesioned mice demonstrate hyperactivity that diminishes after puberty, psychostimulant-induced hypoactivity and deficits in behavioral inhibition. To determine whether DRD4 plays a role in these behavioral phenotypes, Avale et al. (2004) also performed 6-OHDA lesions in DRD4-KO mice. They found that although striatal dopamine and tyrosine hydroxylase-positive midbrain neurons were reduced to the same extent in both wild-type and D4-KO mice, the latter did not develop hyperactivity or behavioral inhibition deficits. Furthermore, a DRD4 antagonist prevented hyperactivity in wild-type mice following 6-OHDA-lesions. These results are interesting in that they suggest DRD4 is essential for the expression of juvenile hyperactivity and impaired behavioral inhibition in 6-OHDA lesioned mice, lending additional support to its' postulated role in ADHD.

2.4. Other ADHD candidate genes

Recently, mice with a deletion encompassing the beta-2 subunit gene of the nicotinic receptor have been proposed as a simple and reliable animal model for ADHD (Granon et al., 2003). It has been demonstrated that nicotinic agonists targeting alpha-4 beta-2 nicotinic receptors alleviate ADHD-like symptoms in these mice (Granon and Changeux, 2006). Interestingly, in the Spontaneously Hypertensive Rat (SHR), one of the best behaviorally validated models of ADHD (see below), alpha-4 beta-2 nicotinic acetylcholine receptor activation ameliorates impairment of spontaneous alternation behavior—one of the ADHD-like symptoms observed in these animals (Ueno et al., 2002). There have been limited studies looking at nicotinic acetylcholine receptor subunit gene polymorphisms in ADHD, and so far no conclusions can be drawn (Kent et al., 2001).

ADHD is a common behavioral phenotype associated with resistance to thyroid hormone (RTH) syndrome (Stein et al., 1995). RTH has been mapped to a mutation in the thyroid receptor beta (TRβ) gene on chromosome 3. McDonald et al. (1998) created a mutant mouse line expressing a mutant TRbeta gene. These transgenic mice show no thyroid abnormalities, but exhibit numerous ADHD-like symptoms including inattention, hyperactivity, and impulsivity (Siesser et al., 2006). As in children with ADHD, these behavioral phenotypes are not seen

after treatment with methylphenidate. While TRβ gene polymorphisms have yet to be investigated in ADHD, these mutant mice suggest that variation in this gene could play a role in the etiology of the disorder.

2.5. Limitations to the use of single-gene mutants

A major limitation in the use of mutant models to discover genes that increase susceptibility to complex diseases is that they do not help in the hunt for novel candidate loci that cause individual differences in traits such as hyperactivity. By definition they contain only one experimental change in otherwise genetically identical animals, and these changes do not reflect the genetic variation seen in normal outbred populations. As a result, the extreme phenotypes observed in mutant animals (both biochemical and behavioral) are unlikely to ever occur naturally, and have thus played little role in shaping the evolution of common behavioral disorders like ADHD. These knockout, knockdown, and transgenetic mutants are often touted as being "models of ADHD" simply because they show elevated locomotor activity. In fact, it is more accurate to describe them as models of ablated gene function. Such a single-gene approach ignores the complexity of the genome—genes do not act in isolation and we know ADHD is caused by numerous interacting genetic and environmental factors.

Such an approach also ignores the complexity of the ADHD phenotype. ADHD is a highly heterogeneous disorder with numerous etiological pathways, and is thus highly unlikely to have one unitary genetic cause. For example, we know there is considerable variation in clinical features among children who meet diagnostic criteria for the disorder (Nigg et al., 2005). Children diagnosed as having ADHD differ with regard to intellectual functioning (Barkley and Russell, 1997), comorbidity with conduct disorder (Jensen et al., 1997), and therapeutic response to stimulant drugs (McGough, 2005). This heterogeneity extends to long-term prognosis; some children's ADHD symptoms remit during adolescence, whereas other children's symptoms persist beyond adolescence (Barkley et al., 2002). Interestingly, there is increasing empirical evidence that much of this heterogeneity may be mediated by specific gene polymorphisms (Cornish et al., 2005; Mill et al., 2006).

