Attention-Deficit Hyperactivity Disorder in the post-genomic era

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2004 European Child and Adolescent Psychiatry, 13, Suppl. 1, 50-70

This is the reformatted manuscript submitted - prior to publication in its final form at (www.springerlink.com) DOI 10.1007/s00787-004-1006-6

Abstract

Background ADHD is a common and complex genetic disorder. Genetic risk factors are expected to be multiple, have small effect sizes when considered individually and to interact with each other and with environmental factors.

Objective To describe the difficulties involved in the genetic investigation of such a complex disorder and give a prospective for the future.

Methods Review based on empirical literature and project description.

Results Considerable progress has been achieved through the association analysis of candidate gene loci. Linkage scans using affected sibling pairs have identified a number of potential loci that may lead to the identification of novel genes of moderate effect size. Quantitative trait locus (QTL) approaches provide powerful complementary strategies that have the potential to link the categorical disorder to continuously distributed traits associated more closely with underlying genetic liability in the general population. Success in identifying some associated genes has been complemented by functional studies that seek to understand the mode of action of such genes.

Conclusion Progress in understanding the mechanisms involved has not been straightforward and many inconsistencies have arisen. In order to take advantage of the potential for progress that stems from the genetic findings it will be important to draw upon a variety of approaches and experimental paradigms. A functional genomic approach to ADHD means that investigation of gene function is carried out at various levels of analysis, not only at the level of molecular and cellular function but also at the level of psychological processes, neuronal networks, environmental interactions and behavioural outcomes.

Key words ADHD – genetics – genomics – IMAGE – children – endophenotype – review

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Introduction

Molecular genetic methods have been highly successful in identifying genes of major effect that co-segregate with single gene disorders. As a result huge strides have been made in our understanding of conditions such as Huntington's disease, fragile-X syndrome, familial Alzheimer's disease and rare familial epilepsies, among many others. Progress in identifying genetic risk factors for more common and complex disorders has however proved to be far more difficult since in most cases the genetic influences result from multiple genetic variants, each conferring only a small additional risk to disease susceptibility. This is the case for attention deficit hyperactivity disorder (ADHD) where familial risks are relatively low, with an estimated sibling risk ratio (λs = risk to siblings of ADHD probands/population risk) for broadly defined ADHD of around 3- to 4-fold [36]. Twin studies support the view that genetic factors are the major influence on familial risk with heritability estimates for ADHD symptom scores consistently reported to be in the region of 60–90% [169]. In general these studies find little evidence of shared environmental influences on familiarity, although the role of environment may still be pivotal, acting through mechanisms of gene-environment interaction [149]. Progress in identifying some of the genes involved in ADHD susceptibility has been relatively fruitful over the past decade by screening genetic variants that lie within or Attention-Deficit Hyperactivity Disorder in the post-genomic era close to genes that regulate neurotransmitter systems, particularly dopamine pathways. At the same time, two studies using affected sibling pair methods have identified several putative target regions that may contain novel ADHD susceptibility genes, although the major findings do not replicate across the two studies and the findings remain speculative. This almost certainly reflects the low power of linkage analysis as compared to association analysis to detect genetic variation conferring relatively small risks to the disorder. Here we review the main methods being adopted to map the genes that influence risk for ADHD and consider the extent to which functional genomic approaches have progressed our understanding of the mechanisms behind genetic susceptibility of ADHD.

Quantitative trait locus (QTL) methods

Until recently gene mapping studies in human have depended on the identification of genetic linkage or association with defined disease categories. However many human phenotypes such as blood pressure, obesity, cholesterol levels, reading ability and general cognitive ability can be perceived as traits that are continuously distributed throughout the population. The key hypothesis that arises from this is whether the same genetic variants that increase risk for a specific disorder also influence symptom scores (levels of the associated trait) across the population, the so-called *quantitative trait locus (QTL)* hypothesis. For example do genetic variants that increase risk for dyslexia also influence levels of reading ability in the general population? The importance of this approach is not simply to answer questions about the size of genetic influences and the relationship between normality and abnormality at the level of etiological genetic factors. Quantitative approaches provide powerful methods to examine the relationship between correlated behavioural traits and experimental measures, test mediation hypotheses that seek to identify the cognitive processes and neuronal networks that mediate genetic influences on behaviour and adopt QTL methods for genetic mapping.

The applicability of the QTL approach to disorders such as ADHD can be tested

using the multiple regression twin method proposed by DeFries and Fulker (DF). DF analysis is based on differential regression to the population mean of MZ and DZ co-twins when proband twins are selected for extreme scores (http://psych.colorado.edu/~willcutt/CLDRC/DFanalysis.htm [27, 28]). If an extreme proband deficit is due to genetic influences then both MZ and DZ co-twin means will regress to the population mean; however the DZ co-twin mean will regress further towards the population mean than the MZ co-twin means. In contrast if proband deficits stem from environmental insults such as early traumatic brain injury or obstetric complications, both MZ and DZ co-twin means should regress to the population mean. Proband deficits that stem from shared environmental risk factors will affect both proband and co-twins equally, so that the means of both MZ and DZ co-twins will regress equally to the population mean. Twin studies that have adopted this approach have all estimated high group heritability for extreme groups defined using thresholds on parent rated ADHD symptoms scales [94, 169] and are consistent with the view that genetic influences on ADHD are the same as those that influence continuous measures of ADHD symptom scores across the population. In another study [144], early ADHD symptom-scores were studied in 6,000 UK twin pairs and ADHD symptom scores at ages 2, 3, and 4 were found to be highly heritable (~90 %), regardless of twin age or the measurement cut-off used. Furthermore, it was found that heritability is equally high at both the low and high ends of the distribution, again suggesting that the trait is continuously distributed (Price et al., unpublished data). Despite these findings, this remains an area that has not been adequately investigated in relation to ADHD, since as yet there are no published reports that estimate group heritability where the extreme group is defined using recognised operational criteria and reliable data capture tools such as standardised interview. Unpublished data, however, of 97 probands with a research diagnosis of DSM-IV combined subtype and their unselected siblings from the IMAGE project dataset (see below) estimated sibling correlations of 0.29 for teacher rated ADHD-symptom scores and 0.27 for parent rated symptoms scores, using a method related to DF analysis (The IMAGE Consortium, unpublished data). In contrast, latent class analysis suggests the existence of both symptom sub-group and severity classes that breed independently, raising the possibility that in ADHD different genetic influences act at different points across symptom dimensions [173]. Nevertheless, available evidence suggests that QTL methods should be applicable to the study of ADHD.

In a series of paradigm studies on dyslexia, QTL linkage methods have successfully been used to identify several loci that increase risk for dyslexia [14, 40, 105, 106]. These studies have used a number of different selection criteria to maximise the power of the analysis and balance this against the cost and effort involved in both sample collection and genotyping. Conventional sibling pair linkage studies aim to collect large series of affected sibling pairs. Following genotyping of sufficient polymorphic genetic markers the analysis seeks to identify short segments of parental chromosomes that are shared by affected siblings more frequently than by chance alone. Sibling pairs are grouped by the number of parental alleles that they share *identical-by-descent (ibd)*, and tested for deviation from the expected ratio of 1:2:1 for 0, 1 or 2 parental alleles shared ibd. However affected sibling pair methods are not very suitable for continuous traits since they restrict ascertainment to the identification of cases and fail to take advantage of the continuous relationship

between genotype and phenotype. For example in the original Haseman-Elston approach [58] ibd score is correlated with the squared difference in phenotypic score, (X1 – X2)2, for each sibling pair. Fulker et al. [43] suggested continuous selection of a proband falling beyond a specific threshold together with an unselected sibling. For analysis, they modified the DeFries and Fulker regression model originally developed for use with twins [27, 28]. Fulker then provided a multipoint procedure solution for the mapping of QTLs for continuous traits, in both selected and unselected samples [44]. Although QTL linkage methods can use population samples with no selection of either probands or siblings, for a given amount of genotyping, selection of extreme concordant and discordant sibling pairs will add considerable power to the analysis. For example, in ADHD far more power will be gained by focusing on individuals with extremely low or extremely high ADHD symptom scores.

A new ADHD linkage study, The International Multicenter ADHD Gene project (IMAGE) takes advantage of QTL methods. IMAGE is a major genetic initiative funded by NIMH that brings together research groups from across Europe, Israel and the United States (Amsterdam, Boston, Dublin, Essen, Göttingen, Jerusalem, London, Nijmegen, Southampton, Tel-Aviv, Valencia and Zurich). The basic study design is similar to that suggested previously by Fulker [43] and applied to the investigation of dyslexia [14]. The main difference is that instead of selecting probands who fall above a particular threshold for ADHD-symptom counts, probands are selected if they have a DSM-IV diagnosis of the combined subtype of ADHD. Siblings on the other hand are unselected for phenotype in the initial ascertainment, but subsequently the most informative pairs are selected for genotyping using their ADHD-symptom counts to derive an index of potential informativeness for linkage for each sib-ship [145].

The initial purpose of the IMAGE project will be to perform quantitative trait locus (QTL) linkage analysis using around 800 sibling pairs selected for maximal informativity from a larger set of sibling pairs. In general siblings whose ADHD-symptom counts fall within the top 10% or bottom 20% of the distribution form the most informative pairings with ADHD probands. Using this sample, a genome scan utilising 400–500 highly polymorphic genetic markers is estimated to have around 80% power to detect linkage regions containing QTLs contributing 10% to phenotypic variance and 50% power for 5% QTLs. The usual approach taken in the fine-mapping stage is to search for association within target regions where there is reasonable evidence of linkage, using dense maps of anonymous markers or by targeting markers located close to or within candidate genes that lie within the region. The IMAGE sample will have considerable power for this type of analysis and is estimated to have 80% power to identify associations contributing as little as 1–2 % to phenotypic variance, or odds ratios around 1.2 for association to the DSM-IV combined subtype diagnosis.

Linkage and association approaches to mapping ADHD genes

Positional cloning strategies such as that outlined above for the IMAGE sample and those already underway by the UCLA/Oxford and Dutch groups (see below), have several potential pitfalls. Chromosomal regions mapped using sibling pair linkage methods are very broad, so that even where significant linkage regions can be established a considerable amount of additional work is required to identify specific genetic variants associated with altered gene function and risk for ADHD.

A potential problem that needs to be considered is the possibility that genes conferring moderate to large genetic risks (> 5% to 10% QTL effect size) may not exist. It is therefore feasible that all susceptibility genes for ADHD will fall below the level of signal detection for feasible linkage studies. Furthermore, even if a few genes of moderately large effect are identified we should still expect there to be many important influences from multiple genes of small effect, acting either alone or in concert with other genetic and environmental factors. In this scenario, it is unlikely that such genes would be easily detected by linkage and we will have to search for association using targeted candidate genes or potentially (in the future) genome wide scanning methods for association. For example we may envisage a time when it will be feasible to obtain the entire genome sequence for all the individuals within an ADHD study sample. The genes that have already been replicated in several independent association studies of ADHD appear to be of this type, with small estimated odds ratios of 1.4–1.9 for DRD4 [38], 1.2 for DAT1 [24], 1.3 for DRD5 [98] and 1.4 for SNAP-25 [121].

A further potential problem is the existence of allelic heterogeneity, in which a few or multiple rare variants of a gene give rise to susceptibility. In this scenario it would be difficult to detect association following a positive linkage result or to detect association if the genetic effects are small. Detection of association in the presence of allelic heterogeneity represents the "high hanging fruit" that will in many cases require further technological advances to be made. The availability of complete sequence data for individuals within a sample suitable for gene mapping, combined with an increased ability to predict functional sequences may provide a solution, by enabling genes to be identified that show a higher level of functional change (but not of one specific functional variant) in cases versus controls.

Contemporary approaches to association mapping

Association methods are particularly powerful in identifying common genetic variants that confer risk for common complex disorders, the "low-hanging fruit". Currently a great deal of effort is going into the development of methods to detect such genes. The availability of sequence data spanning most of the human genome's 3 _ 109 base pairs has been accompanied by the rapid identification of a class of common poly-morphic variants known as single nucleotide polymorphisms (SNPs) that occur on average once every 500 base pairs [107]. The current version of the publicly available SNP database (dbSNP) contains information on almost 6 million SNPs of which around half are likely to represent true SNPs with minor allele frequencies greater than 10% and suitable for testing the common variant common disorder hypothesis (http://www.ncbi.nlm.nih.gov/SNP/). The holy grail of association mapping is to perform genome-wide association tests although the same methods are equally applicable to the analysis of candidate genes and linkage target regions.

One strategy aimed at reducing the amount of genotyping is to generate population specific linkage disequilibrium (LD or marker-marker association) maps [13, 45, 52, 70]. The idea is that adjacent sets of markers are often organised into groups or blocks, with high levels of LD between markers defining a block. Although there are many potential combinations of marker genotypes across individual chromosomes (known as haplotypes), there is usually limited haplotype diversity with only a few haplotypes occurring at frequencies above

5%. It is therefore suggested that a subset of SNPs that tag common haplotypes describing most genetic diversity will be sufficient to identify disease associated functional variants. This, it is argued, will provide a cost-efficient approach by reducing the amount of genotyping, in comparison to single locus studies. This view led to the launch of a large publicly funded effort to generate genome wide LD maps in different population samples (the HapMap project), which will become a major international resource. The DNA samples for the HapMap will come from a total of 270 people from the Yoruba people in Ibadan, Nigeria, Japanese in Tokyo, Han Chinese in Beijing, and the CEPH trios. The numbers of samples (30–45 for each group) is thought to allow the project to find almost all haplotypes with frequencies of 5% or higher (http://www.hapmap.org). Several issues raised by this approach have yet to be resolved including uncertainty over the marker density required to accurately define haplotypes blocks [140], whether haplotype data generated in one population will generalise to other sample populations, and the most efficient method for genotyping [13]. Even when sets of haplotype tagging SNPs are identified, the need to genotype hundreds of individuals for thousands of markers remains prohibitively expensive for many investigators using currently available methods.

One conclusion that all investigators are however agreed upon is the large number of SNPs that need to be analysed, even to exclude the possibility of association to an individual candidate gene. For this reason many journals will no longer publish negative genetic association papers that report on only one or a few SNP markers. One alternative strategy designed to reduce the amount of genotyping is DNA pooling, in which individual samples of DNA are combined together in pools from which allele frequencies are estimated; this can substantially reduce the cost and feasibility of large scale association studies in the initial screening stages [152]. The power of this approach to detect associations is comparable to the haplotype tagging approach where a high-density map of SNPs is available [69]. The basic idea is that because of the increased efficiency in numbers of genotypes performed (several hundred-fold, compared to 4- to 5-fold with the tagging approach) many more markers can be examined. The adoption of DNA pooling strategies based on comprehensive SNP maps of targeted functional regions is therefore an effective alternative approach.

Some groups have adopted an alternative approach by screening within linkage target regions using high-density maps of another class of polymorphisms, microsatellite or simple sequence repeat markers (SSRs) [111].Two studies from Decode in Iceland have been successful in identifying associations to complex disorders using such an approach, the Neuregulin I gene in schizophrenia [159] and the gene encoding phosphodiesterase 4D in ischaemic stroke [56]. The marker maps used are far less dense than those proposed for systematic screening with SNPs, so these studies suggest that at least in some regions association between SSRs and surrounding markers may spread over relatively wide distances in some populations. The success of this approach in these two studies provides a strong argument for adopting a similar approach to the initial screen of linkage target regions in other common complex genetic disorders.

Progress in gene mapping and the functional genomics of ADHD

There has already been considerable progress in the genetics of ADHD. This has arisen largely by good fortune since, until recently, the entire field of molecular

psychiatry research has focused on a relatively small number of candidate genes that regulate the dopamine and serotonin neurotransmitter systems; however these are the most obvious set of candidates to test in ADHD based on the known response of ADHD symptoms to stimulant medication and other evidence implicating monoamine neurotransmitter pathways. The conclusion from numerous studies carried out using the same set of genetic polymorphisms across multiple psychiatric phenotypes is that ADHD is one of the main disorders associated with genetic variation in several of these genes. The genetic data therefore lend considerable support to the a priori hypotheses. In contrast positional cloning approaches have been less fruitful to date, however unlike current candidate gene methods that focus only on one gene at a time, they have the potential to identify novel genes and molecular mechanisms that have a greater influence on risk for ADHD.

Linkage studies

To date there have been two linkage studies of ADHD both using affected sibling pair methods to carry out genome-wide scans [4, 39, 130]. The first set of papers came from a dataset collected in the greater Los Angeles area ascertained mainly through advertisements requesting participation of families with at least two children showing signs of ADHD. The sample reflected ADHD clinical samples ascertained elsewhere with few individuals with the hyperactive/impulsive subtype (6 %), although there was a greater proportion of the inattentive subtype (40%) compared to the combined subtype (54 %) from that seen in most European samples. Using an initial dataset of 126 affected sibling-pairs and a 10-cM grid of simple sequence repeat markers they were able to exclude any loci conferring a sibling relative risk ratio (λs) of ≥ 3 from 96% of the genome and those with a sibling relative risk ratio of ≥ 2.5 from 91%, indicating that there was unlikely to be any genes of moderate to major effect. LOD scores of ≥ 1.5 indicated a number of possible target regions on chromosomes 5p12, 10q26, 12q23, and 16p13. They also used a QTL approach to the analysis of their data using ADHD symptoms counts, suggesting a possible region of linkage on 12p13 with a maximum LOD of 2.6 [39].

In a follow-up study using an extended dataset of 270 affected sibling pairs from 204 nuclear families the same group found increased evidence for linkage on chromosome 17p11 with a combined maximum LOD score of 2.98 compared to only 0.79 after the initial scan alone [130]. Other loci that did not give rise to significant findings in the initial study (LOD ≤1) but did in the extended sample included 5p13 (combined LOD=1.77), 6q14 (1.75), 11q25 (1.27) and 20q13 (1.19). In contrast the locus on chromosome 16p13 identified in the initial study gave a much smaller LOD score in the new samples (0.5) but retained the highest overall evidence for linkage with a combined LOD of 3.73.

The Dutch genome scan employed 164 sibling pairs recruited from clinical centres and through advertisements to patient organisations [4]. In contrast to the US sample 85 % had the combined subtype diagnosis and 13% the inattentive subtype. In addition they included 26 individuals who met full criteria for DSM-IV ADHD but also met criteria for an autism spectrum disorder and 13 siblings who had only 5 out of the 6 required symptoms in one or both symptom domains. Using the same criteria of possible linkage if the LOD score was ≥ 1.5 they found several regions of interest on chromosomes 4p16.3, 7p13, 9q33.3, 13q33.3 and 15q15.1. In addition they had several additional loci with LOD ≥ 1.0 on

chromosomes 3q13.2, 5p13.1, 6q26 and 10cen. Of particular interest was their most promising finding on 15q with a maximum LOD score of 3.54 when all the sibling pairs were included in the analysis; this region having been previously implicated in similar scans of reading disability and autism.

In drawing conclusions from these two linkage studies the most notable observation is the poor correlation between them with only one region of overlap on 5p13. This suggests that genes of moderate to large effect are unlikely to exist but it remains possible that some of the linkage regions highlighted by these studies my contain genes of more minor effect. In this case the marginal power of linkage to detect such loci would suggest that replication of target regions would only rarely occur. As expected neither study had the power to detect linkage to the genes that have already been identified as associated with ADHD. We also do not know the extent to which the differences are due to clinical or genetic heterogeneity. Differences between the two studies include homogeneity of the populations, the sex ratio and the prevalence of the inattentive subtype of ADHD.

Of potential interest is the overlap in both studies with linkage scans of autism. In the US study the three regions of strongest linkage (5p13, 16p13 and 17p11) have all been highlighted in genome scans of autism [130]; the 16p finding in particular has been highlighted in three independent scans [156]. The Dutch group also found that that the region of highest evidence for linkage on 15q showed some overlap with linkage regions for autism [4], suggesting that genes of moderate effect may influence both conditions. This hypothesis appears unlikely given the relatively large size of the pleiotropic gene effects that would have to be involved, but is supported by the occurrence of both autism spectrum and ADHD symptoms in some individuals. Identification of specific genes that give rise to the linkage findings will clarify this issue.

Reading disability (RD) is another phenotype that frequently co-occurs with ADHD and may share genetic influences. Twin studies estimate high bivariate heritability suggesting that one or more common genetic variants may increase susceptibility to both disorders [49, 160, 178]. Direct evidence for this hypothesis came from a QTL study of the known RD linkage region on chromosome 6p that revealed significant bivariate linkage suggesting the influence of a chromosome 6p locus on both disorders [179]. More recently, the CLA/Oxford group adopted a QTL linkage method to reanalyse their genome scan data from 233 ADHD affected sibling pairs, using a composite index of reading ability [95]. Suggestive linkage to RD was found in four chromosomal regions including regions on 16p and 17q that had previously been implicated in ADHD. In addition they found some evidence for unique RD loci on regions of 2p, 8p and 15q (but not 6p) that coincided with those previously reported in studies of RD.

Association studies

This section describes the most prominent findings in the current literature that have been replicated in three or more datasets. Other candidate gene associations reported in one or two studies or not widely replicated, are not described here but may turn out to be important once further data has accrued. The candidate gene approach has been targeted mainly at the dopamine and serotonin systems to date. A few studies have investigated markers in the norepinephrine transporter gene [7, 113] and some in nicotine receptor genes [78, 79, 171] but more work needs to be done

to either include or exclude association with these genes.

The dopamine D4 receptor gene (DRD4)

The association of ADHD with the 7-repeat allele of a variable number tandem repeat (VNTR) polymorphism in exon 3 of DRD4 (DRD4.7) was first described in 1996 in a small sample of 39 children and 39 ethnically matched controls [87]. The association to the 7-repeat allele had previously been reported to novelty seeking in adults [9, 33] although this finding is far from conclusive and has not been established by follow-up studies and meta-analysis [33, 47, 48, 71–73, 90, 91, 99, 101, 129, 133, 135, 139, 142, 150, 161, 162, 192]. In contrast the DRD4 association with ADHD has been found more consistently despite several negative reports and some discrepancies between case-control and within family studies [17, 37, 38, 61, 67, 81, 119, 125, 136, 146, 148, 155, 163, 165, 166].

A number of studies reported a pattern of findings suggestive of population genetic stratification [67, 119, 148]. Each of these studies found a significant excess of the 7-repeat allele in cases compared to independent controls, however none were able to replicate this finding using within family tests of association. These observations are reflected to some extent in the relative strength of association in the meta-analysis of case-control (OR = 1.9, 95% CI = 1.4–2–2, p = 0.00000008) *vs.* within family tests (OR = 1.4, 95% CI = 1.1–1.6, p = 0.02) performed by Faraone et al. [38]. In an attempt to investigate these differences, Holmes et al. [66] examined the possibility that the 7-repeat allele is more strongly associated with the subgroup of children with ADHD and comorbid conduct problems, a hypothesis supported by family and twin studies suggestive of higher genetic loading for the co-morbid subgroup [161]. Using a collaborative UK dataset recruited from clinics in Manchester, Ireland, Birmingham and London a total of 67 children were identified who fulfilled diagnostic criteria for ADHD and displayed conduct disorder symptoms. In this case, TDT analysis that had previously yielded negative results for the total sample showed evidence of association (24 versus 13 transmissions of the 7-repeat allele, one-tailed P=0.05). Although suggestive of subgroup specificity, the co-morbid group was found to have higher hyperactivity and hyperactive-impulsive scores suggesting that hyperactivity rather than conduct disorder might explain the significant finding.

Another question raised by these findings is whether systematic differences between the samples used for case control and within family samples tests of association could account for the observed differences. In contrast to case control studies that require a series of singleton individuals with ADHD, within family tests usually require the analysis of complete trios consisting of an ADHD proband plus both parents available for DNA sampling. The difference in selection procedures might therefore introduce a bias since the severity of parent or proband phenotype might influence family stability and therefore complete parental ascertainment. When this was examined considerable differences were indeed found. Children from duos with one parent missing (usually father) showed a significantly higher frequency of DSM-IV ADHD-combined type, significantly more co-morbid conduct disorder and conduct disorder symptoms, and a trend for higher total ADHD symptom scores [177]. Exclusion of duos and singletons with no parental DNA available could therefore reduce the power of within family tests of association.

Given the amount of evidence supporting the association between ADHD and DRD4 it is perhaps surprising that the

functional role of this gene in ADHD has yet to be established. Analysis of behavioural paradigms in mice suggests that at a genetic level DRD4 appears to be critical for the behavioural expression of reward seeking behaviours. For example DRD4 (–/–) mice exhibit less novel object exploration than DRD4 (+/+) mice, while the C57 mouse strain shows enhanced novel object exploration when treated with a D4 receptor agonist [32, 143], suggesting that increased D4 receptor sensitivity may be associated with reward seeking behaviours. DRD4 (–/–) mice also show reduced spontaneous locomotion and rearing suggesting an association between novel object exploration and activity level that is mediated at least in part by DRD4 function.

Several authors have attempted to demonstrate functional differences between the various repeat sequences of the DRD4 VNTR using in vitro cellular transfection systems but as yet no firm conclusions can be drawn. Asghari et al. [3] concluded that the polymorphic repeat sequence causes only small changes in the ability of the D4 receptor to block cAMP production, after they demonstrated a 2- to 3-fold lower potency for dopamine mediated coupling to adenyl cyclase for the DRD4 7-repeat compared to the DRD4 2-repeat and DRD4 4-repeat alleles. In a further set of experiments by the same group they further concluded that there were no functional differences between the DRD4 2-repeat and DRD4 10-repeat alleles, strongly suggesting that there is no direct relationship between the length of the repeat polymorphism and changes in pharmacology and receptor function [74]. Finally, an independent group found that there were no quantitative differences in G protein coupling between the DRD4 2-repeat, DRD4 4-repeat and DRD4 7-repeat alleles [76]. Their investigations of D4 receptor mutants showed that the regions adjacent to the VNTR sequence were required for G protein coupling; however deletion of the VNTR sequence itself had no impact on receptor function.

While the direct evidence for a functional role of the DRD4 repeats remains uncertain, considerable additional work has gone into delineating in more detail the genetics of this region. This has served to highlight the complexity of the region and suggests that considerable allelic heterogeneity may be contributing to the association with ADHD. It has been known for some time that DRD4 is one of the most variable human genes due to both repeat polymorphism and sequence variation within the 48 base pair VNTR in exon 3. The level of variation enabled the origins of individual alleles to be tracked by re-sequencing the variable region in 600 alleles derived from individuals in North and South American, European, Asian and African populations [29]. In total 56 distinct haplotypes were found composed of 35 distinct 48 base pair motifs and it was possible to show that the origin of the 2 to 6 repeat alleles can be explained by simple one-step recombination/mutation events. In contrast, the 7-repeat allele is not simply related to the other common alleles and strong linkage disequilibrium between the 7-repeat allele and surrounding DRD4 polymorphisms suggests that this allele is at least 5- to 10-fold younger. It appears therefore that the 7-repeat allele arose as a rare mutational event that increased in frequency due to positive selection. As such, it can be inferred that possession of the 7-repeat allele carries some evolutionary advantage providing further support for a functional role associated with this allele. Finally, the same group went on to re-sequence 250 DRD4 alleles obtained from 132 ADHD probands [53] and found that most of the 7-repeat alleles were the same conserved haplotype found in the previous study. Of particular interest however, was the

observation that half of the 24 haplotypes uncovered in ADHD probands were not found in the original study of a worldwide series of control individuals. Over 10 percent of the ADHD probands had these novel haplotypes, a finding that is unlikely to have occurred by chance (p=0.0001). A major conclusion from these studies is the suggestion that allelic heterogeneity at the DRD4 locus may contribute to the observed association with ADHD.

The strength of linkage disequilibrium between the 7-repeat allele and surrounding regions raises the possibility that the association with ADHD may be due to other nearby polymorphisms. Several investigators have examined the promoter region with this in mind since no other coding region variants have been identified at sufficient frequency to explain the observed association. Okuyama et al. [132] identified a functional promoter SNP (–521 C/T) that reduced transcriptional activity by 40% and reported an association between the C allele of this polymorphism and the personality traits of novelty seeking and impulsivity. Subsequent studies however have failed to replicate this finding with ADHD [6, 120]. Another functional promoter polymorphism, a 120-base pair duplication, has been reported to be associated with ADHD in two independent studies [85, 112], although neither study found an association to the 7-repeat allele. While functional studies have shown a strong effect of this repeat on levels of transcriptional activity [25], other groups have been unable to replicate the association with ADHD including studies from Toronto and London using samples that did show association with the 7-repeat allele [6, 120]. The conclusion from these promoter polymorphism studies is that they do not explain the association between ADHD and the DRD4 7-repeat allele; however the possibility exists that they exert small effects on the risk for ADHD independent of the 7-repeat finding.

The dopamine transporter gene (DAT1)

Association between ADHD and the 10-repeat allele of a 40-base pair variable number tandem repeat polymorphism (VNTR) in the 3'-untranslated region of DAT1 has also been reported in numerous studies and meta-analyses; although the net effect across studies appears to be small with an average odds ratio of around 1.2 [8, 19, 21, 24, 26, 50, 67, 85, 125, 148, 170, 176]. Since the high risk allele occurs on around 70% of Caucasian chromosomes and even higher in some other populations [19], it might be better to conceive of the dopamine transporter polymorphism conferring a protective effect against ADHD. Interestingly, there is significant evidence for heterogeneity across the various datasets [24]. Unpublished work reported by Irwin Waldman on the meta-analyses of available datasets suggests that a subtype specific association to the combined subtype may explain the apparent heterogeneity. This is consistent with his earlier report where he concluded that the DAT1 association was especially strong with the combined but not the inattentive subtype [176].

To date there has been no direct demonstration of a functional role for the DAT1 VNTR although the 10-repeat allele has been shown to be associated with increased levels of messenger RNA (mRNA) in postmortem brain tissue [118]. This suggests that the DAT1

10-repeat allele is associated with an increased production or turnover of the dopamine transporter consistent with the main hypo-dopaminergic hypothesis of ADHD. Some in vivo brain SPECT scan studies of DAT density appear to support this finding, reporting increased striatal DAT density among ADHD probands [30, 31, 82] and among individuals carrying two versus one copy of the 10-repeat

allele [62]. This neat set of findings is however far from established since several other studies have failed to find the reported associations with striatal DAT density or DAT1 genotype and we can not yet conclude that the DAT VNTR is associated with altered regulation of the transporter protein [68, 110, 175].

In vitro studies using transient transfection of cell lines have been equally inconclusive with as yet no clear demonstration of functional differences between the common 9 and 10-repeat alleles [42, 55, 114, 123]. Although the DAT1 VNTR lies within the 3' untranslated region (UTR) of the gene and is therefore at the opposite end of DAT1 from the upstream promoter sequences, 3'UTR sequences are known to be important in the regulation of gene expression. It is apparent that 3'UTR sequences can specifically control the nuclear export, polyadenylation status, sub cellular targeting and rates of translation and degradation of mRNA. The 3'UTR may thus be viewed as a regulatory region that is essential for the appropriate expression of many genes and modifications in 3' UTR mediated functions may affect the expression of one (such as a gene carrying a mutation in its 3' UTR) or more (such as by changes in a *trans*-acting factor affecting the fate of different mRNA molecules) genes [20].

Michelaugh et al. [114] demonstrated that the DAT1 VNTR enhances transcription in mouse-embryonic substantia nigra derived cell lines although their study did not compare the effect of the 10 versus the 9 repeat. Fuke et al. [42] examined the effect of the VNTR polymorphism on gene expression using the luciferase reporter system in human COS-7 cells. They found that reporter gene expression was significantly higher in cells transfected with the 10-repeat allele compared to the 7-repeat or 9-repeat alleles. In contrast, Miller & Madras [123] concluded that the 9-repeat allele was correlated with increased expression in human HEK-293 cells, but that expression was further mediated by a SNP also located in the 3'UTR of DAT1, but not within the VNTR itself. However their findings were not totally consistent between experiments with the 9-repeat actually showing no difference to, or lower expression than, the 10-repeat in several replications. Finally, Greenwood & Kelsoe [55] found no effect on transcription of the 9- and 10-repeat alleles in human SN4741 cells; although they found a 1.5 fold difference in regulatory activity between haplotypes of the promoter/ intron 1 region and enhancer elements within introns 9, 12 and 14.

Unlike the DRD4 repeat polymorphism, the DAT1 VNTR is highly conserved with no internal polymorphism identified from sequence analysis [42, 117]. In the absence of any direct evidence for the functional role of the VNTR sequences the possibility of an alternative functional polymorphism in the vicinity needs to be considered. The linkage disequilibrium studies of Greenwood and Kelsoe [54] suggest that any such functional variant must lie in the distal 3' region including exon 9 through to 15 and the 3'UTR sequences, since the genetic variation spanning the gene shows a distinct two-block pattern of linkage disequilibrium. Further studies are now required to isolate the relevant functional sequences and demonstrate the way in which they alter DAT function.

Much has been made of the increased level of activity observed in mice that lack the dopamine transporter gene. These animals are observed to show marked over activity when first exposed to open fields, associated with an approximately 300-fold increase of dopamine in the synapse [51]. However the over activity is

not spontaneous in the sense that they habituate over time and in the home cage they do not appear to be more overactive than wild-type mice; in contrast to children with ADHD who show reduced activity levels when presented with novel situations and become more active as they habituate to the situation [2]. Furthermore as outlined above current human studies suggest that ADHD is associated with increased DAT1 expression and presumably reduced synaptic dopamine, although this remains uncertain.

A more pertinent and naturalistic model of DAT1 function with greater relevance to the hypothesis of altered gene expression is the analysis of mice with altered levels of DAT expression. In an attempt to model altered DAT1 expression, a hyper-dopaminergic mutant mice model with approximately 70% elevated synaptic dopamine was generated by reducing the expression of DAT to 10% of wild-type levels (DAT knockdown) [181]. Unlike the DAT knockout mice they show no growth retardation; however they are similar with regard to activity levels, which are the same as wild-type mice in the home cage and over active compared to wild type mice in novel environments.

Of further interest is the interaction with response to rewards since the knockdown mice appear to show greater incentive salience ("wanting") to a sweet reward. The knockdown mice learned to complete a runway task for a sweet reward in fewer trials and consistently completed the task more quickly each day during training. The faster completion of the runway task was primarily attributable to their avoidance of delays within the runway that distracted wild-type mice [138]. Extrapolating this to humans, incentive salience can be seen as a motivational component of an individual's response to rewards [10] that may in turn be associated with ADHD. For example several investigators have demonstrated that under rewarded conditions and fast presentation of stimuli children with ADHD may perform as well as control children on a variety of cognitive-experimental tasks [80, 154, 174]. One interpretation is that this reflects changes in the allocation of *energetic resources* (i.e. the effortful maintenance of optimal arousal and activation levels) or state-regulation of individuals with ADHD. It therefore seems reasonable to speculate that links may exist between dopamine regulation, incentive salience and response to rewards, and that these in turn may mediate the genetic influences on task performance for some aspects of the ADHD phenotype.

An alternative opportunity to examine the influence of altered DAT1 expression on mouse behaviour comes from the comparison of mice with one copy of DAT1 (heterozygous mice) versus those with two copies (wildtype mice). However, these studies do not appear to show differences between the two types of mice suggesting that the increased activity in open field and other associated behavioural observations may be restricted to large reductions in dopamine re-uptake from the synapse, which does not mirror the relatively minor expression differences related to the human VNTR polymorphisms. Whether these animals would show differences on more subtle behavioural tests such as response to reward and delay of reward paradigms has yet to be investigated. As with all animal models of human behaviour the interpretation of behavioural observations is complicated and is unlikely to be an exact paradigm for the human condition. Nevertheless the observations seem to provide support for the central role of dopamine regulation in relationship to

several core behavioural and cognitive processes related to ADHD.

Synaptosomal-associated protein 25 (SNAP-25)

Interest in SNAP-25 stemmed directly from the mouse behavioural observation that the Coloboma mouse strain displays spontaneous over activity [63]. Coloboma is a radiation mutant mouse strain with a contiguous gene defect that results in several abnormalities including spontaneous over activity, head-bobbing and ocular dysmorphology. Unlike the over activity seen in the DAT1 knockout and knockdown mice, the Coloboma strain shows a 3-fold increase in activity level in the home cage. Moreover the activity of the Coloboma is highly variable, suggesting loss of control of activity rather than a simple increase in the basal motor activity. Coloboma mice exhibit around 50 % reduction in SNAP-25 mRNA and protein levels and it is interesting therefore that the abnormal activity is reversed using a SNAP-25 transgene to increase expression levels; suggesting a dose dependent relationship between gene level, SNAP-25 expression and behavioural response. The SNAP-25 protein is membrane bound and forms part of a complex that mediates the vesicular release of neurotransmitters. Deficits in the expression of SNAP-25 are therefore expected to lead to reductions in the amount of neurotransmitter released. Although the gene is widely expressed in many synapses it appears that under-expression leads to selective deficits, including dopamine release in the dorsal striatum that regulates motor activity and may also impair the regulation of executive functions processed in the pre-frontal cortex [180].

There is now increasing evidence to suggest that genetic variants within SNAP-25 influence the risk for ADHD. While an early study found no linkage between ADHD and markers in the chromosome 20p11-12 region containing SNAP-25 [64] there are to date no negative association-based studies of SNAP-25 markers and ADHD. Barr et al. [5] were the first to investigate two SNPs that they had identified within the 3'UTR of the human gene and found evidence to suggest a specific haplotype (T-C haplotype) was associated with ADHD in a Canadian sample. This finding was partially supported by Kustanovich et al. [86] using a US Caucasian sample, who found evidence for the same haplotype but only when paternal transmissions were considered. Brophy et al. [11] using an Irish sample investigated the same SNPs and while they found no evidence to support biased transmission of the haplotype nominated by Barr et al., they did find that one of the SNPs (T-allele of second SNP) was individually associated with ADHD as well as evidence for increased transmission of the risk allele from paternal chromosomes. Finally, Mill et al. [121] found no association to the individual SNPs, but found significant association to a different allelic combin-ation of the two-marker haplotype (T-T haplotype), as well as further evidence for paternal transmission from the analysis of additional markers (see below).

The inconsistent pattern of findings complicates the interpretation of these data and raises the possibility of multiple type I errors. The overlapping but disparate results suggest that neither of these two SNPs mediate functional effects on SNAP-25 themselves, but they may be in linkage disequilibrium (associated) with other functional markers in or near to this gene. This hypothesis may seem surprising given the recent emphasis on the use of haplotype blocks to map common disease variants based on the premise that marker-marker relationships are relatively stable across broadly similar ethnic populations. However the stability of haplotype blocks is dependent on many

factors including map density and may be sensitive to the precise population under study. As discussed earlier, an alternative approach is to identify and investigate all genetic variants that may confer a functional role so that analysis is less dependent upon the strength of association between selected genetic markers [69].A more in depth analysis of SNAP-25 has been carried out by Mill et al. [121] who screened the coding, UTR and promoter regions for common polymorphisms and identified 12 genetic variants, eight of which had minor allele frequencies of 5% or more. Association analysis in a UK Caucasian sample found associations with three of these markers including a tetra-nucleotide repeat in the first intron of the gene (**Fig. 1**).

The other interesting observation from three out of the four SNAP-25 studies is that the association with ADHD appears to stem mainly from paternally transmitted alleles, suggesting that genomic imprinting may be an important factor mediating the expression of SNAP-25. Genomic imprinting is an epigenetic mechanism that regulates the expression of many genes and can also have dramatic effects on the phenotypic expression depending on whether the variant allele is derived from the maternal or paternal chromosome. As yet there appears to be no investigation of this phenomenon or examples of imprinted genes for the SNAP-25 region in either mouse or human. Further work is required to test directly the hypothesis that genomic imprinting is acting on genes within chromosome 20p11-12, but the fact that three independent groups each find that ADHD is more strongly associated with paternally derived SNAP-25 alleles increases the overall confidence in this interesting finding.

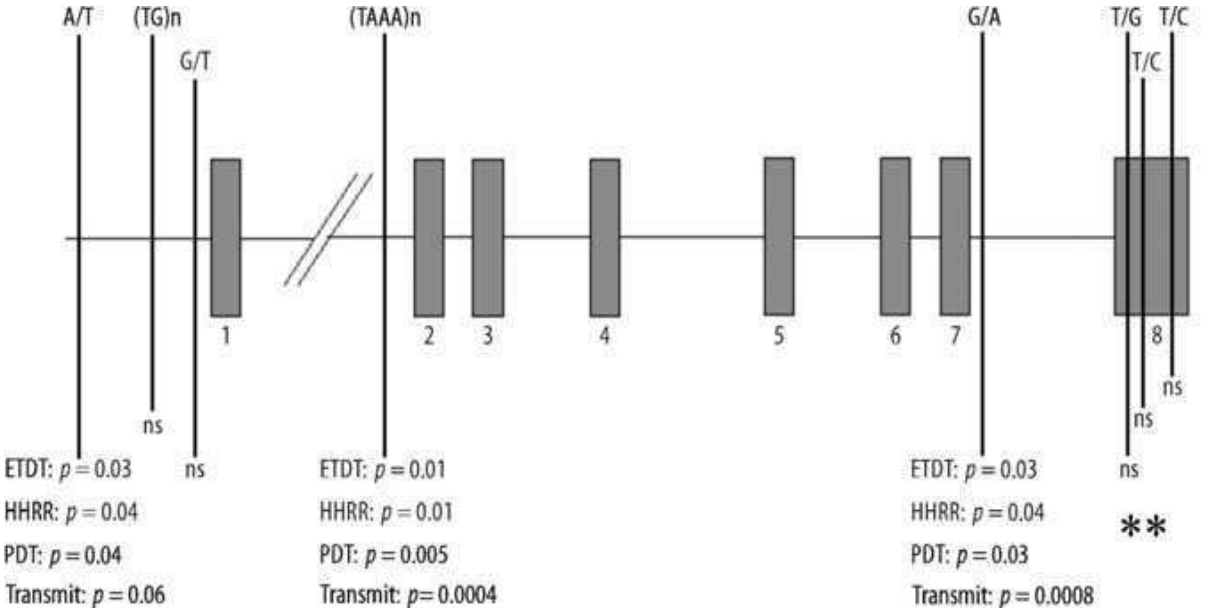

Fig. 1 Global significance values for eight common polymorphisms across SNAP-25 using four different methods for within family association analysis. * Indicate the two SNPs first described by Barr et al. [5]. Adapted from Mill et al. [121]

The dopamine D5 receptor gene (DRD5)

DRD5 is an intron-less gene that shows high structural and functional homology to the dopamine D1 receptor gene. Genetic investigation of DRD5 is complicated by the existence of two pseudogenes present on different chromosomes that have around 98% homology to DRD5 but are thought to have no functional expression [127].Association studies of this gene have focused on a dinucleotide $(CT/GT/GA)_n$ polymorphism [153] that lies within a non-duplicated region around 19,000 base pairs from the start of transcription. Following an initial report of association between the common allele of this polymorphism and ADHD [26] numerous other groups have reported the same significant association or a non-significant trend of association to the same allele. These data have been collated and a formal meta-analysis of 14 independent studies performed that shows a combined association of the DRD5 locus with no evidence of heterogeneity (p=0.00005; OR=1.24, 95% CI 1.12–1.38) [94]. Analysis of ADHD subtypes found similar strength of the association to the DSM-IV combined (CT) and inattentive (I) subtypes but not to the hyperactive impulsive (HI) subtypes; a finding that is in keeping with a twin study that reported cross concordance between the CT and I subtypes but not between these and the HI subtype [173].

Although functional studies of the $(CT/GT/GA)_n$ marker have not been performed, its location at some distance from the coding region of DRD5 suggests it is unlikely to be involved in gene expression and does not alter DRD5 protein structure, receptor binding, or signalling. Attempts to delineate the associated region further using simple sequence repeat markers have been unsuccessful. For example three markers covering a region of ca. 68 kb including the single DRD5 exon were all associated with ADHD, and thus did not provide further localising information [60]. Furthermore, there are as yet no published investigations using putative functional markers apart from [115] who found no association to a $(TC)_n$ repeat marker in the promoter region. Within the coding region five sequence changes have been identified that predict protein alterations, including a 'missense' change that would lead to a prematurely truncated protein and result in a 50 % loss of functional D5 receptor and a non-synonymous SNP (amino acid substitution) that results in an approximately 10-fold decrease in dopamine binding [22, 157]. Although rare and therefore unlikely to explain the observed association with DRD5, these have yet to be investigated in ADHD.

There have as yet been few functional genomic studies of DRD5. An initial report on mice with null mutations (D5–/–) found that under baseline conditions D5–/– mice were generally normal on locomotor activity and several tests of cognitive function, although pharmacological activation of dopamine pathways led to some altered responses relevant to exploratory locomotion, startle, and pre-pulse inhibition [65]. As with mouse models of other genes associated with ADHD, studies investigating more subtle and complex paradigms that better reflect the ADHD phenotype would be interesting.

Serotonin system genes –5HT1B and SERT

In addition to the focus on dopamine system genes there have been a few studies focusing on genes that regulate the serotonin neurotransmitter system. Interaction between the two neurotrans-mitter systems is well recognised and was neatly demonstrated in the paradoxical calming effects of stimulants on the DAT1 knockout mouse through the effects of stimulants on serotonergic transmission [46]. Serotonin regulates dopaminergic

neurotransmission in several areas of the brain via several serotonin receptors including 5-HT1B. Furthermore, animal studies have suggested the involvement of the 5-HT1B receptors in locomotor behaviour [46, 108]. Reduced serotonin activity is related to increased aggression and poor impulse control in both human and animal studies and there is considerable additional evidence implicating its key role in both anxiety and depression [12, 16, 46, 57, 92, 93, 100].

Several investigators have examined polymorphisms in the serotonin transporter gene (SERT) for associations with ADHD. Of particular interest is an insertion/deletion polymorphism in the promoter region and a VNTR within intron 2, both of which appear to have functional effects on SERT expression [97, 124]. Kent et al. [77] investigated both of these polymorphisms and in keeping with two earlier studies [151, 193] found increased transmission of the long allele of the promoter polymorphism from parents to their ADHD offspring, which was significant in a combined analysis of the three datasets (p = 0.008). A Turkish study using a case-control design reported a similar association with a lower frequency of the short homozygous genotype among their ADHD cases compared to controls (p = 0.02) [182].However, in another study of 150 ADHD probands Langley et al. [89] found no evidence for the association with either of the SERT polymorphisms alone or combined as a haplotype. The evidence for association with the intron 2 VNTR is particularly inconsistent, suggesting that this polymorphism is unlikely to have a direct influence on risk for ADHD.

The 5HT1B receptor has also shown evidence of association with ADHD in several datasets. Two studies published at around the same time reported association to the same SNP allele. The first study from Hawi et al. [59] combined datasets from four independent groups in the UK and Ireland and found preferential transmission of a SNP polymorphism (allele 861G; P=0.01). It was therefore of considerable interest that the only other study to look at this particular SNP using a sample of ADHD probands from Canada reported a similar result (p=0.09) [147]. Combining the two studies enhanced the finding (p=0.001) suggesting the possible involvement of this locus in ADHD susceptibility.

Despite the obvious interest in both the SERT and 5HT1B findings, these findings remain uncertain until they have been investigated more widely.

QTL association studies

As discussed above, it has been widely postulated that the categorical diagnosis of ADHD should be seen as the extreme end of a set of traits quantitatively distributed in the general population. A consequence of this is that the genes associated with clinical ADHD should also influence these underlying traits in non-affected individuals. The notion that heritability is constant across the trait distribution has interesting consequences in terms of finding genes that influence levels of hyperactivity. A good test of this hypothesis would be to examine whether specific genetic risk factors, known to have a role in clinical ADHD, correlate with continuous measures of ADHD symptoms in the general population. To date, however, there have been only a few studies investigating this hypothesis directly, or using population samples to contrast individuals who score high and low on ADHD symptom counts. These studies have been largely negative with some inconsistent evidence for the QTL association of the DAT1 10-repeat allele and a possible protective effect of the SNAP-25 microsatellite allele 2. An initial report of association with the DRD4 7-repeat allele using selected individuals

from a population sample [23] was not confirmed when the dataset was further extended (Curran et al., personal communication), and there have been two other negative studies using population samples [122, 172].

Waldman et al. [176] were the first to suggest that DAT1 acts as a QTL when they observed that siblings discordant for the number of DAT1 10-repeat alleles differed markedly in their levels of hyperactive-impulsive and inattentive symptoms, such that the sibling with the higher number of risk alleles had a higher level of symptoms. These data are however inconclusive because they used a selected sample in which one sibling was an ADHD proband, so that a simple association with ADHD diagnosis would predict the pattern of findings they observed (i. e. the sibling who did not have ADHD would be expected to have lower numbers of risk alleles). On the other hand they did find evidence for the QTL association by regressing ADHD symptom scores onto the number of DAT1 risk alleles ($p=0.032$ for hyperactive-impulsive symptoms; $p = 0.116$ for inattentive symptoms).

In another study that used a population sample of 329 male dizygotic twin pairs unselected for phenotype, Mill et al. [116] looked for QTL association with several ADHD risk-alleles (DAT1, DRD4, DRD5, SNAP-25, 5HT1B). Parent rated ADHD-symptom scores had been gathered at ages 2, 3, 4 and 7 years in addition to teacher ratings at age 7-years and combined in various ways to generate a series of composite scores. A major advantage of using sibling pairs is that family-based association methods can compare the between and within pair variation of the trait, using correlations of the sums and differences of the trait value with the sums and differences in the number of the postulated *a priori* risk/protective alleles for the two siblings in a pair [1]. Using this approach they found some evidence for the QTL association with the 10-repeat allele ($p < 0.01$) and the protective effect of the SNAP-25 microsatellite-allele-2 ($p < 0.05$). An interesting observation from these data was that the within pair differences analyses, which are exempt from stratification effects, generally showed the highest degree of association, whereas the between pair analyses were often negative, suggesting that stratification effects could be hiding some positive effects. In this particular sample the stratification were more likely to be phenotypic rather than genetic and could have resulted from the fact that, at least for parent ratings, it is easier to rate one child against another within a family than compare a child to some pre-calculated childhood norm.

Two additional studies, using population samples, compared allele frequency differences between individuals with high and low scores on continuous measures of ADHD symptoms. Although one of the studies found a trend in favour of the DAT1 association in a small sample of 50 high and 42 low scoring individuals [137], the other larger study failed to find any evidence for the association (Curran et al., personal communication). Finally, Todd et al. [170] used 100 randomly selected families and 413 families in which at least one twin passed an initial screening interview for ADHD; of these, 219 had a full DSM-IV diagnosis. Using conventional within family tests of association to analyse both latent class criteria and DSM-IV ADHD subtypes they failed to find any significant association or trend for association with the 10-repeat allele.

Attempts to replicate the association reported with clinical ADHD in population samples or using QTL methods have therefore failed for most genes and been

inconclusive for others, although several of the studies described above remain relatively underpowered for this type of analysis. This raises the possibility that the phenotypic measures being used to define quantitative ratings of ADHD symptoms in general population samples may not accurately reflect an underlying distribution of genetic liability. One example that relates to this issue is the use of parent or teacher ratings that correlate only to a modest degree, around 0.3 in most studies. Twin analyses using ADHD items from the Conners' scales indicated that only 31% of the variance in teacher and parent ratings of ADHD symptoms were due to genetic effects common to both ratings, whereas 41% of the variance in parent ratings and 50% of the variance in teacher ratings were due to additional genetic effects that were unique to each [109]. Therefore the ratings by parents and teachers reflect only partially overlapping phenotypes and genotypes. This finding is further supported by the observation that performance on cognitive-experimental tasks associated with ADHD correlate strongly with teacher ratings of ADHD symptoms in a general population sample, but not parent ratings [83]. Oosterlaan et al. [134] also found that only teacher ratings of ADHD symptoms predicted performance on cognitive tasks that were sensitive to ADHD with parent ratings not contributing to the association, in this case using a clinical sample with research diagnoses of pervasive ADHD and control children. Whether other types of continuous variables, such as cognitive-experimental *endophenotypes*, will provide a better representation of underlying genetic liability and relate more strongly to ADHD genetic risk alleles is considered in the next section.

The genetic analysis of cognitive endophenotypes

In addition to molecular analysis of behavioural phenotypes there is a growing interest in the analysis of cognitive endophenotypes [18]. For example, there have been several papers reporting on the association between the DRD4 repeat polymorphism and neurocognitive performance in children with ADHD and controls. By focusing on tasks that are known to be associated with clinical ADHD or have theoretical links with the disorder, these studies hope to provide direct evidence for the influence of genetic variation on underlying cognitive phenotypes that may mediate the genetic risk on the behavioural phenotype.

Identification of suitable measures for this type of approach remains a matter of some considerable debate. One approach is to use classical family and twin study designs to identify cognitive-experimental measures that show a familial relationship. Family studies, like the IMAGE sample that consists of ADHD probands plus their unaffected siblings, can be supplemented with control data to estimate shared familial influences. Although this would establish the familial link between the two sets of measures (behavioural and cognitive-endophenotype), it remains feasible that this could arise from non-genetic influences acting on pairs of siblings. Twin studies that adopt multivariate approaches can partial the familial influences into shared genetic and environmental factors and establish more precisely the degree to which shared genetic influences are acting on both sets of measures. To date there have been only a few studies in this area and these have been greatly underpowered, so that only very tentative conclusions can be drawn at this time.

Kuntsi et al. [84] used a twin design to investigate whether the same genes that influence ADHD-symptom scores also affected performance on tasks that

discriminated between ADHD and control children. The only variable for which they obtained evidence of shared genetic effects was the variability in the speed of responding on a reaction time task, suggesting that a state regulation problem could be the psychological process that mediates the genetic effects on hyperactivity. Another study investigated biological and adoptive parents of ADHD probands [128]. Biological parents of ADHD boys (n=16) were found to have slower reaction times to un-cued left visual field targets than to right visual field targets and slower response to invalidity cued targets in the right visual field; a finding that they had also observed in children with ADHD compared to controls. These lateral effects were not observed in adoptive parents of ADHD boys (n = 12) or biological parents of comparison boys (n = 14), suggesting a possible link between ADHD and abnormal hemispheric asymmetry mediated by genetic factors. Other family and twin studies of cognitive-experimental endophenotypes are currently underway and these studies should provide critical information on some of the psychological processes that mediate the genetic effects on behaviour.

The first publication to look directly at the influence of ADHD risk alleles on cognitive-endophenotypes in relation to ADHD, investigated children with ADHD grouped on the presence of one or two copies of the DRD4 7-repeat allele (n = 13) versus absence of the 7-repeat allele (N = 19) plus a control group (n = 21) [164]. Using neuropsychological tests with reaction time measures designed to probe attentional networks, they found surprisingly that although reaction times in the 7-repeat absent group were slow and variable, the 7-repeat present group showed normal speed and accuracy compared to controls opposite to primary predictions for the study. However, the very small sample sizes meant that only a cautious conclusion could be made.

A further study investigating 200 adults with ADHD, used a battery of tasks designed to measure separately three anatomically defined attentional networks relating to alerting, orientating and executive control, the Attention Network Test (ANT). Although robust evidence for the strength of genetic influences on the measures used is lacking, a pilot study of 26 MZ and 26 DZ adult twin pairs suggested that genetic variation contributes to normal individual differences in higher order executive attention [35]. In the molecular genetic analysis, the DRD4 7-repeat allele was found to have no influence on the measures used. There was however modest association with the 4-repeat allele and a functional promoter SNP (–521) with reduced executive efficiency [41].A potentially interesting conclusion from their analysis was that all the genetic alleles they investigated that were predicted to lead to higher levels of extra-synaptic dopamine or dopamine signal transduction, showed less efficient executive attention scores – this included the DAT1 10-repeat allele and functional polymorphisms in Monoamine Oxidase A (MAOA) and Catecholamine-O-methyl transferase (COMT).

Another study of 178 ADHD probands from Israel also failed to find association between the DRD4 7-repeat allele and cognitive-experimental measures from the Test Of Variables of Attention (TOVA). However they did report a positive association between the 4-repeat allele and errors of commission [104].Cognitive deficits associated with the 7-repeat allele in ADHD probands have however been reported by one group. Langley et al. [88] recently reported on the investigation of 133 drug naive children with ADHD aged 6–13 years using several tests known to be

associated with attention, impulse control and response inhibition; the continuous performance task, matching familiar figures test, go/no go task and stop task. In contrast to the findings from the earlier two studies, they found that DRD4 7-repeat was positively associated with an inaccurate and impulsive style of responding, as well as increased activity levels measured by actigraphs that is not explained by ADHD symptom severity. Although interesting, some discrepancies remain to be explained. For example, on the go/no go task the ADHD 7-repeat group had particularly slow mean reaction times, whereas on the stop task the same group was particularly fast compared to both the ADHD non-7-repeat group and controls.

There have also been a few studies investigating correlations between the DAT1 10-repeat allele and neurocognitive performance. As described above the study of Fossella et al. [41] found only minor effects with modestly lower executive function scores for the rare 9/9 homozygous genotype compared to the pooled score for the more common 9/10 and 10/10 genotype group. In another study from the United States significant association was reported between the 10/10 genotype and poor performance on a sustained attention [96]. This finding was not however supported in a study of 44 children with ADHD from Korea using the TOVA where the 10-repeat allele was associated with less commission errors and had no effects on response time and variability [131]. In another study [96] the authors reported that within their sample of ADHD probands the 10/10 genotype was associated with the combined subtype of ADHD and an increase in neuronal activity in response to treatment with methylphenidate, measured by the ratio of theta to beta waves on electroencephalography (EEG). This last finding may reflect differing neuronal activation states consistent with the state regulation model, described above.

Whether these various studies can be reconciled remains unclear. The first set of studies suggests that the DRD4 7-repeat allele is not associated with a loss of attentional efficiency but may be associated with other dimensions that underlie the development of ADHD. On the other hand the findings from Langley et al. appear to contradict this and are more consistent with the reported association between ADHD and the DRD4 7-repeat allele, their main a priori hypothesis. Similar discrepancies appear for the DAT1 VNTR polymorphism. One message is that extreme caution must be introduced in the interpretation of these data. The odds of true association need to be adjusted in some way to take into account the prior probability of association, which will be lower for associations that are not consistent with prior hypotheses based on theoretical considerations. A second important message is that the use of endophenotype measures will not necessarily be the *phenotypic panacea* that some investigators hope for. While it is pertinent to search for the cognitive mechanisms that underlie ADHD, we cannot be certain that cognitive experimental measures will necessarily provide an improved way to find the genes involved; by parsing clinical and genetic heterogeneity into more homogenous compartments with larger, more easily detectable, gene effects or providing more accurate measures of underlying genetic liability.

One issue that needs to be addressed is the reliability and consistency of different neurocognitive measures both within and between studies. For example many groups use slightly different formats and protocols for similar tasks and reliability is not always formally tested. Factors such as

the sensitivity of measures to rewards and the presentation rate of stimuli need to be considered when making comparisons between different studies. A more interesting theoretical issue is whether single or multiple independent genetic pathways can explain the various associations between performance on cognitive-experimental measures and ADHD. Although independent neural networks and cognitive processes that relate to different aspects of task performance can be identified, these are often correlated within general population samples. Furthermore, genetic influences can combine together to influence several different neuronal networks and cognitive processes. For this reason we might expect there to be only one or a few common genetic pathways that influence a range of correlated cognitive outcomes. This type of common pathway model is similar to that proposed for "g", the most heritable phenotype for general cognitive ability derived from factor analysis of multiple aspects of cognition [141]. Although this model has yet to be formally tested in ADHD, it remains feasible and would imply that the best phenotype to map the genes involved would be derived by combining multiple correlated cognitive measures together, reducing the effects of measurement error and forming a heritable multivariate index. Such a composite index would be predicted to be more strongly associated with specific gene variants than any single test alone. Following a similar line of argument, it may be the multivariate nature of the ADHD behavioural phenotype that has led to the relative success in identifying ADHD associated genes to date.

Model fitting approaches using family and twin studies can help to unravel the various processes involved by examining the genetic and environmental contributions to covariance between behavioural ratings and experimental task variables, as well as estimating the level of shared genetic influences between different cognitive experimental measures. Multivariate analyses can be used to compare multiple independent versus single common pathway models to investigate the extent to which the different cognitive experimental measures can be used alone or in combination to provide one or more heritable common factors. The extension of DF analysis to examine the association of group membership, for example DSM-IV ADHD combined type, to cognitive performance in co-siblings can also be used to investigate whether there are shared familial/genetic influences on both the clinical group and task performance. In combination with molecular genetic data, these types of approaches can provide an empirical basis for the use of endophenotypes in the search for ADHD susceptibility genes, as well as providing a functional understanding of specific genetic variants.

Gene-environment interaction

Despite considerable progress in discovering genetic risk factors for ADHD, even the strongest associations have not been ubiquitously replicated and definitive 'ADHD genes' have yet to be discovered. As discussed above, this problem is usually explained by concluding that psychiatric disorders such as ADHD are caused by a large number of genes, each with a very small effect size. To routinely detect such small effects, it is argued, extremely large samples will be needed. Another possibility, however, comes from the fact that the heritability coefficient indexes not only the direct effects of genes but also the effects of interactions between genes and environments, where genes confer sensitivity or susceptibility to specific risk environments [141, 149]. Gene x environment interaction (G x E) may provide one possible explanation for the

discrepancies seen between clinical and population studies where clinical ADHD samples have been much more successful in detecting associations than population-based samples [116]. It is possible, for example, that specific environmental factors that are over-represented in clinical samples may play an important role via gene-environment interactions.

The importance of gene-environment interactions in behavioural disorders has been highlighted by two recent studies using the Dunedin Multidisciplinary Health and Development Study, where functional polymorphisms in candidate genes have been shown to have an effect only in groups subjected to specific environmental stressors. The first study describing MAOA as a moderating influence on the effects of maltreatment on antisocial behaviour [15, 141] and the second describing the serotonin transporter gene (5-HTT) as a moderator of stressful life events on depression [16]. Importantly, neither of the two genes investigated showed main effects with the behavioural phenotypes, so that the genetic associations would have been missed entirely if the environmental risk factors had not been taken into account. Similar types of interactions are likely to be relevant to the development of ADHD and may be particularly useful for prediction of high-risk groups and targeting of therapeutic interventions.

To date there have been few G x E studies in relation to ADHD. A recent example, however, was the report of 161 children followed from the age of 6 months up to 5-years of age. Child hyperactivity-impulsivity and oppositional behaviour was found to be associated with the DAT1 10-repeat allele, but only when the child was also exposed to maternal prenatal smoking [75]. Further studies in this area are needed with the focus on environmental risks already known to be associated with ADHD including low birth weight, in utero alcohol and nicotine exposure and expressed emotion [158, 167]. More sophisticated genetic designs will be required to unravel the direct influences of identified risk factors from passive environmental correlations: For example, distinguishing the toxic effects of smoking during pregnancy, from the association between maternal smoking, maternal ADHD and shared genes between mother and offspring.

Concluding remarks

ADHD is a complex disorder and we must adopt research strategies that can best embrace such complexity. It will be important to draw various approaches and experimental paradigms together. The functional genomic approach to ADHD means that gene function is understood at various levels of analysis, not only at the level of molecular and cellular function but also at the level of psychological processes, neuronal networks, environmental interactions and behavioural outcomes. Human studies will need to be complemented by animal models of ADHD and more sophisticated animal behavioural paradigms that better reflect the human condition need to be developed, especially in mouse which is the main functional genomic tool. The investigation of cognitive endophenotypes will be central to this process as it is likely to identify genetically determined cognitive experimental measures that can be more easily modelled in animal behaviour. Neuroscience will increasingly focus on the use of genetically modified animals to investigate the effects of genetic variation and environmental interaction on the development of behaviour. Experimental protocols are currently being established that will enable us to investigate both molecular and behavioural expression of genetic

constructs in vivo, so that we can follow these processes throughout development. Taken together these multiple approaches hold the promise of developing a far more detailed knowledge of the developmental origins of ADHD with increased benefits for future generations.

Acknowledgements Philip Asherson is currently supported by grants from National Institute of Mental health (NIMH), the Wellcome Trust and the UK Medical Research Council for research on genetic aspects of ADHD. The IMAGE Consortium is supported with a grant from NIMH.

References

1. Abecasis GR, Cardon LR, Cookson WO (2000) A general test of association for quantitative traits in nuclear families. Am J Hum Genet 66:279–292
2. Antrop I, Roeyers H, Van Oost P, Buysse A (2000) Stimulation seeking and hyperactivity in children with ADHD. Attention Deficit Hyperactivity Disorder. J Child Psychol Psychiatry Allied Disciplines 41:225–231
3. Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH (1995) Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochemistry 65: 1157–1165
4. Bakker SC, van der Meulen EM, Buitelaar JK, Sandkuijl LA, Pauls DL, Monsuur AJ, van't Slot R, Minderaa RB, Gunning WB, Pearson PL, Sinke RJ (2003) A whole-genome scan in 164 Dutch sib pairs with attention-deficit/ hyperactivity disorder: suggestive evidence for linkage on chromosomes 7p and 15q.Am J Hum Genet 72:1251–1260
5. Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, Schachar R, Tannock R, Kennedy JL (2000) Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. Mol Psychiatry 5: 405–409
6. Barr CL, Feng Y, Wigg KG, Schachar R, Tannock R, Roberts W, Malone M, Kennedy JL (2001a) 5'-untranslated region of the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Am J Med Genet 105:84–90
7. Barr CL, Kroft J, Feng Y, Wigg K, Roberts W, Malone M, Ickowicz A, Schachar R, Tannock R, Kennedy JL (2002) The norepinephrine transporter gene and attention-deficit hyperactivity disorder. Am J Med Genet 114:255–259
8. Barr CL, Xu C, Kroft J, Feng Y, Wigg K, Zai G, Tannock R, Schachar R, Malone M, Roberts W, Nothen MM, Grunhage F, Vandenbergh DJ, Uhl G, Sunohara G, King N, Kennedy JL (2001b) Haplotype study of three polymorphisms at the dopamine transporter locus confirm linkage to attention-deficit/hyper-activity disorder. Biol Psychiatry 49:333–339
9. Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH (1996) Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nat Genet 12:81–84
10. Berridge KC, Robinson TE (2003) Parsing reward. Trends in Neuroscience 26: 507–513
11. Brophy K, Hawi Z, Kirley A, Fitzgerald M, Gill M (2002) Synaptosomal-associated protein 25 (SNAP-25) and attention deficit hyperactivity disorder (ADHD): evidence of linkage and association in the Irish population. Mol Psychiatry 7:913–917
12. Cadoret RJ, Langbehn D, Caspers K, Troughton EP, Yucuis R, Sandhu HK, Philibert R (2003) Associations of the serotonin transporter promoter polymorphism with aggressivity,

attention deficit, and conduct disorder in an adoptee population. Compr Psychiatry 44:88–101
13. Cardon LR, Abecasis GR (2003) Using haplotype blocks to map human complex trait loci. Trends Genet 19: 135–140
14. Cardon LR, Smith SD, Fulker DW, Kimberling WJ, Pennington BF, DeFries JC (1994) Quantitative trait locus for reading disability on chromosome 6. Science 266:276–279
15. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R (2002) Role of genotype in the cycle of violence in maltreated children. Science 297:851–854
16. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R (2003) Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301: 386–389
17. Castellanos FX, Lau E, Tayebi N, Lee P, Long RE, Giedd JN, Sharp W, Marsh WL, Walter JM, Hamburger SD, Ginns EI, Rapoport JL, Sidransky E (1998) Lack of an association between a dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. Mol Psychiatry 3:431–434
18. Castellanos FX, Tannock R (2002) Neuroscience of attention-deficit hyperactivity disorder: the search for endophenotypes. Nat Rev Neurosci 3:617–628
19. Chen CK, Chen SL, Mill J, Huang YS, Lin SK, Curran S, Purcell S, Sham P, Asherson P (2003) The dopamine transporter gene is associated with attention deficit hyperactivity disorder in a Taiwanese sample. Mol Psychiatry 8:393–396
20. Conne B, Stutz A, Vassalli JD (2000) The 3' untranslated region of messenger RNA: A molecular 'hotspot' for pathology? Nat Med 6:637–641
21. Cook EH, Stein MA, Krasowski MD, Cox NJ, Olkon DM, Kieffer JE, Leventhal BL (1995) Association of attention deficit disorder and the dopamine transporter gene. Am J Hum Genet 56: 993–998
22. Cravchik A, Gejman PV (1999) Functional analysis of the human D5 dopamine receptor missense and non-sense variants: differences in dopamine binding affinities. Pharmacogenetics 9: 199–206
23. Curran S, Mill J, Sham P, Rijsdijk F, Marusic K, Taylor E, Asherson P (2001a) QTL association analysis of the DRD4 exon 3 VNTR polymorphism in a population sample of children screened with a parent rating scale for ADHD symptoms. Am J Med Genet 105: 387–393
24. Curran S, Mill J, Tahir E, Kent L, Richards S, Gould A, Huckett L, Sharp J, Batten C, Fernando S, Ozbay F, Yazgan Y, Simonoff E, Thompson M, Taylor E, Asherson P (2001b) Association study of a dopamine transporter polymorphism and attention deficit hyperactivity disorder in UK and Turkish samples. Mol Psychiatry 6:425–428
25. D'Souza, Russ C, Tahir E, Browes C, Mill J, McGuffin P, Asherson P, Craig IW (2002) Functional effects of a tandem duplication polymorphism in the 5'flanking region of the DRD4 gene. Am J Med Genet (114):P349
26. Daly G, Hawi Z, Fitzgerald M, Gill M (1999) Mapping susceptibility loci in attention deficit hyperactivity disorder: preferential transmission of parental alleles at DAT1, DBH and DRD5 to affected children. Mol Psychiatry 4: 192–196
27. DeFries JC, Fulker DW (1985) Multiple regression analysis of twin data. Behav Genet 15:467–473 28. DeFries JC, Fulker DW (1988) Multiple regression analysis of twin data: etiology of deviant scores versus individual differences. Acta Genetica Medica

Gemellologica (Roma.) 37:205–216
29. Ding YC, Chi HC, Grady DL, Morishima A, Kidd JR, Kidd KK, Flodman P, Spence MA, Schuck S, Swanson JM, Zhang YP, Moyzis RK (2002) Evidence of positive selection acting at the human dopamine receptor D4 gene locus. Proc Natl Acad Sci USA 99:309–314
30. Dougherty DD, Bonab AA, Spencer TJ, Rauch SL, Madras BK, Fischman AJ (1999) Dopamine transporter density in patients with attention deficit hyperactivity disorder. Lancet 354: 2132–2133
31. Dresel S, Krause J, Krause KH, LaFougere C, Brinkbaumer K, Kung HF, Hahn K, Tatsch K (2000) Attention deficit hyperactivity disorder: binding of [99mTc]TRODAT-1 to the dopamine transporter before and after methyl-phenidate treatment. Eur J Nuclear Med 27:1518–1524
32. Dulawa SC, Grandy DK, Low MJ, Paulus MP, Geyer MA (1999) Dopamine D4 receptor-knock-out mice exhibit reduced exploration of novel stimuli. J Neurosci 19:9550–9556
33. Ebstein RP, Belmaker RH (1997) Saga of an adventure gene: novelty seeking, substance abuse and the dopamine D4 receptor (D4DR) exon III repeat polymorphism. Mol Psychiatry 2:381–384
34. Ebstein RP, Segman R, Benjamin J, Osher Y, Nemanov L, Belmaker RH (1997) 5-HT2C (HTR2C) serotonin receptor gene polymorphism associated with the human personality trait of reward dependence: interaction with dopamine D4 receptor (D4DR) and dopamine D3 receptor (D3DR) poly-morphisms. Am J Med Genet 74:65–72
35. Fan J, Wu Y, Fossella JA, Posner MI (2001) Assessing the heritability of attentional networks. BMC Neurosci 2:14
36. Faraone SV, Biederman J, Monuteaux MC (2000) Toward guidelines for pedigree selection in genetic studies of attention deficit hyperactivity disorder. Genet Epidemiol 18:1–16
37. Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, Spencer TJ, Wilens TE, Frazier J, Cleves M, Sakai J (1999) Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 156:768–770
38. Faraone SV, Doyle AE, Mick E, Biederman J (2001) Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. Am J Psychiatry 158:1052–1057
39. Fisher SE, Francks C, McCracken JT, McGough JJ, Marlow AJ, MacPhie IL, Newbury DF, Crawford LR, Palmer CG, Woodward JA, Del'Homme M, Cantwell DP, Nelson SF, Monaco AP, Smalley SL (2002) A genome wide scan for loci involved in attention-deficit/hyper-activity disorder. Am J Hum Genet 70: 1183–1196
40. Fisher SE, Marlow AJ, Lamb J, Maestrini E, Williams DF, Richardson AJ, Weeks DE, Stein JF, Monaco AP (1999) A quantitative-trait locus on chromo-some 6p influences different aspects of developmental dyslexia. Am J Hum Genet 64: 146–156
41. Fossella J, Sommer T, Fan J, Wu Y, Swanson JM, Pfaff DW, Posner MI (2002) Assessing the molecular genetics of attention networks. BMC Neurosci 3:14
42. Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. The Pharmacogenomics Journal 1:152–156
43. Fulker DW, Cardon LR DeFries JC Kimberling WJ Pennington BF Smith SD (1991) Multiple regression analysis of sib-pair data on reading to detect quantitative trait loci. Reading and

Writing. An Interdisciplinary Journal 1991:299–313
44. Fulker DW, Cherny SS, Cardon LR (1995) Multipoint interval mapping of quantitative trait loci, using sib pairs. Am J Hum Genet 56:1224–1233
45. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D (2002) The structure of haplotype blocks in the human genome. Science 296:2225–2229
46. Gainetdinov RR, Wetsel WC, Jones SR, Levin ED, Jaber M, Caron MG (1999) Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. Science 283:397–401
47. Gebhardt C, Leisch F, Schussler P, Fuchs K, Stompe T, Sieghart W, Hornik K, Kasper S, Aschauer HN (2000) Non-association of dopamine D4 and D2 receptor genes with personality in healthy individuals. Psychiatr Genet 10:131–137
48. Gelernter J, Kranzler H, Coccaro E, Siever L, New A, Mulgrew CL (1997) D4 dopamine-receptor (DRD4) alleles and novelty seeking in substance dependent, personality-disorder, and control subjects. Am J Hum Genet 61: 1144–1152
49. Gilger JW, Pennington BF, DeFries JC (1992) A twin study of the etiology of comorbidity: attention-deficit hyperact-ivity disorder and dyslexia. J Am Acad Child Adolesc Psychiatry 31:343–348
50. Gill M, Daly G, Heron S, Hawi Z, Fitzgerald M (1997) Confirmation of association between attention deficit hyperactivity disorder and a dopamine transporter polymorphism. Mol Psychiatry 2:311–313
51. Giros B, Jaber M, Jones SR, Wightman RM, Caron MG (1996) Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 379: 606–612
52. Goldstein DB (2001) Islands of linkage disequilibrium. Nat Genet 29:109–111
53. Grady DL, Chi HC, Ding YC, Smith M, Wang E, Schuck S, Flodman P, Spence MA, Swanson JM, Moyzis RK (2003) High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. Mol Psychiatry 8:536–545
54. Greenwood TA, Alexander M, Keck PE, McElroy S, Sadovnick AD, Remick RA, Shaw SH, Kelsoe JR (2002) Segmental linkage disequilibrium within the dopamine transporter gene. Mol Psychiatry 7:165–173
55. Greenwood TA, Kelsoe JR (2003) Promoter and intronic variants affect the transcriptional regulation of the human dopamine transporter gene. Genomics 82:511–520
56. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andrason H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjornsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K, Gulcher JR (2003) The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 35:131–138
57. Halperin JM, Sharma V, Siever L, Schwartz ST, Matier K, Wornell G, Newcorn JH (1994) Serotonergic function in aggressive and nonaggressive boys with attention deficit hyperactivity disorder. Am J Psychiatry 151:243–248

58. Haseman JK, Elston RC (1972) The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3–19
59. Hawi Z, Dring M, Kirley A, Foley D, Kent L, Craddock N, Asherson P, Curran S, Gould A, Richards S, Lawson D, Pay H, Turic D, Langley K, Owen M, O'Donovan M, Thapar A, Fitzgerald M, Gill M (2002) Serotonergic system and attention deficit hyperactivity disorder (ADHD): a potential susceptibility locus at the 5-HT(1B) receptor gene in 273 nuclear families from a multi-centre sample. Mol Psychiatry 7:718–725
60. Hawi Z, Lowe N, Kirley A, Gruenhage F, Nothen M, Greenwood T, Kelsoe J, Fitzgerald M, Gill M (2003) Linkage disequilibrium mapping at DAT1, DRD5 and DBH narrows the search for ADHD susceptibility alleles at these loci. Mol Psychiatry 8:299–308
61. Hawi Z, McCarron M, Kirley A, Daly G, Fitzgerald M, Gill M (2000) No association of the dopamine DRD4 receptor (DRD4) gene polymorphism with attention deficit hyperactivity disorder (ADHD) in the Irish population. Am J Med Genet 96:268–272
62. Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, Lee KS, Linnoila M, Weinberger DR (2000) Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology 22:133–139
63. Hess EJ, Collins KA, Wilson MC (1996) Mouse model of hyperkinesis implicates SNAP-25 in behavioral regulation. J Neurosci 16:3104–3111
64. Hess EJ, Rogan PK, Domoto M, Tinker DE, Ladda RL, Ramer JC (1995) Absence of linkage of apparently single gene mediated ADHD with the human syntenic region of the mouse mutant Coloboma. Am J Med Genet 60:573–579
65. Holmes A, Hollon TR, Gleason TC, Liu Z, Dreiling J, Sibley DR, Crawley JN (2001) Behavioral characterization of dopamine D5 receptor null mutant mice. Behav Neurosci 115:1129–1144
66. Holmes J, Payton A, Barrett J, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Gill M, Kirley A, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mill J, Gould A, Taylor E, Kent L, Craddock N, Thapar A (2002) Association of DRD4 in children with ADHD and comorbid conduct problems. Am J Med Genet 114:150–153
67. Holmes J, Payton A, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Thapar A (2000) A family based and case-control association study of the dopamine D4 receptor gene and dopamine transporter gene in attention deficit hyperactivity disorder. Mol Psychiatry 5:523–530
68. Jacobsen LK, Staley JK, Zoghbi SS, Seibyl JP, Kosten TR, Innis RB, Gelernter J (2000) Prediction of dopamine transporter binding availability by genotype: a preliminary report. Am J Psychiatry 157:1700–1703
69. Jawaid A, Sham P, Makoff A, Asherson P (2004) Is haplotype tagging the panacea to future association mapping studies? Eur J Med Genet 12:259–262
70. Johnson GC, Esposito L, Barratt BJ, Smith AN, Heward J, Di Genova G, Ueda H, Cordell HJ, Eaves IA, Dudbridge F, Twells RC, Payne F, Hughes W, Nutland S, Stevens H, Carr P, Tuomilehto-Wolf E, Tuomilehto J, Gough SC, Clayton DG, Todd JA (2001) Haplotype tagging for the identification of common disease genes. Nat Genet 29:233–237
71. Jonsson EG, Ivo R, Gustavsson JP, Geijer T, Forslund K, Mattila-Evenden M, Rylander G, Cichon S, Propping P, Bergman H, Asberg M, Nothen MM (2002) No association between dopamine D4 receptor gene variants

and novelty seeking. Mol Psychiatry 7:18–20
72. Jonsson EG, Nothen MM, Gustavsson JP, Neidt H, Brene S, Tylec A, Propping P, Sedvall GC (1997) Lack of evidence for allelic association between personality traits and the dopamine D4 receptor gene polymorphisms. Am J Psychiatry 154:697–699
73. Jonsson EG, Nothen MM, Gustavsson JP, Neidt H, Forslund K, Mattila-Evenden M, Rylander G, Propping P, Asberg M (1998) Lack of association between dopamine D4 receptor gene and personality traits. Psychol Med 28:985–989
74. Jovanovic V, Guan HC, Van Tol HH (1999) Comparative pharmacological and functional analysis of the human dopamine D4.2 and D4.10 receptor variants. Pharmacogenetics 9:561–568
75. Kahn RS, Khoury J, Nichols WC, Lanphear BP (2003) Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. J Pediatr 143: 104–110
76. Kazmi MA, Snyder LA, Cypess AM, Graber SG, Sakmar TP (2000) Selective reconstitution of human D4 dopamine receptor variants with Gi alpha subtypes. Biochemistry 39:3734–3744
77. Kent L, Doerry U, Hardy E, Parmar R, Gingell K, Hawi Z, Kirley A, Lowe N, Fitzgerald M, Gill M, Craddock N (2002) Evidence that variation at the serotonin transporter gene influences susceptibility to attention deficit hyperactivity disorder (ADHD): analysis and pooled analysis. Mol Psychiatry 7:908–912
78. Kent L, Green E, Holmes J, Thapar A, Gill M, Hawi Z, Fitzgerald M, Asherson P, Curran S, Mills J, Payton A, Craddock N (2001a) No association between CHRNA7 microsatellite markers and attention-deficit hyperactivity disorder. Am J Med Genet 105:686–689
79. Kent L, Middle F, Hawi Z, Fitzgerald M, Gill M, Feehan C, Craddock N (2001b) Nicotinic acetylcholine receptor alpha4 subunit gene polymorphism and attention deficit hyperactivity disorder. Psychiatr Genet 11:37–40
80. Konrad K, Gauggel S, Manz A, Scholl M (2000) Lack of inhibition: a motivational deficit in children with attention deficit/hyperactivity disorder and children with traumatic brain injury. Neuropsychology, Development and Cognition Section C: Child Neuropsychology 6:286–296
81. Kotler M, Manor I, Sever Y, Eisenberg J, Cohen H, Ebstein RP, Tyano S (2000) Failure to replicate an excess of the long dopamine D4 exon III repeat polymorphism in ADHD in a family-based study. Am J Med Genet 96:278–281
82. Krause KH, Dresel SH, Krause J, Kung HF, Tatsch K (2000) Increased striatal dopamine transporter in adult patients with attention deficit hyperactivity disorder: effects of methylphenidate as measured by single photon emission computed tomography. Neuroscience Letters 285:107–110
83. Kuntsi J, Andreou PM, Borger J, Van der Meere J (2004) Testing assumptions for endophenotype studies in ADHD: Reliability and validity of tasks in a general population sample. MS submitted for publication
84. Kuntsi J, Stevenson J (2001) Psychological mechanisms in hyper-activity: II. The role of genetic factors. J Child Psychol Psychiatry and Allied Disciplines 42:211–219
85. Kustanovich V, Ishii J, Crawford L, Yang M, McGough JJ, McCracken JT, Smalley SL, Nelson SF (2004) Transmission disequilibrium testing of dopamine-related candidate gene polymorphisms in ADHD: confirmation of association of ADHD with DRD4 and DRD5. Mol

Psychiatry (in press)
86. Kustanovich V, Merriman B, McGough J, McCracken JT, Smalley SL, Nelson SF (2003) Biased paternal transmission of SNAP-25 risk alleles in attention-deficit hyperactivity disorder. Mol Psychiatry 8:309–315
87. LaHoste GJ, Swanson JM, Wigal SB, Glabe C, Wigal T, King N, Kennedy JL (1996) Dopamine D4 receptor gene polymorphism is associated with attention deficit hyperactivity disorder. Mol Psychiatry 1:121–124
88. Langley K, Marshall L, Van Den BM, Thomas H, Owen M, O'Donovan M, Thapar A (2004) Association of the dopamine d(4) receptor gene 7-repeat allele with neuropsychological test performance of children with ADHD. Am J Psychiatry 161:133–138
89. Langley K, Payton A, Hamshere ML, Pay HM, Lawson DC, Turic D, Ollier W, Worthington J, Owen MJ, O'Donovan MC, Thapar A (2003) No evidence of association of two 5HT transporter gene polymorphisms and attention deficit hyperactivity disorder. Psychiatr Genet 13:107–110
90. Lee HJ, Lee HS, Kim YK, Kim L, Lee MS, Jung IK, Suh KY, Kim S (2003a) D2 and D4 dopamine receptor gene polymorphisms and personality traits in a young Korean population. Am J Med Genet 121B:44–49
91. Lee HJ, Lee HS, Kim YK, Kim SH, Kim L, Lee MS, Joe SH, Jung IK, Suh KY, Kim S (2003b) Allelic variants interaction of dopamine receptor D4 polymorphism correlate with personality traits in young Korean female population. Am J Med Genet 118B:76–80
92. Lesch KP, Mossner R (1998) Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disorders? Biol Psychiatry 44: 179–192
93. Lesch KP, Zeng Y, Reif A, Gutknecht L (2003) Anxiety-related traits in mice with modified genes of the serotonergic pathway. Eur J Pharmacol 480:185–204
94. Levy F, Hay DA, McStephen M, Wood C, Waldman I (1997) Attention-deficit hyperactivity disorder: a category or a continuum? Genetic analysis of a large scale twin study. J Am Acad Child Adolesc Psychiatry 36:737–744
95. Loo SK, Fisher SE, Francks C, Ogdie MN, MacPhie IL, Yang M, McCracken JT, McGough JJ, Nelson SF, Monaco AP, Smalley SL (2004) Genome-wide scan of reading ability in affected sibling pairs with attention-deficit/hyperactivity disorder: unique and shared genetic effects. Mol Psychiatry 9:485–493
96. Loo SK, Specter E, Smolen A, Hopfer C, Teale PD, Reite ML (2003b) Functional effects of the DAT1 polymorphism on EEG measures in ADHD. J Am Acad Child Adolesc Psychiatry 42:986–993
97. Lovejoy EA, Scott AC, Fiskerstrand CE, Bubb VJ, Quinn JP (2003) The serotonin transporter intronic VNTR enhancer correlated with a predisposition to affective disorders has distinct regulatory elements within the domain based on the primary DNA sequence of the repeat unit. Eur J Neurosci 17:417–420
98. Lowe N, Kirley A, Hawi Z, Sham P, Wickham H, Kratochvil CJ, Smith SD, Lee SY, Levy F, Kent L, Middle F, Rohde LA, Roman T, Tahir E, Yazgan Y, Asherson P, Mill J, Thapar A, Payton A, Todd RD, Stephens T, Ebstein RP, Manor I, Barr CL, Wigg KG, Sinke RJ, Buitelaar JK, Smalley SL, Nelson SF, Biederman J, Faraone SV, Gill M (2004) Joint Analysis of the DRD5 Marker Concludes Association with Attention-Deficit/Hyperactivity Disorder Confined to the Predominantly Inattentive and Combined Subtypes. Am J Hum Genet 74:348–356
99. Luciano M, Zhu G, Kirk KM, Whitfield

JB, Butler R, Heath AC, Madden PA, Martin NG (2004) Effects of dopamine receptor D4 variation on alcohol and tobacco use and on novelty seeking: multivariate linkage and association analysis. Am J Med Genet 124B:113–123

100. Lucki I (1998) The spectrum of behaviors influenced by serotonin. Biol Psychiatry 44:151–162
101. Lusher JM, Chandler C, Ball D (2001) Dopamine D4 receptor gene (DRD4) is associated with Novelty Seeking (NS) and substance abuse: the saga continues. Mol Psychiatry 6:497–499
102. Malhotra AK, Virkkunen M, Rooney W, Eggert M, Linnoila M, Goldman D (1996) The association between the dopamine D4 receptor (D4DR) 16 amino acid repeat polymorphism and novelty seeking. Mol Psychiatry 1: 388–391
103. Manor I, Eisenberg J, Tyano S, Sever Y, Cohen H, Ebstein RP, Kotler M (2001) Family-based association study of the serotonin transporter promoter region polymorphism (5-HTTLPR) in attention deficit hyperactivity disorder. Am J Med Genet 105:91–95
104. Manor I, Tyano S, Eisenberg J, Bachner-Melman R, Kotler M, Ebstein RP (2002) The short DRD4 repeats confer risk to attention deficit hyperactivity disorder in a family-based design and impair performance on a continuous performance test (TOVA). Mol Psychiatry 7:790–794
105. Marlow AJ, Fisher SE, Francks C, MacPhie IL, Cherny SS, Richardson AJ, Talcott JB, Stein JF, Monaco AP, Cardon LR (2003) Use of multivariate linkage analysis for dissection of a complex cognitive trait. Am J Hum Genet 72: 561–570
106. Marlow AJ, Fisher SE, Richardson AJ, Francks C, Talcott JB, Monaco AP, Stein JF, Cardon LR (2001) Investigation of quantitative measures related to reading disability in a large sample of sib-pairs from the UK. Behav Genet 31: 219–230
107. Marth G, Yeh R, Minton M, Donaldson R, Li Q, Duan S, Davenport R, Miller RD, Kwok PY (2001) Single-nucleotide polymorphisms in the public domain: how useful are they? Nat Genet 27: 371–372
108. Martin KF, Webb AR, Marsden CA (1987) The behavioural response to the 5-hydroxytryptamine 1B (5-HT1B) receptor agonist – RU-24969 may exhibit a circadian variation in the mouse. Chronobiol Int 4:493–498
109. Martin N, Scourfield J, McGuffin P (2002) Observer effects and heritability of childhood attention-deficit hyperactivity disorder symptoms. Br J Psychiatry 180:260–265
110. Martinez D, Gelernter J, Abi-Dargham A, van Dyck CH, Kegeles L, Innis RB, Laruelle M (2001) The variable number of tandem repeats polymorphism of the dopamine transporter gene is not associated with significant change in dopamine transporter phenotype in humans. Neuropsychopharmacology 24:553–560
111. Matise TC, Sachidanandam R, Clark AG, Kruglyak L, Wijsman E, Kakol J, Buyske S, Chui B, Cohen P, de Toma C, Ehm M, Glanowski S, He C, Heil J, Markianos K, McMullen I, Pericak-Vance MA, Silbergleit A, Stein L, Wagner M, Wilson AF, Winick JD, Winn-Deen ES, Yamashiro CT, Cann HM, Lai E, Holden AL (2003) A 3.9-centimorgan-resolution human single-nucleotide polymorphism linkage map and screening set. Am J Genet 73: 271–284
112. McCracken JT, Smalley SL, McGough JJ, Crawford L, Del'Homme M, Cantor RM, Liu A, Nelson SF (2000) Evidence for linkage of a tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4) with attention deficit hyperactivity

disorder (ADHD). Mol Psychiatry 5: 531–536
113. McEvoy B, Hawi Z, Fitzgerald M, Gill M (2002) No evidence of linkage or association between the norepinephrine transporter (NET) gene polymorphisms and ADHD in the Irish population. Am J Med Genet 114:665–666
114. Michelhaugh SK, Fiskerstrand C, Lovejoy E, Bannon MJ, Quinn JP (2001) The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. J Neurochem 79:1033–1038
115. Mill J, Curran S, Richards S, Taylor E, Asherson P (2004a) Polymorphisms in the dopamine D5 (DRD5) receptor and ADHD. Am J Med Genet B 125B:38–42
116. Mill J, Xu X, Ronald A, Curran S, Price T, Knight J, Craig I, Sham P, Plomin R, Asherson P (2005) Quantitative trait locus analysis of candidate gene alleles associated with attention deficit hyperactivity disorder (ADHD) in five genes: DRD4, DAT1, DRD5, SNAP-25, and 5HT1B. Am J Med Genet B 133B: 68-73
117. Mill J (2004) The Genetic Investigation of ADHD. University of London
118. Mill J, Asherson P, Browes C, D'Souza U, Craig I (2002a) Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. Am J Med Genet 114:975–979
119. Mill J, Curran S, Kent L, Richards S, Gould A, Virdee V, Huckett L, Sharp J, Batten C, Fernando S, Simonoff E, Thompson M, Zhao J, Sham P, Taylor E, Asherson P (2001) Attention deficit hyperactivity disorder (ADHD) and the dopamine D4 receptor gene: evidence of association but no linkage in a UK sample. Mol Psychiatry 6: 440–444
120. Mill J, Fisher N, Curran S, Richards S, Taylor E, Asherson P (2003) Polymorphisms in the dopamine D4 receptor gene and attention-deficit hyperactivity disorder. Neuroreport 14: 1463–1466
121. Mill J, Knight J, Curran S, Taylor A, Asherson P (2004c) Haplotype analysis of SNAP-25 suggests a role in the aetiology of ADHD. Mol Psychiatry: in press
122. Mill JS, Caspi A, McClay J, Sugden K, Purcell S, Asherson P, Craig I, McGuffin P, Braithwaite A, Poulton R, Moffitt TE (2002b) The dopamine D4 receptor and the hyperactivity phenotype: a developmental-epidemiological study. Mol Psychiatry 7:383–391
123. Miller GM, Madras BK (2002) Polymorphisms in the 3'-untranslated region of human and monkey dopamine transporter genes affect reporter gene expression. Molecular Psychiatry 7: 44–55
124. Mortensen OV, Thomassen M, Larsen MB, Whittemore SR, Wiborg O (1999) Functional analysis of a novel human serotonin transporter gene promoter in immortalized raphe cells. Mol Brain Res 68:141–148
125. Muglia P, