The genetics of attention deficit hyperactivity disorder

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Attention deficit hyperactivity disorder (ADHD) is a highly heritable, disruptive, childhood-onset condition, the aetiology and pathogenesis of which is poorly understood. There have been relatively few genome-wide linkage studies, and no chromosomal region has yet been unequivocally implicated. In contrast, evidence from pharmacological, neuroimaging and animal studies has suggested the involvement of specific neurotransmitter systems, notably dopaminergic pathways, in ADHD and these aetiological clues have inspired a fruitful application of the candidate gene association approach. Meta-analyses or pooled data analyses have supported association between ADHD and polymorphisms in DRD4, DRD5 and SLC6A3 which encode dopamine D4 and D5 receptors and the dopamine transporter, respectively. A weaker, but nevertheless replicated, body of evidence also supports associations with SNAP-25 (synaptosomal-associated protein, 25 kDa) and SLC6A4 (serotonin transporter). There is increasing research interest in gene-phenotype links, clinical phenotypic markers of heterogeneity and gene–environment interaction, which are likely to be important in the next generation of genetic studies.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) was first described in 1902 by George Still, a paediatrician. It is now recognized as a common, highly disruptive, disabling neurodevelopmental disorder that affects up to 6% of children and which results in adverse sequelae in adult life. ADHD has its onset in childhood and is characterized by symptoms of inattention, over-activity and impulsiveness of a degree that is both severe and impairing and that must be present in more than one setting to meet internationally accepted diagnostic criteria (Table 1). Although affected individuals show neurocognitive deficits including disruption of learning and memory, there is no measure of cognitive performance that identifies cases reliably and ADHD remains a clinical diagnosis on the basis of reported symptoms. However, the widespread use of structured assessment tools combined with internationally accepted operationalized diagnostic criteria (Table 1) have resulted in very high rates of diagnostic reliability and international uniformity in research diagnostic approaches.

Despite being an important clinical problem, the aetiology and pathogenesis of ADHD is poorly understood. ADHD is a complex disorder influenced by genetic and environmental factors. However, most of the variation is attributable to genetic factors, with genetic epidemiological studies, including more than 14 published twin studies and five adoption studies, consistently demonstrating high heritability in the range of 75–91% (1). As a result, there has been growing interest in the molecular genetic basis of ADHD. In this review, current progress in identifying specific susceptibility genes and newly emerging evidence are considered.

FUNCTIONAL CANDIDATE GENE ASSOCIATION STUDIES

To date, most published molecular genetic studies of ADHD have been based on a functional candidate gene approach (2,3). The choice of candidate genes has mainly, but not exclusively, focussed on genes encoding proteins involved in dopaminergic, and to a lesser extent serotonergic, pathways. This reflects a convergence of evidence from pharmacology, neuroimaging and animal studies suggesting, in particular, that ADHD reflects an abnormality of dopaminergic neurotransmission. Although such studies are sometimes criticized on the basis of low prior probability for the involvement of any gene, in ADHD, this approach has been remarkably fruitful, particularly in comparison with other psychiatric phenotypes.

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ADHD is one of the few childhood neuropsychiatric disorders for which there is well-established evidence of good response to medication. Seventy to eighty per cent of children with ADHD show immediate symptomatic improvement when treated with stimulant medication such as methylphenidate (4). Methylphenidate crosses the blood–brain barrier rapidly with most marked uptake in the striatum. There is now considerable evidence to suggest that a key mechanism through which methylphenidate exerts its therapeutic effects in ADHD is by increasing the functional availability of extracellular dopamine (5), although other pathways are also likely to be involved (4,6). The mechanism by which treatment response is believed to occur is by the inhibition of the dopamine transporter (DAT1, encoded by \textit{SLC6A3}) which is responsible for re-uptake of dopamine from the synaptic cleft into presynaptic terminals (4) (Fig. 1). Neuroimaging studies of ADHD, including structural MRI (7,8), SPECT and PET studies (9,10), are also beginning to provide direct evidence for the involvement of brain regions rich in dopaminergic innervation and more specifically increased DAT binding in the striatum (9,11) and lower DAT binding in the mid-brain (10), although findings have not been completely consistent (10,12).

Findings from animal studies converge with those from neuroimaging and pharmacology in implicating dopaminergic mechanisms in ADHD. Thus, mouse \textit{Drd3} and \textit{Slc6a3} homozygous knockouts show increased activity (13–15). Moreover, studies of \textit{Drd1}–/– and \textit{Drd5}–/– mice also suggest the involvement of these receptors in locomotor activity, and \textit{Drd4}–/– mice show decreased novelty-related exploration (14,16). Although these findings point to the involvement of dopamine, the efficacy of stimulant medication in inhibiting hyperactivity of \textit{Slc6a3}–/– mice, which lack DAT, suggests the possibility of a therapeutically relevant action independent of DAT blockade. One such action appears to be dependent on serotonergic and glutamatergic functions (17,18).

There have been numerous published association studies of ADHD on many different candidate genes and the findings are reviewed in considerable detail elsewhere (2,19). Here, we focus on those genes that, by virtue of meta-analyses or pooled analyses, have either robust support or sufficient support to have a reasonable probability of involvement in ADHD.

**ASSOCIATIONS CONFIRMED IN PUBLISHED META-ANALYSES/POOLED DATA**

The dopamine D4 receptor gene (\textit{DRD4})

Since its discovery (20), \textit{DRD4}, which maps to 11p15.5, has been the focus of intense interest as a candidate gene for aspects of normal and abnormal human behaviour. In particular, candidate gene studies have focussed on a 48 bp variable number tandem repeat in exon 3 of \textit{DRD4}. Alleles contain between two (2R) and 11 (11R) repeats, which correspond

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**Table 1. Symptoms of ADHD from the DSM-IV research diagnostic criteria**

<table>
<thead>
<tr>
<th>A. Inattention (six or more symptoms required)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Often fails to give close attention to details or makes careless mistakes in school work, work or other activities</td>
</tr>
<tr>
<td>Often has difficulty sustaining attention in tasks or play activities</td>
</tr>
<tr>
<td>Often does not seem to listen when spoken to directly</td>
</tr>
<tr>
<td>Often does not follow through on instructions and fails to finish schoolwork, chores or duties in workplace (not due to oppositional behaviour or failure to understand)</td>
</tr>
<tr>
<td>Often has difficulty organizing tasks and activities</td>
</tr>
<tr>
<td>Often avoids, dislikes or is reluctant to engage in tasks that require sustained mental effort</td>
</tr>
<tr>
<td>Often loses things necessary for tasks or activities</td>
</tr>
<tr>
<td>Is often easily distracted by extraneous stimuli</td>
</tr>
<tr>
<td>Is often forgetful in daily activities</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>B. Hyperactivity–impulsivity (six or more symptoms required)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperactivity</td>
</tr>
<tr>
<td>Often fidgets with hands or feet and squirms in seat</td>
</tr>
<tr>
<td>Often leaves seat in classroom or in other situations in which remaining seated is expected</td>
</tr>
<tr>
<td>Often runs about or climbs excessively in situations in which it is inappropriate (subjective feelings of restlessness for adolescents or adults)</td>
</tr>
<tr>
<td>Often has difficulty playing or engaging in leisure activities quietly</td>
</tr>
<tr>
<td>Often ‘on the go’ or often acts as if ‘driven by a motor’</td>
</tr>
<tr>
<td>Often talks excessively</td>
</tr>
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<table>
<thead>
<tr>
<th>Impulsivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Often blurts out answers before questions have been completed</td>
</tr>
<tr>
<td>Often has difficulty awaiting turn</td>
</tr>
<tr>
<td>Often interrupts or intrudes on others (e.g. butts into conversations or games)</td>
</tr>
</tbody>
</table>

Symptoms must have persisted for at least 6 months, must be developmentally inappropriate and present before the age of 7. Symptoms are of a degree that is maladaptive, associated with impairment in functioning that is present in two or more settings.

**Figure 1.** Schematic diagram representing a dopaminergic terminal.
to a protein polymorphism of 32–176 amino acids in the third intra-cytoplasmic loop of the receptor, a region that couples to G proteins and mediates signal transduction via alterations in intracellular cAMP levels (21). Allele frequencies vary considerably between populations (22,23).

Initial studies suggested that the 7R allele was associated with novelty seeking (24) and ADHD (25). Although at least superficially, one might consider the two as related phenotypes, the former association is controversial and has not Withstood meta-analysis of follow-up studies (26). This is in marked contrast with findings of association between ADHD and the 7R allele that have now been extensively replicated. Moreover, in a published meta-analysis, reported odds ratios were 1.9 (95% CI 1.5–2.2; \( P = 0.0000008 \)) for seven case–control studies and 1.4 (95% CI 1.1–1.6; \( P = 0.02 \)) for 14 family-based studies (27).

It has been reported that the 7R variant exhibits blunted ability to reduce cAMP in comparison with the common 4R variant (28). Moreover, the 7R allele displays much more extensive linkage disequilibrium to adjacent polymorphisms than the 4R allele suggesting that it arose more recently. It has been proposed that its high frequency is likely due to positive selection, supporting the hypothesis that it is functional and has an important adaptive role for humans, possibly by influencing a behavioural characteristic (22,23).

Virtually all association studies of the DRD4 VNTR in ADHD have defined alleles based on repeat number inferred from PCR-product length, but it has been known, for some time, that the repeat sequences show considerable variation in terms of base composition as well as length (22,29,30). To date, the only study that has sequenced the repeats in ADHD (30) not only confirmed an excess of the 7R variant but also found that most of the associated 7R alleles consisted of the common, conserved haplotype in keeping with the common variant–common disorder hypothesis. However, 11% of cases had one of a number of rare, novel haplotypes, most of which were 7R derivatives. The high frequency of amino acid changing variants in these rare haplotypes (>90%) and the low probability that they were detected by chance (\( P < 0.0001 \)) suggests that allelic heterogeneity is also playing a role in the association of DRD4 and ADHD (30).

**The dopamine D5 receptor gene (DRD5)**

DRD5, which maps to 4p16.3, was first implicated in ADHD by Daly et al. (31) who reported association with a dinucleotide repeat located almost 20 kb 5' to the start of transcription. A small meta-analysis of five studies (32) and a larger international joint analysis of almost 2000 probands and 3000 of their parents derived from 14 groups (33) showed significant association with a reported odds ratio of 1.24 (95% CI 1.12–1.38; \( P = 0.00005 \)) for the 148 bp allele. However, although the case for DRD5 as a susceptibility gene is fairly strong, there is no evidence that the dinucleotide repeat is functional, nor have analyses of other markers in the gene permitted resolution below the gross level of the gene (34). Although containing only a single exon, analysis of DRD5 is obstructed somewhat by the presence of expressed pseudogenes with very high levels of homology at the nucleotide level (35).

**Dopamine transporter gene (SLC6A3)**

SLC6A3 (also known as DAT1) encodes the DAT and maps to 5p15.3. Given the widely perceived mode of action of stimulant drugs in ADHD, it is not surprising that SLC6A3 has been among the best-studied genes in this disorder (Fig. 1). Following up the first report (36), nearly all published studies have focussed on a VNTR in the 3'-UTR of the gene with reported significant evidence for association to the 480 bp (10R) allele. There have been three published pooled and meta-analyses of the data (19,32,37). The first two sets of analyses based on nine and eleven studies, respectively (664 and 824 informative transmissions), showed evidence of heterogeneity across samples but only trends for association (OR = 1.16, \( P = 0.063 \) and OR = 1.27, \( P = 0.06 \)). The more recent analysis based on an extension of the samples from the previous meta-analyses found a small but significant association (OR = 1.13, 95% CI 1.03–1.24) (38).

There is some evidence that the associated VNTR is associated with the amount of DAT1 measurable by SPECT neuroimaging (39), with the putative ADHD 10-repeat risk allele being associated with more DAT1 protein. This finding is compatible with the hypothesis of excess dopamine clearance in ADHD. At present, there are no replication data to confirm the finding and even if it is correct, it may reflect indirect association because reporter gene analyses in vitro do not confirm that the repeat per se influences mRNA steady state (40,41). Intriguingly, the former study (40) suggested the presence of alleles in the proximal promoter, intron 1 and intron 14 exerting an effect on transcription in vitro. However, their relevance to ADHD is as yet unsupported by any consistent evidence for association between ADHD and any other variant in SLC6A3, including the proximal promoter region (42,43).

**INDEPENDENT REPLICATED FINDINGS**

**SNAP-25 (synaptosomal-associated protein) (44)**

The coloboma mouse mutant shows marked motor hyperactivity which is alleviated by stimulant medication (D-amphetamine). The phenotype results from hemizygous deletion of 2 cM of chromosome 2 which contains, among other genes, SNAP-25. As over-expression of SNAP-25 rescues the hyperactivity phenotype (45), low SNAP-25 appears to be required for the phenotype, although deletion of another gene is likely to be contributory because pure SNAP-25 knockouts are not hyperactive (46).

In humans, SNAP-25 maps to 20p11.2. A number of findings suggest possible association between ADHD and SNAP-25 markers (47–50). However, as yet, the body of evidence is still not fully convincing, as groups have studied different markers and detailed studies of markers spanning the locus are only beginning to emerge (51). Interestingly, some of these have reported fairly specific parent-of-origin effects with association being attributable to over-transmission of paternal alleles (49,50).

Although not yet convincing, findings related to SNAP-25 may be of particular value for understanding ADHD, because this gene was selected purely on the basis of animal work without regard to any preconceptions concerning ADHD.
pathophysiology. In fact, \textit{SNAP-25} is a highly plausible functional candidate gene because it is localized on the cytosolic side of the synaptic plasma membrane and is, along with VAMP/synaptobrevin and syntaxin, one of the SNAPRE proteins that forms part of the molecular machinery required for exocytosis of synaptic vesicles (52) during neurotransmission (Fig. 1). Given this function, it is at least possible that variation in \textit{SNAP-25} might influence susceptibility to ADHD by influencing the release of dopamine as well as other neurotransmitters, or indeed as has been proposed in the coloboma mouse, altering the ratio of noradrenaline to dopamine (53).

**Genes encoding the serotonin transporter (\textit{SLC6A4}) and the serotonin receptor 5HT1B (\textit{HTR1B})**

Interest in the serotonergic system has again been fuelled by animal studies. Independent replicated association findings have been reported in connection with \textit{SLC6A4} and \textit{HTR1B} but in neither case are there sufficient data for meta-analysis. With respect to \textit{SLC6A4}, which is often referred to as \textit{SERT}, \textit{HTR} or 5-HTT and maps to 17p11.1-q12, association has mainly been reported to a 44 bp insertion/deletion polymorphism (usually referred to as 5-HTTLPR) involving six to eight repeat elements in the promoter region (chromosome 17p11.1-q12). The long and short allelic variants are associated with differences in transcriptional efficiency, with the short form being associated with lower transporter expression (54), as well as reduced serotonin uptake in platelets (55). Pooled analysis (56) of data from three published studies (57–59) showed an increased frequency of the long allele in children with ADHD ($\chi^2 = 7.14, 1df, P = 0.008$) and over-representation of the long/long genotype (OR = 1.33, 95% CI 0.11–1.66; $P = 0.01$) (56). Although the effect size is small and we ourselves found no evidence for association in our sample (60), this finding has since been replicated in a small case–control study (61) and two population-based studies that have examined sub-clinical symptoms related to ADHD rather than frank clinical disorder (62,63). Thus, there is a mounting body of evidence suggesting this allele as a susceptibility allele for ADHD.

The second serotonin related gene of topical interest is \textit{HTR1B} which encodes the 5HT1B receptor and maps to chromosome 6q13. In addition to more general reasons for postulating serotonin related genes as being of relevance in ADHD, specific support comes from the finding that mice lacking this receptor show motor hyperactivity (64). However, the findings to date have only been partially replicated and are inconclusive (65,66).

**LINKAGE**

There have been only three whole-genome linkage studies, two affected sib pair (ASP) linkage studies (67,68) from the USA and the Netherlands and one study of multiplex families from Colombia (69) (Table 2). In the Dutch study of 164 ASPs (68), two regions on chromosomes 7p and 15q showed suggestive evidence of linkage (maximum MLSs = 3.04 and 3.54). In the US (UCLA) study of 270 ASPs (67), significant genome-wide linkage was obtained on chromosome 16p13 (MLS = 3.73) and suggestive linkage on 17p11 (MLS = 2.98).

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Total cases</th>
<th>Netherlands ASPs ($n = 164$)</th>
<th>Colombian isolate ($n = 16$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5p13</td>
<td>1.77* (2.55)*</td>
<td>1.43f</td>
<td></td>
</tr>
<tr>
<td>6q12</td>
<td>(1.3)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7p13</td>
<td>3.04*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17p11</td>
<td>2.98** (3.63)**</td>
<td>1.42 ($\alpha = 0.10$)</td>
<td></td>
</tr>
<tr>
<td>16p13</td>
<td>3.73**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15q15.1</td>
<td>3.54**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a*Published genome-wide scans to 1.6.05.

**GENE-PHENOTYPE LINKS**

ADHD, like all complex disorders, is phenotypically and aetiologically heterogeneous. As a result, there has been increasing interest in distinguishing specific variants of ADHD that may index underlying aetiological heterogeneity and for examining links between associated gene variants and potential intermediate phenotypes (such as measures of neurocognitive functioning) as well as specific clinical symptoms. In addition, it has been suggested that identifying susceptibility genes for intermediate or alternative phenotypes rather than clinical ADHD may prove a more fruitful approach. In most cases, the data are as yet inconclusive, thus we focus on findings that have been independently replicated with the purpose of illustrating potential future directions.
Clinical symptoms

The presence of antisocial behaviour is an important clinical marker of heterogeneity in ADHD, indexing greater clinical severity, poorer outcome (72,73) persistent problems in adult life, a stronger association (71) with neurocognitive deficits (74) and higher genetic loading (75). Thus, there are a priori reasons for undertaking analyses that take antisocial behaviour in ADHD into account. For example, a re-analysis of combined data from the UK and Eire showed evidence of association with the DRD4 7R allele in a sample of children with both ADHD and antisocial behaviour that was not present in ADHD alone (76). A further study, on the basis of an expanded sample from Eire, obtained similar findings (77). Other studies have examined different phenotypic features; for example, demonstrating the association of specific ADHD subtypes, defined according to the preponderance of inattention or hyperactive–impulsive symptoms (Table 1), with DRD5 (78) and the association of ADHD symptom persistence with the DRD4 7R (79).

Neurocognitive measures and other potential intermediate phenotypes

There remains considerable uncertainty as to the core neurocognitive deficits in ADHD (80,81). Thus, genetic studies that have examined neurocognitive deficits have used a variety of different measures which renders comparison difficult. Some studies have found faster responses and greater neurocognitive impairment in children with ADHD, who carry the DRD4 7R repeat allele (82,83). In contrast, others have failed to find deficits in 7R carriers (84,85). Neuroimaging has been strongly advocated as a useful strategy both for identifying susceptibility genes for ADHD and for more studying gene-phenotype links (86,87). Such studies are underway but remain expensive and thus by necessity are based on small samples. Thus, replication and adequate sample size, which are crucial for genetic studies, have been difficult to achieve thus far.

A number of genetic studies have been aimed at identifying quantitative trait loci (QTLs) for ADHD symptom scores in non-clinical populations. What evidence is available suggests that some, but not all, of the association findings from clinical samples are replicated in population-based QTL studies of ADHD (DAT1 and SLC6A4) (41,63,88). However, so far, results from population-based QTL association studies have been weaker than that from clinical case-based studies (41) and the case for using this approach as a replacement to a diagnostic approach for molecular genetic studies has not been made.

GENE–ENVIRONMENT INTERACTION

The aetiology of ADHD like all complex disorders is not entirely explained by genes; environmental factors also contribute. The most consistent and robustly associated of these are exposure to maternal smoking in utero (89,90) with a pooled odds ratio of 2.38 (95% CI 1.61, 3.52) (Langley et al., manuscript in preparation) and low birth weight/gestational age with a reported relative risk of 2.64 (95% CI 1.85–3.78) from a meta-analysis (91).

Not all individuals exposed to environmental risk factors develop disorder, that is, individuals differ in their response to specific environmental factors. Equally, for complex diseases, not all individuals carrying susceptibility gene variants will develop the disorder unless they are exposed to environmental risk factors. This phenomenon of gene–environment interaction is now increasingly recognized as an important contributor to complex diseases and behaviour such as cardiovascular disease (92), neuropsychiatric disorders (93) and antisocial behaviour (94) and may contribute to non-replication across molecular genetic studies. Although including environmental covariates can enhance the power of linkage studies (95), to date this approach has not been used in linkage studies of ADHD. In one association study of a non-clinical sample (ADHD itself was not examined), the SLC6A4 480 bp allelic variant was shown to be associated with hyperactive–impulsive symptom scores only in those who were exposed to maternal smoking in pregnancy (96) but this finding need replication.

CONCLUSIONS AND WAYS FORWARD

The genetic contribution to ADHD is well established and as a result, in recent years, there has been an emergence of molecular genetic studies. Functional candidate gene association studies of ADHD have yielded a number of replicated findings. To date, nearly all of the associated genes have been functional candidates nominated, at least in part, by animal studies, suggesting that such an approach may be valid for ADHD. Whole-genome linkage studies are at an early stage and further studies are needed if susceptibility genes are to be identified by positional cloning.

The associated variants are of small effect size (reported OR from meta-analyses of 1.13–1.9), at least when considered in isolation. However, genetic epidemiological and clinical study findings suggest that ADHD is heterogeneous and that gene–environment and gene–gene interactions are likely to be important (97). Potential gene–gene interaction in ADHD might be informed by animal studies using modifier screens based on random mutagenesis (98) or from greater understanding of the neurobiology of dopamine neurotransmission. It will be also important that the next generation of linkage and association studies incorporate readily assessed (and therefore easy to replicate) measures of environmental risk and clinical, and possibly neurocognitive indicators of heterogeneity. ADHD is a disorder that can be diagnosed with high reliability and which shows neurobiological validity. The molecular genetic evidence that has been generated so far further reinforces the utility of focusing on the phenotype of clinical ADHD. Results from ongoing and new genetic studies will be crucial in now elucidating the pathogenesis of this condition.

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Conflict of Interest statement. None declared.
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