Furthermore, single gene mutants, in whom the function of a gene is totally eliminated, are unrealistic models of the more subtle polymorphic variation observed in normal populations, which act to quantitatively alter gene expression or function. These animals harbor their mutations through the entire developmental process, and it is possible that the aberrant phenotypes observed in adulthood result not from the gene *per se*, but from developmental defects or adaptations of the organism. In fact, the process of entirely 'knocking out' the function of a gene often produces non-specific global physical and behavioral effects that have nothing to do with any known aspect of ADHD. DAT-KO mice, for example, have severe developmental deficits, and the coloboma mouse is virtually blind and demonstrates constant head-bobbing. Furthermore, these animal models often demonstrate elevated locomotion in novel environments, but not in their home-cages (e.g. Zhuang et al., 2001). This is the opposite of the symptoms seen in the human condition where childhood hyperactivity is generally more pronounced in constant, unstimulating situations.

3. Using rodent models to identify novel ADHD candidate genes

In addition to providing information about the dysregulation of known candidate genes, rodents are excellent tools for the identification of novel ADHD candidate genes. Unlike the study of known candidate genes in single-gene mutants, where animals are selected on the basis of recognized genetic abnormalities and the goal is to identify the functions of that particular gene (i.e. gene → phenotypes), gene hunting strategies generally select animals on the basis of behavior and aim to identify the genes mediating phenotypic variance (i.e. phenotype → genes). In this regard, accurate phenotyping and behavioral assessment of the animals used is of vital importance. The behavioral paradigms employed must tap into the neuropsychological pathways known to be influencing aspects of the human ADHD phenotype. Several approaches for gene mapping using rodents exist. One method is to take validated models of the ADHD phenotype, and to compare these (behaviorally and genetically) with control animals that do not show ADHD-like behavior, to identify regions of the genome that differ between the two strains. This is analogous to gene association studies in human ADHD samples, where the frequency of gene polymorphisms are compared between groups of affected and unaffected individuals. Another method is to take a quantitative mapping approach, for example, using recombinant inbred (RI) strains, to identify regions of the genome shared in common between animals displaying certain behavioral characteristics. This is more analogous to the whole-genome genetic linkage studies that have been performed in humans, examining the co-segregation of genetic markers with ADHD. A third approach that is gaining popularity, but has not yet been widely used in relation to the ADHD-phenotype, is the mutagenic screening of aberrant behavioral phenotypes in mice using N-ethyl-N-nitrosourea (ENU). These approaches to gene mapping in rodents provide geneticists with the means to identify novel loci that may have important influences on a behavior of interest, but which would not be investigated a priori based on what is known about the trait.

3.1. The spontaneously hypertensive rat (SHR)—a validated rodent model of ADHD

Several criteria need to be met before an animal can be considered to truly model a psychiatric disorder like ADHD: (i) high face validity, (ii) high construct validity, (iii) strong predictive validity and (iv) developmental relevance (Sagvolden et al., 2005). The SHR is perhaps the most widely validated animal models of ADHD, meeting most of these criteria (Sagvolden et al., 2005). The SHR, selected from outbred Wistar Kyoto (WKY) rats for high blood pressure (Okamoto and Aoki, 1963), shows a number of behaviors that closely parallel those seen in children with ADHD including motor hyperactivity, increased impulsiveness and deficient sustained attention

(Sagvolden, 2000; Sagvolden et al., 2005). Furthermore, like children with ADHD, the SHR is more sensitive to immediate behavioral reinforcement and less sensitive to delayed reinforcement than non-hypertensive WKY control rats (Sagvolden, 2000; Sagvolden et al., 2005). The behavioral and cognitive deficits in the SHR are responsive to stimulants, including d-amphetamine and d, l-methylphenidate (Sagvolden et al., 1992). Finally, several studies have shown that dopaminergic and noradrenergic neurotransmission is altered in the SHR compared to the WKY, strongly implicating these systems in the etiology of ADHD (Russell, 2003; Russell et al., 1998, 2005) and suggesting that genes in these systems may be good candidate for mediating the SHRs' behavioral phenotypes. Recently Mill et al. (2005b) examined three candidate dopaminergic genes (*Drd2*, *Drd4*, and *Dat1*) in the SHR and WKY to identify between-strain sequence differences. No between-strain sequence differences were found in either *Drd2* or *Drd4*, but several variations were found in the *Dat1* gene. These included a synonymous single base change (T > C) within the coding sequence of exon 3, and a 160 bp section of sequence immediately upstream of exon 3 present in SHR but not WKY. It is plausible that DNA sequence changes in the Dat1 gene account for some of the behavioral differences observed between the SHR and WKY strains. SHR strains have been shown to exhibit elevated DAT expression in mesocortical projections (Viggiano et al., 2002; Watanabe et al., 1997), and the WKY strain, often used as a model for depression, also demonstrates an unusual DAT profile compared to non-depressive control strains (Jiao et al., 2003). It is interesting that these findings are partially mirrored in studies on human psychiatric patients. Whilst individuals with ADHD have been shown to exhibit increased DAT density in the brain (Dougherty et al., 1999), depressive patients were found to have overall decreased levels of DAT (Meyer et al., 2001). Future work will focus on elucidating the functional effects of the observed polymorphisms, and investigating sequence changes in other genes.

