Dopamine system genes and ADHD: a review of the evidence

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The search for genes influencing the development of attention-deficit/hyperactivity disorder (ADHD) has identified a number of associated genes within, or influencing, the dopamine neurotransmitter system. The focus on this system as the site of genetic susceptibility was prompted by information from animal models, particularly transgenics, as well as the mechanism of action of the psychostimulants, the primary pharmacological treatment for ADHD. Thus far, genes in the dopamine system reported as associated with ADHD, by at least one study, include the dopamine transporter, the dopamine receptors D1, D4 and D5, as well as genes encoding proteins that control the synthesis, degradation and release of dopamine. For some of these genes, replication across studies provides evidence supporting the relationship; however, for others, the data is far from conclusive and further work is needed. The quick progress in the genetic findings was initially surprising given the complexity of the phenotype and the relatively small sample sizes used in the initial studies. However, the high heritability of ADHD, as indicated by twin studies, may have contributed to the success. The genes studied so far are estimated to contribute only weakly or moderately to the risk for the development of ADHD. This may be because these genes, in fact, make only a small contribution. However, few studies have comprehensively examined the genetic information across the gene. This will lead to underestimates of risk if the polymorphism(s) tested is/are not the functional change(s) actually contributing to the genetic susceptibility and if linkage disequilibrium between tested marker(s) and causal variant(s) is weak, or if there is substantial allelic heterogeneity. While the studies thus far are very promising, virtually nothing is known on precisely how genetic variation in these genes actually contributes to risk; thus, functional studies are now required.

Keywords
ADHD + animal models + association + dopamine + dopamine receptors + genes + genetics + hyperactivity + impulsivity + inattention

Attention-deficit/hyperactivity disorder
Attention-deficit/hyperactivity disorder (ADHD) is a prevalent condition, being the most commonly diagnosed psychiatric disorder in children [1]. Characterized by developmentally inappropriate and impaired levels of inattention and hyperactivity/impulsivity, ADHD is defined by the American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) [2] as having three subtypes: inattentive, hyperactive/impulsive and combined. These subtype designations are established according to a checklist of nine possible symptoms within each of two domains, inattentive and hyperactive/impulsive. The predominantly inattentive subtype is defined by the presence of at least six of nine possible inattentive symptoms. Similarly, the hyperactive/impulsive subtype is defined by at least six of nine hyperactive/impulsive symptoms. The combined type meets both of the subtype criteria. The current criteria also stipulate the presence of some symptoms before the age of 7 years, the persistence of symptoms for at least 6 months and evidence for impairment, due to these symptoms, in two or more settings. The current criteria further stipulate that the symptoms do not occur exclusively during the course of a pervasive developmental disorder, schizophrenia or other psychotic disorder, and are not better accounted for by another mental disorder, such as mood disorder, anxiety disorder or a personality disorder.

Developmental studies of ADHD in children have repeatedly documented a change in behavioral symptoms over time, with hyperactivity/impulsivity appearing earlier, being more prominent in preschool and school-age children, with symptoms declining as a person matures into adulthood. Inattention problems become evident later, at around the time of school entry, and tend to persist beyond adolescence [3–4]. Symptoms often diminish significantly in adults, and although usually not completely asymptomatic, many such individuals no longer meet criteria for the disorder [5].

The focus of this review is genetics of the dopamine system in ADHD. We recognize that there are many factors influencing the genetics of
ADHD that, in turn, may influence our ability to identify the genes involved. Because of space limitations we can only touch on some of these complicated issues, but this is not intended to downplay their importance. For the same reason, we are unable to review, for each genetic association study, the details of the study design (case–control vs family-based control and population-based vs clinical samples) or characteristics of the subjects (ethnicity, age, comorbidities, gender, age, informant and inclusion/exclusion criteria). These factors may have an impact on the findings of genetic studies and the conclusions that can be made from them. For some of these factors, there is a clear indication that these could influence the outcome and power of genetic studies (e.g., ethnicity, as discussed below). For others, it may not be critical (e.g., gender, as discussed below). Comorbidities are common in ADHD; however, the genetic relationships between ADHD and comorbid disorders are, for the most part, unclear. Twin studies can be informative in determining heritable phenotypes that should be used for genetic studies, and multivariate analyses can provide information on the genetic relationships between different phenotypes, such as comorbid disorders. However, currently only a few studies have been published on the relationship of ADHD to comorbid disorders, and thus, information is still lacking as to which phenotypes will be the most informative for genetic study. While the current review is focused on the contribution of the dopamine system to the genetic risk of developing ADHD, we also point out that pharmacogenetic aspects of the disorder will likely prove useful in the future in the prediction and/or improvement of medication response, the prevention of side effects, and the development of novel therapeutics. Pharmacogenetics research in ADHD is still in its infancy, and while this topic is outside the scope of the present review, the reader is referred to recent publications that have covered this in some detail [6–8].

**Genetics of ADHD**

Attention-deficit/hyperactivity disorder is highly heritable, with twin studies, to date, finding heritability of ADHD, and ADHD symptoms in the population, in the range of 70 to 90% [9–17]. Despite the high heritability estimates, there are a number of characteristics of ADHD that can be problematic for genetic studies and may impede the identification of genes. Foremost is that ADHD is a complex trait, that is, it does not follow a simple Mendelian pattern of inheritance. Multiple genes and environmental factors are likely involved. Studies of ADHD are also complicated by the fact that ADHD diagnosis relies on the reporting of behavioral symptoms. This can be problematic, as there may be varying expectations of age- and situation-appropriate behavior, depending on the informant (e.g., parent, self, teacher or clinician), and this can influence the reporting of symptoms. Furthermore, children can behave differently in different environments. Additionally, as noted above, there are several aspects of the phenotype with potential to influence genetic studies, including developmental changes in symptoms, gender differences in prevalence of ADHD, and uncertainties regarding genetic relationships with comorbid disorders. Regarding the developmental changes, it is not clear how the developmental shift in symptoms occurs, but it is possible that genes play a part [18]. A number of neurotransmitter systems relevant to ADHD are known to change during development, particularly the dopaminergic and serotonergic systems [19,20]. For example, the expression of the dopamine transporter gene decreases with age [21], and thus, reductions in symptoms over time may, in part, be related to changes in gene expression. As a result, the age of subjects used for genetic studies may have an impact on the association findings. This is particularly relevant when comparing studies of ADHD in childhood cases to studies of adults with ADHD, the latter of which may involve a subset of genes contributing to the persistence of ADHD symptoms.

The relationship between gender and genetic susceptibility to ADHD is not yet known. More males than females are affected by ADHD, with sex ratios reported in the range of 3:1 to 9:1 [22]. Boys and girls have the same ADHD symptoms; however, there are differences in the frequency of some symptoms and subtypes, as well as comorbidities. Girls are more likely to have the predominantly inattentive subtype, and are less likely to have certain comorbidities (learning disability in reading or maths, comorbid depression, conduct disorder or oppositional defiant disorder) [23]. The reasons for these gender differences are unclear, however, there is little evidence from twin studies for either genetic or environmental contributions to the differences between boys and girls [10,14,24–28]. This indicates that, although biological differences are likely to be involved, genetic variation does not contribute to these differences and it is unlikely that there will be different risk genes in boys versus girls.
Interestingly, a number of genes in the dopaminergic system are influenced by estrogens and this may provide a protective role in females. Estrogens have effects on dopaminergic transmission [29–31], including regional and temporal effects on striatal dopamine receptor expression [29], and there is evidence that estrogen inhibits the dopamine transporter [29]. This may provide a protective effect in females by increasing synaptic dopamine. The mechanism underlying the differences in receptor expression is poorly understood, however for some genes, such as the dopamine receptor D1 (DRD1) gene, increased transcription is due to an estrogen response element in the promoter [32]. Thus, despite the lack of evidence for different risk genes in boys versus girls, there might be differences in terms of gene dosage. For example, if a DRD1 allele that reduces D1 receptor expression were a risk factor for development of ADHD, then it is conceivable that females might be more likely to require two copies, rather than just one copy, of the allele in order for this influence to be realized. The rationale for this is that the upregulation of DRD1 expression in response to estrogen in females might compensate, to some extent, for the otherwise downregulatory effect of the risk allele.

**Role of dopamine in ADHD**

The hypothesis that dysregulation of neurotransmitter systems, particularly the catecholamines, contributes to ADHD has been a major driving influence behind neurobiological models of this disorder, and has guided research in this field for the past three decades [33–35]. Both ‘hypo’ and ‘hyper’ dopaminergic theories have been put forward and both may be correct [34,36–38]. The dysregulation of dopamine neurotransmission may arise in multiple ways, involving, for example, alterations in dopamine synthesis, synaptic release, cellular responses originating through receptor signaling processes and/or second-messenger activity, or processes during brain development that alter dopamine neuronal innervation. Genes with the potential to contribute to dopamine dysregulation could, thus, influence any of these processes. Support for the dopaminergic hypothesis is substantial and is gained from a number of lines of evidence, including neuroimaging [39,40], the mechanism of pharmacological interventions [39–41], animal models [42–47] and, most recently, the findings from molecular genetic studies, as reviewed here. We note that while the focus of this review is the dopaminergic system and ADHD, support for the involvement of other neurotransmitter systems is equally convincing.

In particular, the adrenergic, glutamatergic, nicotinic and serotonergic systems are strongly supported [48–51]. The complex interactions and balance of the neurotransmitter systems makes it difficult to dissect cause and effect. It is quite possible that multiple interacting and/or independent perturbations, genetic as well as environmental, may disturb neurotransmitter regulation to create a common observable outcome.

**Animal models supporting the involvement of the dopaminergic system in ADHD**

A number of clues to the mechanisms involved in hyperlocomotion have been gained from the study of naturally occurring, selected, and genetically engineered animals [29–31]. In particular, a number of transgenic/knockout mouse models exhibit hyperlocomotion, including those involving manipulation of the genes for the dopamine transporter [42,54], the dopamine receptors D1 [43–45] and D4 [46], and a G-protein subunit involved in D1 and D5 signaling, Gαolf (GNAL) [55].

Several animal models of genes involved in neurotransmitter release also show a hyperlocomotor phenotype, suggesting these genes as possible contributors. For example, the mouse mutant *Coloboma* displays spontaneous hyperactivity and is hemizygous for a 2 centimorgan (cM) deletion that includes the gene for the synaptic vesicle docking fusion protein, synaptosomal-associated protein of 25 kDa (Snap25) [56]. The Snap25 gene encodes a protein involved in the vesicle docking and fusion machinery, mediating regulated release of neurotransmitters [57]. Besides hyperlocomotion, another feature of this mouse strain that makes it an appealing model is that the mice exhibit developmental delays in motor skills, but eventually catch up with their normal littermates [58]. Problems in developmental coordination, particularly in fine motor control, are often noted in ADHD children and some estimates indicate that the number of ADHD children that meet criteria for developmental coordination disorder may be as high as 50% [59,60]. Also of interest is that the *Coloboma* strain is responsive to dextroamphetamine but not to methylphenidate [56].

Another animal model that implicates mechanisms controlling the release of neurotransmitters in hyperlocomotion is the transgenic mouse engineered for overexpression of the Calcyon gene (official nomenclature is *Caly*; calcyon neuron-specific vesicular protein) in the forebrain [61]. Calcyon has been implicated in clathrin-mediated
endocytosis ([62,63]), a process critical to the recycling of releasable pools of neurotransmitters and contributing to synaptic plasticity. This transgenic mouse line exhibits spontaneous hyperactivity as well as reduced anxiety [61].

While an animal model can never fully represent the complex behavioral and cognitive features of ADHD, these mouse models have provided clues that have led to the identification of ADHD-associated genes. These animal models, in combination with clues from the pharmacological and imaging data, provide a starting point for gene identification.

Dopamine transporter
The dopamine transporter gene, DAT1 (official nomenclature is SLC6A3; solute carrier family 6, neurotransmitter transporter, dopamine, member 3) was the first candidate tested for involvement in ADHD. The transporter functions in regulating synaptic dopamine levels, through reuptake, and is the primary target for methylphenidate, supporting this gene as a prime candidate. Notably, both DAT-knockout mice (expressing no DAT) and DAT-knockdown mice (expressing 10% of the normal level) exhibit novelty-induced hyperactivity with impaired habituation, providing further support for the involvement of this gene in ADHD ([42,54]). Interestingly, despite the absence of DAT, hyperactivity in the knockout mice can still be inhibited by methylphenidate, a finding that has subsequently proved useful in shedding light on other pathways (e.g., serotonergic and glutamatergic) that, as noted earlier, may also be involved in ADHD ([64,65]).

In 1995, Cook and co-workers reported a significant association of ADHD with the 10-repeat (480 bp PCR fragment) allele of the variable number of tandem repeats (VNTR) polymorphism located in the 3′-untranslated region (3′-UTR) of the DAT1 gene [66]. A number of independent studies have supported the association of this allele ([67–73]), although an equal number of nonreplications have also been reported ([70,74–95]).

Despite the number of replicated studies, meta-analyses and analyses of pooled odds ratios across ADHD studies indicate the contribution of this gene to be from none to modest, with odds ratios in the range of 1.04–1.27 ([96–100]). However, these analyses are based solely on association studies of the 3′-UTR VNTR alleles. Such analyses will underestimate the effect of this gene if the 3′-UTR VNTR polymorphism is not, itself, the causal variant and linkage disequilibrium with the susceptibility allele(s) is not complete. Supportive evidence from studies of additional polymorphisms and haplotypes indicates that this is indeed the case ([72,89,101–104]). Other polymorphisms reported to be associated with ADHD include polymorphism rs27072 ([104,105]), located 442 bp 5′ of the VNTR, in a region reported to influence gene regulation [106], a VNTR in intron 8 ([72,103]), the alleles of which show differential response to stimuli (forskolin and cocaine hydrochloride) in in vitro transcription studies ([107]), two promoter polymorphisms, −839C/T and −67A/T ([88,108]), and a polymorphism located in the fourth intron of the gene ([109]). The analysis of haplotypes of the intron 8 VNTR and 3′-UTR VNTR polymorphisms across three samples has provided an overall odds ratio of 1.4, much higher than that provided by analysis of the VNTR alone ([103]).

The predominant use of a single DAT1 marker for ADHD studies, as well as imaging ([110–112]), pharmacogenetic ([113–115]) and gene–environment interaction studies ([72,116], has been a major limitation in the interpretation of association findings for this gene. The 3′-UTR VNTR is the most studied polymorphism for this gene, owing to previous evidence that alleles of this polymorphism affect transporter protein levels ([117,118]). However, these studies using in vitro reporter construct expression assays found different effects of the alleles. Fuke et al. observed higher expression from constructs containing the 10-repeat allele than the 9-repeat allele [117], whereas Miller and Madras observed higher levels with the 9-repeat allele [118]. Additional studies have not confirmed either finding ([119,120]).

A number of factors can influence the outcome of in vitro reporter assays, including the cell lines used, use of the endogenous promoter of the gene of interest in the construct, and the inclusion/exclusion of additional sequences in the construct that may function in gene regulation. For the most part, in vitro studies of this type can correctly identify regulatory elements. However, by artificially removing such elements from the normal chromosomal environment that contains the correct arrangement of regulatory elements responsible for controlling temporal and cell-type specific expression, it is possible that the function of some regulatory elements will be disrupted, and hence, their effect might be missed in in vitro assays. It is also important to note that some regulatory sequences can function as repressors and enhancers, depending on the cell type and/or induction stimulus used.
Likewise, genetic studies that examine in vivo function, in other words, studies assessing function in relation to genotype in the organism, do not allow for the dissection of a particular element from the context of other polymorphisms located on the same chromosome. For example, a study examining mRNA levels of \( \text{DAT1} \) in lymphocytes from individuals with different VNTR alleles identified higher expression in lymphocytes from individuals with the 10-repeat allele than in those without this allele. Similar results were also obtained in an examination of postmortem brain tissue [121]. This type of study cannot, however, distinguish a direct effect of the VNTR from the effect of a polymorphism located on the same chromosome in linkage disequilibrium with the VNTR. In a related follow-up study, the 3-repeat allele of the intron 8 VNTR was also found to be associated with increased \( \text{DAT1} \) expression in postmortem brain, but whether this was best accounted for by a haplotype containing this allele, together with the 3′-UTR VNTR 10-repeat allele, could not be distinguished [122].

Based on the current data, the functional role of the 3′-UTR VNTR polymorphism is far from conclusive and requires further study. While there is no clear picture yet as to the role of this polymorphism in influencing DAT density in the brain, neuroimaging approaches may prove useful in the future. In this regard, it is noteworthy that several, although not all, neuroimaging studies have found evidence for higher DAT availability in ADHD patients relative to healthy controls [39,40,123–125]. What might account for the variability among studies has been the subject of some discussion, and we refer the reader to recent publications that provide a more comprehensive coverage of this topic than is possible here [39,40,123–126]. Also a matter of some debate in the neuroimaging literature are the implications of findings that methylphenidate can lower DAT expression. Placement of the 7-repeat allele within the 3′-UTR segment of a luciferase reporter construct resulted in a decrease in luciferase expression relative to that seen when either the 2- or 4-repeat allele was used [127]. This was suggestive of a post-transcriptional effect on gene expression, possibly through a decrease in mRNA stability or translation efficiency. Whether this allele exerts a similar effect in its normal context (i.e., a coding exon) is unknown. Nevertheless, these findings suggest the possibility that a combination

**Dopamine receptor D4**

Another gene supported as an ADHD susceptibility gene by multiple studies and meta-analyses is the gene for dopamine receptor D4 (\( \text{DRD4} \)). \( \text{DRD4} \) is a complicated gene with multiple polymorphisms and rare DNA variants that change the coding region. These include the most studied variants located within exon 3. This complex polymorphic region consists of 2–10 repeats of a 48 bp motif, with further sequence variation within the repeats [128,129]. The repeat is located in the third intracellular loop of the receptor, and has been reported to influence dopamine-receptor affinity [130] as well as intracellular signaling [131]. Specifically, the 7-repeat allele was reported to be slightly less sensitive to dopamine than the 4-repeat allele, leading to the suggestion that the 48 bp VNTR polymorphism is the functional change contributing to the ADHD phenotype. Nevertheless, the effect of the VNTR repeats on the function and/or expression of the D4 receptor appears to be quite modest [130,131], and thus, the potential to contribute to a phenotype such as ADHD is questionable. Functional differences among the other repeat lengths have also been documented, but there is no simple correlation between allele length and pharmacology or functional activity [132]. For example, whereas the longer (7-repeat) allele is slightly less sensitive to dopamine than the shorter (4-repeat) allele, the longest (10-repeat) allele is slightly more sensitive compared with the shortest (2-repeat) allele [132]. Thus, studies that have combined ‘long’ versus ‘short’ alleles for association analyses have combined functionally dissimilar alleles.

In addition to the functional effects of the 7-repeat allele described above, there is some evidence that this allele may also have an effect on receptor expression. Placement of the 7-repeat allele within the 3′-UTR segment of a luciferase reporter construct resulted in a decrease in luciferase expression relative to that seen when either the 2- or 4-repeat was used [133]. This was suggestive of a post-transcriptional effect on gene expression, possibly through a decrease in mRNA stability or translation efficiency. Whether this allele exerts a similar effect in its normal context (i.e., a coding exon) is unknown. Nevertheless, these findings suggest the possibility that a combination
of slightly lower receptor sensitivity and reduced receptor expression may compound the effect of this polymorphism [133]. Other DRD4 mutations/polymorphisms have been identified that also change the coding sequence. These include variants in the first exon of the gene: a 12 bp repeat [134], a 13 bp deletion [135] and a 21 bp deletion [136]. There are also a number of variants in the promoter region that are reported to change the expression levels in *in vitro* transcription assays. These include a 120 bp repeat [137–139] and a -521C/T polymorphism [140]. A recent study, however, did not find evidence for differential effects of the -521C/T alleles in an *in vitro* transcription assay [141], as had previously been reported [140]. As noted earlier, a number of factors may influence the results of *in vitro* transcription assays and confound the identification of functional elements.

**DRD4** association with ADHD was first reported for the 7-repeat allele of the exon 3 polymorphism [142], and has since been replicated in a number of studies [74,78,143–150]. Other studies, however, have failed to replicate this association [79,86,93,151–155]. Association with other alleles of the exon 3 repeat (i.e., other than the 7-repeat allele) have been identified in different ethnic groups [155,156,157]. Several meta-analyses published for this gene support the association between the **DRD4** 7-repeat allele and ADHD [98,100,158], with pooled analyses for studies of this polymorphism, up to 2005, indicating a significant association for case–control (OR: 1.45; 95% CI: 1.27–1.65) and family-based (OR: 1.16; 95% CI: 1.03–1.30) association studies [97]. Furthermore, meta-analyses indicate a protective effect of the 4-repeat allele, with an OR of 0.90 (95% CI: 0.84–0.97) [100].

At present, a number of factors complicate the ability to make firm conclusions as to the role of the 48 bp repeat in ADHD. Studies have, for the most part, used small sample sizes. Ethnic variation is also likely to be a complicating factor, as there are dramatic differences in allele frequencies, both of the 48 bp VNTR itself and of sequence variants within the repeat units, across populations [129]. Thus far, the possible contribution of sequence variation within the repeat units to receptor function has not been tested. Association analyses using the number of repeats alone may obscure an effect of functional differences within the repeats.

Several ADHD studies have investigated the inheritance of **DRD4** polymorphisms other than the exon 3 repeat, some of which have provided further evidence for association with different polymorphisms, although there is no agreement across studies as to the associated markers or alleles [91,139,159–166]. While haplotype studies have consistently yielded stronger results than analyses of single markers, such studies have not all used the same markers, and thus, consensus across studies is not possible based on the current published data [160,161,167]. For example, our ADHD studies of either three markers (48 bp VNTR in exon 3, 12 bp repeat in exon 1, and a (G)n mononucleotide repeat in intron 1) or four markers (48 bp VNTR and three promoter polymorphisms, the 120 bp repeat, -616C/G and -521C/T) have shown evidence for association with a particular 7-repeat allele-containing haplotype of the markers analyzed in each case. Evidence for a protective effect of a particular 4-repeat allele-containing haplotype was also seen with each of the marker sets analyzed [160,167]. We note, however, that in their haplotype analysis of all five of these markers, Mill and co-workers found evidence for ADHD association with a particular 4-repeat allele-containing haplotype [161]. Together, such studies indicate that risk for ADHD conferred by the **DRD4** gene is unlikely to be simply due to the inheritance of the 7-repeat allele, but rather that risk will likely depend on the particular combination of variants making up the haplotype.

In addition to haplotype considerations, alleles at **DRD4** may not actually be sufficient, on their own, to create risk, but may be influenced by other genes elsewhere in the genome. This idea is consistent with the notably mild hyperlocomotive phenotype of the **Drd4**-knockout mouse that only exhibits hyperactivity in the first 5 min of a novel open-field test [168]. The knockout mice also did not differ in measures of impulsivity compared with their wild-type counterparts [168]. While knockout of the **Drd4** gene does not appear to create a strong phenotype on its own, further studies using this knockout have found the D4 receptor to be critical for hyperlocomotion in an established lesioning model of hyperactivity. Neonatal lesioning of dopaminergic pathways with 6-hydroxydopamine (6-OHDA) results in hyperactivity after puberty. However, 6-OHDA lesioning in the **Drd4**-knockout does not result in a characteristic hyperactive phenotype, indicating that the presence of D4 is necessary for hyperactivity in the 6-OHDA-lesioned model [46].

**Dopamine receptor D5**

The gene for dopamine receptor D5 (**DRD5**) is also supported as a susceptibility gene for ADHD. The first study of an association between ADHD...
and DRD5 reported association of a 148 bp allele of a microsatellite repeat polymorphism located 18.5 kilobases (kb) upstream of the gene [67]. Later studies replicated this finding [169] or detected trends for biased transmission of this allele [91,93,146,165,170]. The most convincing data have come from combined analyses, published in 2004, using all available data at the time [171]. That study reported association of the 148 bp allele in 14 samples (p = 0.00005; OR: 1.24) [171]. More recent meta-analyses and pooled analyses of this polymorphism also support involvement of this gene in ADHD [97,98,100], with the 2006 study indicating a significant relationship with the 148 bp allele (p = 8 x 10^-8; OR: 1.34; 95% CI: 1.21–1.50), as identified before, as well as the suggestion of a protective effect of the 136 bp allele (OR: 0.57; 95% CI: 0.34–0.96) [100].

In terms of examining additional markers, this gene has been relatively understudied, with only two studies examining other polymorphisms [102,172]. One complication, in terms of gene screening and testing of polymorphisms within the gene, is the existence of two pseudogenes with high (~94%) homology to DRD5 [173].

Dopamine receptor D1
In addition to the evidence from transgenic mouse models showing hyperlocomotion [43–45], support for the dopamine receptor D1 gene (DRD1) as a candidate for ADHD can be garnered from its expression pattern, specifically the prevalent expression in prefrontal cortex and striatum, both of which are regions implicated in ADHD [174,175]. In our sample of ADHD families we found suggestive evidence for association in single marker analyses of four markers across the gene. However, the most significant finding was with a haplotype of these four markers, D1P.5/rs35916350(G), D1P.6/rs265981(T), D1.1/rs4532(G) and D1.7/rs686(C) [176]. Interestingly, we also found that the association was only with inattention symptoms, but not with hyperactive/impulsive symptoms [176], a result not predicted from the mouse model that displays hyperlocomotion. We have recently confirmed this association to inattention symptoms, in an independent sample of families participating in a study of reading disabilities [177]. Support for DRD1 association with ADHD has also been obtained in a case–control study in which two of the above polymorphisms were analyzed. Consistent with the findings from our laboratory, higher frequencies of the D1P.6/rs265981(T) and D1.1/rs4532(G) alleles were found among individuals with ADHD, compared with controls [99]. We note, however, that associations with these markers were not replicated in two family-based samples by the same [99] and other investigators [154], and a recent population-based study of five polymorphisms in or near the gene (including D1P.6/rs265981 and D1.7/rs686) did not find evidence for association with ADHD [94]. Notwithstanding the negative findings (which might be related to small sample sizes, clinical versus population-based ascertainment difference, and/or analyses of individual markers but not haplotypes), it is notable that one of the markers on our ADHD-associated haplotype has recently been implicated in regulation of the gene. This marker, rs686, is located in the DRD1 3’-UTR. Placing this 3’-UTR downstream of the luciferase gene in a reporter construct resulted in a downregulation of luciferase expression, with this effect being more pronounced using the rs686 C allele than the T allele [178]. Together, the findings suggest that altered D1 expression, possibly mediated through a transcriptional or post-transcriptional effect of the 686C allele, may be related to attentional ability. It will be of interest to investigate this possibility further in future studies.

Dopamine receptor D2
Two studies, thus far, have found support for the DRD2 gene in ADHD. The first of these was a case–control study in which association of the A1 allele of the Taq1A polymorphism was observed [179]. This polymorphism is located downstream of the gene and was originally thought to be intergenic. Later studies determined that this marker is actually located within the coding sequence of another gene, ankyrin repeat and kinase domain containing 1 (ANKKT), and alters the amino acid sequence (Glu713Lys) of the encoded protein [180]. The second study to report association identified modest evidence for association with three markers and with the A1 allele of Taq1A, as well as with haplotypes of three markers (including Taq1A), in males [94]. No additional studies of ADHD using family-based methods and/or more comprehensive coverage of the gene have found evidence of association [91,154,181–183, Misener VL, Wigg K, Couto J M E T A L., Unpublished Data]

The A1 allele of the Taq1A polymorphism has been associated with reduced D2-receptor binding potential in several neuroimaging (PET) [184,185] and postmortem [186,187] studies, with this reduction being attributable to a lowering of receptor density but not receptor affinity [185,186]. Although another neuroimaging (single photon emission computed tomography [SPECT]) study did not
observe this effect [188], the weight of evidence from these studies supports a relationship between the TaqI A polymorphism and density of the D2 receptor. In addition, given that a number of neuropsychiatric disorders have been reported to be associated with the TaqI A marker [179,189], the mechanism by which this polymorphism could influence DRD2 function warrants comment. For example, the association could result from the ANKK1 gene conferring risk, possibly from the Glu713Lys variant. However, the association could stem from linkage disequilibrium between the TaqI A marker and an unidentified risk allele in DRD2. For example, the A1 allele is in linkage disequilibrium with another marker allele, 957T, the latter of which has been found to influence the predicted folding structure of the DRD2 mRNA and to cause a decrease in mRNA stability and translation [190]. Interestingly, the presence of a particular allele of another polymorphism, 1101A, annuls the effect of the 957T allele [190]. Together, these findings point to the importance of examining haplotypes of markers, and suggest that positive association findings for the A1 allele may be reflecting changes in function created by the 957T allele. It is also entirely possible that the TaqI A change, while located in the coding region of ANKK1, could regulate DRD2 expression. A relevant example of such a scenario is the locus control region (LCR) of the TH2 cytokine locus, which regulates expression of the genes for IL-4, IL-5 and IL-13. The LCR is located in the 3’ end of Rad50, an unrelated and ubiquitously expressed gene. The LCR regulates these interleukin genes by looping to interact with the three promoters, but does not regulate the Rad50 gene itself [194]. Thus, DNA variation may affect genes located a large distance from the physical location of the marker, without affecting the nearest gene. Caution should therefore be taken in the interpretation of function with regard to gene position. Functional studies will be required to determine the relationship, if any, of the TaqI A polymorphism with the function of DRD2.

Dopamine receptor D3

Studies of the DRD3 gene in ADHD have been limited, both in terms of study number and gene coverage. The primary focus has been on the Ser9Gly nonsynonymous coding polymorphism, with few studies testing additional markers. Thus far, no evidence for DRD3 association has been reported in any study [94,154,165,192–194], including the more comprehensive studies that have investigated multiple markers across the gene [94, 194].

Genes regulating neurotransmitter synthesis & degradation

Genes that encode enzymes involved in neurotransmitter synthesis and degradation were also considered prime candidates for ADHD, and some of these have received support in association studies. Genes tested include those for tyrosine hydroxylase (TH), dopa decarboxylase (DDC) and dopamine β-hydroxylase (DBH). Also tested are genes for proteins involved in the degradation of neurotransmitters, catechol-O-methyltransferase (COMT) and monoamine oxidases A and B (MAOA and MAOB). We briefly review the results here.

Regarding DDC, which converts l-DOPA to dopamine, only a few studies have been reported to date; however, significant evidence for DDC association with ADHD has been identified [154,195,196]. A further study in Han Chinese also identified association, but only with the inattentive subtype [194]. The polymorphisms investigated are not known to be functional, and thus, further exploration of the association with DDC is needed.

In the studies of TH, which converts tyrosine to l-DOPA (the rate-limiting step in dopamine biosynthesis), two groups have investigated the TH01 polymorphism, a tetrameric repeat located in the first intron of the TH gene. This polymorphism has been reported to have an influence on transcriptional regulation of the gene [197]; however, neither of the ADHD studies found evidence for association of this marker with the disorder [165,167]. Another study has reported some evidence for association with a marker of the TH gene, but only in paternal transmissions [154].

For COMT, an initial positive finding examining the Val158Met polymorphism [198] did not hold up once the sample size was increased [199]. This particular polymorphism has been the focus of a number of studies of psychiatric and cognitive traits because it influences the activity of the enzyme, with the valine allele reported to have high activity and the methionine allele reported to have low activity [200]. Activity of the enzyme is reduced by three- to four-fold in individuals homozygous for the methionine allele [201]. No subsequent studies detected evidence for association of this polymorphism with ADHD [146,154,165,202–206] and a meta-analysis of 11 published studies found no support for this gene (OR: 0.99; 95% CI: 0.88–1.12) [207]. Of note, however, is that all studies of COMT and ADHD, thus far, have focused on a single functional polymorphism, Val158Met, ignoring the complexity of the gene. Specifically, additional
genetic variation contributing to differential expression of COMT has recently been identified [208], and it is notable that in studies of both schizophrenia and cognitive phenotypes, haplotypes of these markers with the Val158Met polymorphism have provided more significant association results than the Val158Met marker alone [208, 209]. Thus, the study of only a single functional marker of the COMT gene may hinder the identification of association, by neglecting functionally relevant haplotypes.

Results for the gene encoding DBH, which, in noradrenergic neurons converts dopamine to norepinephrine, have been mixed. Positive results have been reported for the A2 allele (T) of the TaqLA polymorphism located in intron 5 of the DBH gene [67, 210, 211]. However, other studies observed only trends for this allele [212] or found association with the opposite (A1 or C) allele [79]. Negative studies of other polymorphisms have also been reported, including studies of the -1021C/T promoter polymorphism [165, 213, 214], a marker highly correlated with DBH serum levels [215]. Furthermore, additional markers genotyped on the sample of Daly and colleagues did not reveal any other associated markers [202]. One study identified association with one marker in intron 12, out of seven markers tested, but no evidence was found for association of the intron 5 polymorphism [94].

If plasma levels of DBH are the critical factor contributing to risk, then more consistent results across studies would be expected because plasma levels strongly correlate with multiple polymorphisms located across the gene. These include the single nucleotide polymorphisms TaqLA (rs2519152), 1603C/T (rs6271) and -1021C/T (rs1611115), and the insertion/deletion and microsatellite polymorphisms located in the 5´ region [215–218]. It is not clear which, if any, of these polymorphisms is/are the functional change(s) controlling DBH levels. The polymorphism at -1021 exhibits the strongest correlation with plasma levels, with the T allele being associated with lower expression. However, the addition of a nonsynonymous polymorphism, R535C, to this analysis increased the correlation by 0.02 (p = 0.0024) [215], indicating that combinations of more than one polymorphism may contribute to the overall levels.

As with DBH, several polymorphisms in or near the MAOA gene also correlate with activity or transcription levels. A polymorphism in exon 8, 941T/G, is associated with altered enzymatic activity of the MAOA protein [219], and in the 5´ region, alleles of a 30 bp VNTR appear to alter transcriptional activity in in vitro reporter assays [220–222]. The 5´ VNTR polymorphism is located 1.2 kb upstream of the gene and is characterized by at least 5 alleles (2-, 3-, 3.5-, 4- and 5-repeats) of an imperfect 30 bp repeat. For the 5´ VNTR, in vitro transcription assays from one study indicated that the 3.5- and 4-repeat alleles have two- to ten-times more transcriptional activity than the 3- or 5-repeat alleles [220]. However, a later study obtained different results for the 5-repeat allele, finding it to be similar in activity to the 3.5- and 4-repeat alleles [221]. A study of this polymorphism in relation to both mRNA expression and enzyme activity in post-mortem brain found no relationship with either measure; however, analyses of haplotypes found a particular 3-repeat-containing haplotype to be associated with low enzyme activity [222].

The initial studies of association of the MAOA gene and ADHD focused on a nearby microsatellite marker (DXS7). The first study reported evidence of association with the 157 bp allele of this dinucleotide repeat polymorphism in a Chinese sample [224]. However, Lowe and colleagues noticed that two paternal alleles had been included in that analysis, when for X-linked markers there is only a single allele. After reanalyzing the results of Jiang et al., the association with MAOA was found to be maintained, but with reduced significance [225]. In the study of this same polymorphism in an Irish sample, no association was observed [225]. Jiang and co-workers followed up their analysis of DXS7 by testing a dinucleotide repeat polymorphism in the MAOA gene, finding significant evidence for association with the 114 bp allele (global p < 0.05) [226]. Analysis of this same polymorphism in a sample of families from the UK showed a trend for association of a different allele, the 122 bp allele, but it was not significant after correction for the number of alleles of the marker, and yielded a global result that was not significant [165].

Alleles of the MAOA 30 bp VNTR have been tested for association with ADHD, with association reported in several studies, however the association was with different alleles. The association in an Israeli study was with the 4- and 5-repeat alleles combined [227], and in an Indian study the 3-repeat allele was associated in males (designated as 3.5-repeat allele by the authors) [228]. In a UK study, no associations were found for ADHD; however, in a subset of the males with comorbid conduct disorder, association with the ‘low activity’ (3-repeat) allele was reported [229]. This finding is interesting in view of previous reports of this marker being associated with aggressive/impulsive behavior [230].
Alleles of the MAOA exon 8 (941T/G) synonymous coding region variant correlate with low (T) and high (G) activity levels [219]. Some ADHD studies have found evidence for association of the 941G allele with the disorder [231,232], although this was not found by others [229]. The most recent study used 12 markers across the MAOA gene and reported association [194], although we note that the 941T/G (rs6323) polymorphism was not included in that study.

The association of ADHD with the MAOA ‘high-activity’ alleles, and thus increased degradation of monoamines, is consistent with multiple lines of evidence supporting a hypo-dopaminergic state as contributing to ADHD [34], and suggests a plausible explanation by which this gene could contribute to the disorder. However, much like DBH, there is no consistent finding for association to either high activity- or low activity-correlated alleles, or to haplotypes composed of these alleles. For example, the study of Xu et al. found association to haplotypes containing the 941G (high activity) and the 3-repeat (low activity) alleles [232]. Furthermore, some indication of a more complex relationship between genotype and activity has also emerged from a recent PET study that failed to find a correlation between genotypes of the MAOA VNTR alleles and brain MAOA activity in adult males [233]. This could indicate that the VNTR has little effect on protein levels in vivo.

Other genetic variation, either within the MAOA gene or in other genes that could regulate MAOA protein levels, may influence the overall activity levels. Expression of the gene may be modulated by multiple mechanisms, such as compensation by other genes, epigenetic down- or upregulation, or to haplotypes composed of these alleles. For example, the study of Xu et al. found association to haplotypes containing the 941G (high activity) and the 3-repeat (low activity) alleles [232]. Furthermore, some indication of a more complex relationship between genotype and activity has also emerged from a recent PET study that failed to find a correlation between genotypes of the MAOA VNTR alleles and brain MAOA activity in adult males [233]. This could indicate that the VNTR has little effect on protein levels in vivo.

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Dopamine-receptor signaling

The findings of association with the dopamine receptors suggested the possibility that genetic variation in the respective signaling pathways could also play a role in ADHD. Following on from the findings for association of DRD4, the G-protein α subunits Ga, Gaα, and Gaα, were tested based on their role in D4 signaling, however, no evidence for association was detected [253].

Our group tested the gene for Gαolf (GNAL), because of its role in D1 and D5 signaling, and because the G(olf)-knockout mouse displays a hyperactive phenotype, further supporting this gene as a candidate [55]. We obtained evidence for GNAL association with ADHD that included a parent-of-origin effect, with biased transmission found for maternal, but not paternal, transmissions [254]. A parent-of-origin effect is consistent with previous evidence from genetic linkage studies of bipolar disorder and schizophrenia, which had suggested parent-of-origin effects for the chromosome 18p region encompassing GNAL [255–257].

Conclusion

The hypothesis that dopamine dysregulation would underlie the genetic susceptibility to ADHD was the first line of inquiry in genetic studies of ADHD, and this approach has been successful in identifying genes associated with the disorder. Findings for a number of the genes have been replicated across multiple studies. However, for the majority of genes, the findings are far from conclusive. Studies thus far have been limited by relatively small sample sizes for a complex trait. Larger samples will allow for more rigorous tests of association. A number of factors, in addition to sample size, can influence power to identify genetic associations, and these should also be kept in mind when interpreting the genetic literature. These include the effect size of the gene in relation to the phenotype, the degree of linkage disequilibrium between the marker(s) tested and the susceptibility allele(s), as well as locus and allelic heterogeneity.

A major issue for the majority of genes studied thus far is that only a limited number of markers were studied for each gene, resulting in less than comprehensive coverage. Frequencies of the marker alleles will influence power, for example, if the susceptibility allele is rare and the marker allele frequency is common. Furthermore, differences in allele frequencies in different ethnic groups can result in both Type I and II errors in case–control association studies if the ethnic composition of cases and controls is not exactly matched. A number of polymorphisms in key genes of the dopaminergic system have differences in allele frequencies across populations. For example, the allele frequencies of the exon 3-repeat in DRD4 are dramatically different across populations, with the frequency of the 7-repeat allele varying by 25-fold [259]. There are also differences in the distribution of the sequence differences within the repeat units across populations [259].

Similar, for DRD2, allele frequency of the Taq1A polymorphism is highly variable, with frequencies ranging from 0.09 in Yemenite Jews to 0.75 in Muskoke Amerindians [259]. Thus, the ethnic composition of the samples is an important consideration for the study of these markers, and the ethnic composition of case–control studies must be carefully controlled or a family-based control approach should be used.

Future perspective

Despite the complexities described above, we are beginning to see some consensus across studies for some of the ADHD-associated genes. The challenge now is to move the studies forward by identifying the functional changes that actually contribute to the phenotype. Although labor-intensive, gene screening for DNA variation is relatively easy, given current methodology. However, the interpretation and understanding of precisely how a particular genetic change contributes to risk is not straightforward. Other than large deletions or obviously deleterious amino acid changes, attributing a change in gene function to a given variant is generally not simple or clear cut. The DNA variants of interest require assays that can probe for functional changes, usually in a cellular system, followed by the construction of animal models when appropriate. Animal models can also be complicated by measurement and modeling of the phenotype, and by the genetic background of the animal. For example, studies of the hyperlocomotor phenotype of the Coloboma mouse strain originally appeared to indicate a straightforward relationship with Snap25. This mouse is characterized by the single copy loss of a 2 cM chromosomal region that includes Snap25 (deletion of both copies results in nonviable offspring), resulting in a complex phenotype with head bobbing and ocular dysmorphology, in addition to hyperlocomotion. The insertion of a Snap25 transgene into this Coloboma line, the genetic...
background of which is C3He/SnJ, rescued the hyperactive phenotype but not the other phenotypes, indicating that the hyperactivity was directly attributable to this single gene [56]. However, there was no indication of a hyperactive phenotype in an engineered knockout of Snap25 on the C57Bl/6/129 genetic background, thus pointing to the existence of some additional genetic factor(s) (present in the C3He/SnJ strain but not in the C57Bl/6/129 strain), that must also be contributing to the phenotype [260].

Modeling of appropriate phenotypes, particularly for common ADHD comorbidities such as specific reading disabilities (developmental dyslexia), oppositional defiant disorder and conduct disorder, may be difficult to achieve in animals, although for some phenotypes a reasonable proxy can be measured. A recent example of this is the measurement of auditory processing and visuospatial memory in rats after disruption of the expression of a gene implicated in dyslexia, DYSXIC1 [261]. Difficulties in temporal auditory processing of speech sounds and complex nonspeech sounds have been observed in some individuals with dyslexia [262–266], and thus, auditory processing provides a measurable phenotype for studies in rodent models of this complex trait.

Further complicating the interpretation of functional studies is the possibility that multiple DNA changes, in combination, create risk. These could be variants in different genes, as suggested by the example of Snap25 and the Coloboma mouse model above, and/or could be different variants located on the same haplotype of a particular gene. For example, as indicated above for the DRD2 gene, while the 957T allele appears to contribute to a less stable mRNA, this effect is annulled by the presence of the 1101A variant on the same haplotype. It is conceivable that in the case of DRD4, a combination of multiple promoter polymorphisms, reducing gene expression, together on the same haplotype with the exon 3 VNTR 7-repeat allele, reducing receptor function, could increase risk further than either of these two polymorphism types alone. Fortunately, it is possible to investigate such effects by investigating variants within the context of their naturally occurring haplotypes, as has been demonstrated by Drysdale and colleagues in their comprehensive study of the adrenergic β2-receptor gene and asthma [267]. Functional studies of ADHD-associated genes will, therefore, need to take into account the association findings for haplotypes.

It is likely that, in many instances, risk will result from genetic changes influencing gene transcription instead of gene changes that alter the code of the protein [268–272]. Sequences that control gene expression are located in the promoter region, the region of the start of transcription, but can also be located within any region of the gene or can be located at large distances from the gene, and even within other genes, as noted above. Thus, finding these regulatory regions and then further identifying the DNA variation within these regions contributing to risk is labor-intensive. A recent development that streamlines the identification of regulatory elements is the use of modified histones to identify the position of such elements [273, 274]. These techniques allow for the efficient identification of regulatory regions that can then be targeted for functional studies and screened for risk alleles. We have recently used these techniques to identify a 2.5 kb region marked by acetylated histones in the 5¢ region of the KIAA0319 gene [Couto JM, Livne-Bar I, Xu Z et al., unpublished data]. Markers in this region, which spans the promoter region, first untranslated exon and part of the first intron, have been associated with reading disabilities in six independent studies [275–280]. Thus, the identification of this putative 5¢ regulatory region suggests a possible location of functional risk alleles.

Environmental risk factors have also been implicated in ADHD [281–284] and much more research is required in this area. Particular focus has been on prenatal and perinatal risk factors, with complications in pregnancy, delivery and infancy (PDICs), and chronic exposure during pregnancy (smoking, alcohol and illicit drugs), reported to increase risk for ADHD [281, 283]. Perhaps the most studied risk factor is maternal smoking during pregnancy [283–289]. However, the association between these factors and ADHD has not been found in all studies [283, 290, 291], and it is not clear how they influence risk. In one study, the relationship with PDICs was strongest for ADHD with comorbidities, and for nonfamilial cases, suggesting that these factors may produce risk independent of genetic risk [290]. A number of recent molecular genetic studies of ADHD have incorporated a gene–environment interaction effect into the analyses [72, 116, 292–294]. At the present time, however, twin studies that support an interaction effect (i.e., genetic sensitivity or susceptibility to the environment) are lacking [295], and thus, this is an area of research that should be expanded, so that the molecular genetic studies can be modeled appropriately.
Some of the prenatal and perinatal factors associated with ADHD may affect the dopamine system. Animal models of hypoxia/ischemia at birth have found long-term abnormalities in dopamine levels, dopamine turnover and expression of dopamine receptors, some of which normalize over time. For example, in one model of perinatal hypoxia in rats, there are early reductions in D1 and D2 dopamine receptor mRNA, however, by early adulthood, normal levels are recovered [286]. Animal models of prenatal and perinatal exposure to nicotine also show alterations in multiple neurotransmitter systems, some of which normalize over time [282].

Reducing environmental risk factors for ADHD, by changing behavior such as maternal smoking, is more desirable than managing symptoms after development of the disorder, and provides great promise in the realm of prevention. Thus, the clear identification of both genetic and environmental risk factors and an understanding of the relationships between them should be the focus of future research.

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<tr>
<td><strong>Attention-deficit/hyperactivity disorder</strong></td>
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<td>▪ Attention-deficit/hyperactivity disorder (ADHD) is a prevalent childhood disorder characterized by developmentally inappropriate and impairing levels of inattention and/or hyperactivity/impulsivity.</td>
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<td>▪ Developmental shifts in ADHD symptomology have been well documented, with hyperactivity/impulsivity being prominent in very young children, inattention becoming apparent later and persisting through adolescence, and symptoms often diminishing (although usually not completely) in adulthood.</td>
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<td>▪ ADHD is a highly heritable and complex trait, with multiple genes and environmental factors likely to be involved.</td>
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<td>▪ Genetic studies of ADHD can be influenced and complicated by a number of factors, such as the reliance on informant-based reporting of behavioral symptoms, shifts in symptomology with age, and uncertainties regarding genetic relationships with comorbid disorders. These, in addition to other subject characteristics (e.g., ethnicity and gender) and study design features (e.g., case–control vs family-based and population-based vs clinical) must be kept in mind when interpreting the genetic literature.</td>
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<th>Role of dopamine in ADHD</th>
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<td>▪ Support for a role of the dopamine system in ADHD is substantial and can be gained from a number of lines of evidence, including neuroimaging, pharmacological interventions and animal models.</td>
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<td>▪ Other neurotransmitters are also likely to be involved, however, the dopaminergic system has received the most attention thus far.</td>
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<td>▪ The evidence supporting the dopaminergic system has guided the search for genes contributing to ADHD and this approach has created a very successful starting point.</td>
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<th>Animal models supporting the involvement of the dopaminergic system in ADHD</th>
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<td>▪ Naturally occurring and genetically engineered hyperlocomotive mouse models have provided clues to genes contributing to ADHD.</td>
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<td>▪ These include the genes for the dopamine transporter (DAT1), the dopamine receptors D1 (DRD1) and D4 (DRD4), and a G-protein subunit, Gaq (GNAL), involved in D1- and D5-receptor signaling.</td>
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<td>▪ Mouse models also implicated genes involved in the controlled release of neurotransmitters (SNAP25 and Calcyon) as ADHD-susceptibility genes.</td>
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<td>▪ The dopamine transporter functions to regulate synaptic dopamine levels, through reuptake, and is the primary target for methylphenidate, supporting this gene as a prime candidate.</td>
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<td>▪ The first studies of this gene found support for association using a single genetic marker (variable number of tandem repeats [VNTR] in the 3'-untranslated region [UTR]) for which there had been some indication of functionality.</td>
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<td>▪ Most studies have focused on the 3’-UTR VNTR, and studies both supporting and not supporting association of this marker have been published. However, a few studies investigating additional DNA markers have identified more significant evidence for association. The predominant use of a single marker for this gene in genetic studies, to date, may underestimate the gene’s contribution to ADHD.</td>
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<td>▪ The DRD4 gene is supported as an ADHD-susceptibility gene by multiple studies and meta-analyses.</td>
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<td>▪ The majority of studies have focused on a single polymorphism, specifically, the alleles of a 48 bp repeat located in the third exon, and have found evidence for association with the 7-repeat allele.</td>
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<td>▪ The 7-repeat allele was reported to be slightly less sensitive to dopamine than the 4-repeat allele, leading to the speculation that risk could be attributed to a hypodopaminergic state created by the inheritance of this allele. An additional study indicates that the repeat may also influence mRNA stability or translation efficiency.</td>
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<td>▪ Additional genetic studies indicate that risk is more complicated than inheritance of the 7-repeat alone, and that risk for ADHD will likely involve the combination of multiple DNA changes across the gene.</td>
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**Executive summary**

**Dopamine receptor D5**
- The *DRDS5* gene is also supported as a susceptibility gene for ADHD by multiple studies. The association reported, thus far, is with a polymorphism located 18.5 kb upstream of the gene.

**Dopamine receptor D1**
- Some studies support an association of the *DRD1* gene with ADHD, specifically with symptoms of inattention.
- One of the associated polymorphisms (rs686 in the 3’-UTR) has been implicated as a regulatory variant that may influence gene expression.

**Dopamine receptor D2**
- While evidence for association of the *DRD2* gene with ADHD is quite limited, one of the polymorphisms commonly studied in ADHD (and in other disorders), namely the Taq1A marker, is deserving of some comment.
- Although originally known as a *DRD2* marker, the Taq1A polymorphism is actually located several kb downstream of *DRD2*, and is now known to be a nonsynonymous variant (Glu713Lys) within the coding sequence of a neighbouring gene, *ANKK1*. This must now be taken into consideration in the interpretation of studies that use this marker.

**Dopamine receptor D3**
- Studies of the *DRD3* gene have been limited, and thus far, there is no support for association of this gene with ADHD.

**Genes regulating neurotransmitter synthesis & degradation**
- Associations with genes encoding proteins that control the synthesis, degradation and release of dopamine have been identified in ADHD.
- Genes supported by more than one study are the genes for dopa decarboxylase (*DDC*), which converts L-DOPA to dopamine, dopamine β-hydroxylase (*DBH*), which, in noradrenergic neurons, converts dopamine to norepinephrine, and the monoamine oxidases (*MAOA* and *MAOB*), which are involved in neurotransmitter degradation. While some of the polymorphisms studied have been correlated with enzymatic activity levels (e.g., *DBH* -1021C/T, *MAOA* 941T/G and *MAOB* intron 13 G/A), there is still no clear picture as to which, if any, of these might be causal in terms of susceptibility to ADHD.
- The gene for catechol-O-methyltransferase (*COMT*), also involved in degradation, has been analyzed in numerous ADHD studies, the vast majority of which have not found evidence for association. However, such studies have ignored the complexity of the gene by focusing exclusively on a single marker (Val158Met), and thus, firm conclusions cannot yet be made.

**Genes regulating neurotransmitter release**
- Several genes involved in the controlled release of neurotransmitters have been implicated in ADHD, the most studied of which is the synaptosomal-associated protein of 25 kd (*SNAP25*) gene that was selected for study based on the mouse model, *Coloboma*.
- Multiple studies have found evidence for *SNAP25 association with ADHD; however, causal variants have yet to be identified.

**Dopamine receptor signaling**
- Genetic variation in intracellular signaling pathways may also play a role in ADHD. Thus far, association for only one gene, *GNAL*, a gene involved in D1 and D5 signaling, has been reported.

**Conclusion**
- The hypothesis that dopamine dysregulation would underlie the genetic susceptibility to ADHD was the first line of inquiry in genetic studies of ADHD, and this approach has been successful in identifying genes associated with the disorder.
- While studies have supported a number of the candidate genes as contributors to ADHD, conclusions are limited by the number of studies conducted thus far and the relatively small sample sizes used in the majority of studies. Genes implicated include those for the dopamine receptors, genes involved in dopamine transport and release as well as synthesis and degradation, and genes involved in dopamine signaling.
- For many of the genes, only a limited number of markers were used, resulting in less than comprehensive coverage of the gene. This will lead to underestimates of risk if the polymorphism(s) tested is/are not the functional change(s) actually contributing to the genetic susceptibility and if linkage disequilibrium between tested marker(s) and causal variant(s) is weak.

**Future perspective**
- The challenge now is to move the association findings forward by identifying the functional changes that actually contribute to the phenotype.
- Identification of the genetic variants that contribute to risk for ADHD and determination of the mechanisms by which they contribute to the disorder are complicated by the existence of multiple gene variants within the same gene, as well as the possibility of gene–gene and/or gene–environment interactions.
- Genetic changes influencing gene regulation are likely to contribute to risk. However, the identification of gene-regulatory elements, as well as the genetic variation within these regions contributing to risk, can be difficult. Novel strategies using histone modifications as markers of regulatory elements will streamline this process.
- The delineation of more refined phenotypes useful for the genetic study of ADHD, and the establishment of multisite collaborative networks that can capitalize on the collection of large sample sizes, are also promising approaches for future genetic studies of ADHD.
A number of approaches hold considerable promise for facilitating the identification of ADHD-susceptibility genes in the future. One approach is the establishment of multisite collaborative networks, such as the International Multi-Centre ADHD Gene (IMAGE) program. It is anticipated that collaborative programs such as this will be able to capitalize on the collection of very large sample sizes that are likely to be crucial in future gene-finding efforts [297,298]. In addition, refinements in the ADHD phenotype, both behavioral and cognitive, may prove useful. For example, we have used this approach successfully in our investigations of the DRD1 gene (described above), using quantitative measures of the two symptom dimensions of ADHD, both of which are known to be highly heritable [176,177]. Furthermore, it has been proposed that genome-wide association studies may now be useful in the identification of quantitative trait loci (QTLs) for sustained and spatial attention, QTLs that, in turn, may be involved in ADHD [299]. The pursuit of endophenotypes, in other words, intermediate phenotypes that bridge the gap between the clinical manifestations of ADHD and the underlying genes, is also of intense interest [297,300].

In summary, the selection of the dopaminergic system as the starting point for study of the genetic susceptibility to ADHD has provided the field with a promising beginning. However, we have just barely scratched the surface in understanding the precise roles of these genes in ADHD, and years of studies await us.

**Financial & competing interests disclosure**

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

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This investigation of the DAT1 gene was the first to report association of a candidate gene with attention deficit hyperactivity disorder and, thus, was of major importance in promoting the molecular genetic studies of the disorder.

Dopamine system genes & ADHD Review


**This study surveyed, in detail, the exon 3 VNTR repeat originally identified by Van Tol and colleagues. The authors documented sequence differences within the repeats, as well as substantial diversity in sequence and repeat length across populations. This striking illustration of the coding region diversity for this gene had important implications for genetic studies of *DRD4*.**


**This paper was the first to report association of the *DRD4* gene with ADHD. The successful identification of *DRD4* by these authors, in combination with the DAT1 findings by Cook and colleagues (1995), generated intense interest in the molecular genetics of ADHD.**


This study revealed that a polymorphism that had long been known as a DRD2 marker (the TaqI A marker) was actually a non-synonymous single nucleotide polymorphism located within the coding region of a neighbouring gene, ANKK1.

This new knowledge had important implications for the interpretation of genetic studies using this marker. This discovery also demonstrated, more generally, the importance of understanding, as fully as possible, the nature of the polymorphisms under analysis in any genetic study.


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