3.2. ADHD linkage scans

As discussed, most ADHD genetic studies have taken a candidate-gene association-based approach. To date this tactic has been relatively successful, with replicated findings in several genes (Faraone et al., 2005). In contrast, only four independent genome-wide linkage scans for ADHD have been published to date (Arcos-Burgos et al., 2004; Bakker et al., 2003; Hebebrand et al., 2006; Ogdie et al., 2004). These studies have generally used an affected sib-pair approach, looking for evidence of increased allele-sharing at specific markers in siblings concordant for ADHD. Despite several chromosomal regions being highlighted across studies, including regions of chromosomes 5p, 6q, 7p, 11q, 12q and 17p, the observed linkage patterns are generally not consistent, and no genes accounting for the observed linkage peaks have yet been identified.

3.3. ADHD category or continuum?

ADHD, as defined by operational criteria, is a dichotomous trait making up a distinct diagnostic category (American Psychiatric Association, 1994). Measures of activity, impulsivity and inattention, however, are continuously distributed in the general population. Many studies have found an excellent correspondence between quantitative measures of these traits and the categorical diagnosis of ADHD (e.g. Biederman et al., 1993; Boyle et al., 1997). These studies do not report any obvious bimodality separating ADHD children from non-ADHD children and it has thus been argued that clinical ADHD should be regarded as the extreme end of these quantitative traits rather than as a discrete category (Levy et al., 1997). Most twin studies examine variations in symptom scores across the entire range and find them to be almost equally heritable when assessed by parents or teachers. Several studies have employed the DeFries and Fulker extremes method of analysis (DeFries and Fulker, 1988) and find that the genetic contribution to symptoms at the upper end of the distribution is generally the same as for the entire distribution (Thapar, 2002). The notion that heritability is constant across the trait distribution has interesting consequences in terms of finding genes that influence ADHD symptom scores. A good test of this conclusion would be to examine whether specific genetic risk factors, known to have a role in clinical ADHD, correlate with continuous-ratings of ADHD symptoms in the general population. To date, few studies have investigated the role of ADHD candidate genes in causing variation in the normal distribution of underlying phenotypes, although there is some emerging data to support a role of specific ADHD candidate genes in mediating quantitative trait scores (Cornish et al., 2005; Mill et al., 2005a).

3.4. Using rodents to map QTLs

It has been recently proposed that QTL mapping strategies may increase our power to detect ADHD risk genes by linking the categorical disorder to continuously distributed traits associated more closely with underlying genetic liability in the general population. The use of rodents, particularly mice, to map the QTLs involved in complex behavioral disorders such as ADHD offers a viable alternative to linkage studies in humans. There are several advantages of performing genome-wide QTL scans in rodents compared to humans. In particular, it is possible to control numerous experimental parameters, including external environmental factors, so that much 'cleaner' linkage peaks can be generated. Furthermore, rodents are relatively inexpensive to maintain, and easy to phenotype, allowing geneticists to dramatically increase experimental sample sizes and more easily detect genes of small effect.

Conventional QTL mapping approaches generally rely upon recombinant inbred (RI) mice strains. RI strains are produced by crossing two inbred parental strains and repeatedly mating the resulting siblings for >20 generations to ensure that they are at least 99% inbred (Silver, 1995). A major strength of using RI strains for the mapping of complex phenotypes is that, once created, they only need to be genotyped once, but can be phenotyped indefinitely. For 'noisy' behavioral traits, the ability to repeatedly phenotype is invaluable. The BXD set of RI strains are the most widely utilized mice for QTL mapping. They were derived by crossing C57BL/6J (B) and DBA/2J (D) and then inbreeding

progeny for over 21 generations. The BXD RI strains provide a powerful tool because of the large volume of genetic, behavioral, and biochemical data that are available for each strain. Many of the strains have been extensively phenotyped, and the two parental strains have been extensively sequenced. These mice are known to differ at ∼1.8 million SNPs, making them ideal for QTL mapping. WebQTL (www.webqtl.org) is a freely accessible suite of databases and online analysis software that contains phenotype, genotype, and gene expression data for 99 BXD RI strains (Chesler et al., 2004) allowing *in silico* analyses to be easily performed.

Before rodents can be used for fine-scale QTL mapping, it needs to be shown that strain differences, assumed to be caused by genetic differences, exist for the particular trait being studied. In this regard, several groups have begun to test for differences between strains on a range of behavioral tests postulated to measure aspects of the ADHD phenotype. For example, Patel et al. (2006) assessed attentional performance in the 5-choice serial reaction time task of C57BL/6 and DBA/2 mice, the progenitor strains for the BXD recombinant inbred panel. They found that both strains were able to perform the task, but they differed significantly in the levels of performance achieved. DBA/2 mice appeared to be less accurate, and to make more impulsive responses. It is known that the two strains differ markedly in their dopamine systems (Cabib et al., 2002), and it is likely that these differences are due to genetically determined neurobiological processes. Furthermore, sequence analysis has shown that DBA/2 mice have a single-nucleotide polymorphism in their tryptophan hydroxylase-2 gene that significantly lowers frontal cortical and striatal serotonin levels (Zhang et al., 2004).

As discussed above, dysregulation in DAT expression is postulated to be one of the major neurobiological processes in ADHD. Janowsky et al. (2001) examined DAT density in C57BL/6 and DBA/2 mice, along with 21 BXD RI strains and uncovered significant strain differences. Following genotyping and QTL mapping, they uncovered a large effect QTL, accounting for over half of the genetic variation in DAT density, on mouse chromosome 19. Interestingly, this region does not contain the murine *Dat1* gene, but spans an area containing the proopiomelanocortin pseudogene, *Pomc-ps1*. This study provides an elegant example of how previously unknown modifier loci, not directly linked to systems thought to be dysregulated in ADHD, can be identified using powerful QTL mapping approaches in BXD mice strains.

QTL mapping for ADHD traits is not restricted to mice. As described previously, the SHR is the best-validated rodent model of ADHD. It has been previously shown that the hyperactivity in these animals is not genetically linked to the hypertensive phenotype that they were originally selected for. From a WKY X SHR intercross Hendley et al. (1986) were able to produce two new strains with isolated hypertension, the Wistar Kyoto Hypertensive (WKHT) strain, and with isolated hyperactivity, the Wistar Kyoto Hyperactive (WKHA) strain. Moisan et al. (1996) used a WKY X WKHA (distinct for their low and high activity scores in a novel environment, respectively) intercross to map a QTL on chromosome 8 linked to locomotor activity in a novel environment. This QTL has a fairly major effect, accounting for 29% of the variance in activity level. Ramos et al. (1998) studied a Lewis X SHR intercross and found two QTL linked to locomotor activity in the centre of the open field, on chromosomes 4 and 7. Mormede et al. (2002) selected animals from this intercross on the basis of genotype at these two loci, identifying a 'high line' in which rats have the alleles associated with increased hyperactivity, and a 'low line' with the alleles associated with lower activity levels. Interestingly, these two loci are also associated with performance on a behavioral inhibition task (Mormede et al., 2002). Recently, Vendruscolo et al. (2006) demonstrated that the QTL on chromosome 7 also modulates prepulse inhibition, another neuro-behavioral trait associated with ADHD, in these animals.

One potential limitation of traditional QTL mapping approaches is that the mapping resolution is coarse because the chromosomes of the F2 animals have undergone very little recombination. Much finer resolution QTL mapping in rodents can be achieved by using an outbred stock of animals for which the entire genealogy is known. An example of these are the heterogeneous stock (HS) mice, a systematically outbred stock established over 30 years ago from an eight-way cross of C57BL/6, BALB/c, RIII, AKR, DBA/2, I, A/J and C3H inbred mouse strains (McClearn et al., 1970). As well as vastly increasing the genetic variation amongst the experimental animals, such stocks are also more representative of a general population in terms of behavioral traits, with individual genetic differences mapping onto individual behavioral differences. If differences in ADHD-like traits can be demonstrated in these animals then they should provide a valuable tool for QTL identification. Mill et al. (2002b), for example, undertook a pilot study investigating home-cage activity in HS mice, and found reliable individual differences in home-cage behavior, mapping strongly onto a stable general activity factor. The aim is to use these measures in to identify novel mouse activity QTLs that can inform future human studies.

Conventional QTL mapping, based on natural, polygenic variation between different trains of mice, is undoubtedly a powerful tool for identifying loci associated with ADHD-like traits. There are disadvantages to this approach, however, and the process can be extremely laborious. Whilst the initial stages of QTL mapping have been made easy via the free availability of genotype and phenotype data, considerable verification work needs to be performed once a linkage peak is identified. Single QTLs have to be genetically isolated into congenic strains and then mapped via genomic sequencing. Because a single QTL linkage peak can contain numerous genes, locating the precise functional variant can be time consuming and costly. The problem of fine mapping QTLs in rodents is highlighted in a recent review by Flint et al. (2005). While several thousand QTLs have been identified in crosses between inbred strains of mice and rats over the last couple of decades, less than 1% have been actually characterized at a molecular level.

3.5. ENU mutagenesis screening

A powerful alternative to investigating natural, polygenic variation in rodents (e.g. via QTL mapping) is chemical muta-

genesis (Nadeau and Frankel, 2000). *N*-Ethyl-*N*-nitrosourea (ENU) is a potent alkylating agent that acts as a powerful mutagen in mouse spermatogonial stem cells. The mutation rate following ENU is very high affecting approximately 1 in 1000 gametes screened (Hitotsumachi et al., 1985). The effect of ENU is genome-wide and non-specific—all genomic regions have an equal chance of acquiring a novel mutation. One of the strengths of ENU mutagenesis is that it can induce many different types of alleles, thus, better representing the action of most human gene polymorphisms than the full loss-of-function effects in knockout models. Several centers around the world are performing systematic high-throughput phenotype screens of these ENU-created mutant lines (Nolan et al., 2000). Many of the behavioral test batteries given to these animals involve paradigms that approximate to endophenotypes associated with human psychiatric disorders, and contain several that can be used to assess ADHD-like behavior. Following screening, the phenotype of interest is confirmed via inheritance testing and the location of the mutant detected via the generation of a genetic backcross, positional cloning and sequencing. Many of these mutant animals are available to other researchers, with phenotype and genotype information readily available on public databases (e.g. http://www.neuromice.org/). One of the strengths of the ENU mutagenesis screening approach compared to previous *targeted* gene disruption methods, is that no prior assumptions are made about the genes involved in any pathway, so novel genes and pathways can be identified. The promise of ENU mutagenesis for the elucidation of genes involved in behavior has been widely recognized (Godinho and Nolan, 2006), and this method should provide a powerful tool for identifying novel candidate genes likely to be involved in the pathogenesis of ADHD.

4. Conclusions

There is undoubtedly a strong genetic component to the aetiology of ADHD, and to date polymorphisms in several candidate genes have been associated with the disorder. As with other complex psychiatric disorders, rodent models have proven extremely useful in helping understand more about the genetic basis of ADHD in humans. Single-gene mutant rodents have been invaluable in elucidating the function of these loci, especially DAT1, DRD4, and SNAP-25, and informing us about the behavioral consequences of severely disrupting their expression. There are, however, limitations to these mutant rodent models. Specifically they ignore many of the phenotypic and polygenic complexities of ADHD, and cannot be used to nominate novel candidate genes for use in human studies. Furthermore, single gene mutants, in whom the function of a gene is totally eliminated, are unrealistic models of the more subtle polymorphic variation observed in normal populations, which acts to quantitatively alter gene expression or function. In addition to providing information about the dysregulation of known candidate genes, rodents can be excellent tools for the identification of novel ADHD candidate genes. While not yet widely used to map ADHD-like behavior in rodents, QTL mapping approaches using recombinant inbred strains, heterogeneous stock mice, and ENU mutated animals have the potential to revolutionize our understanding of the genetic basis of ADHD.

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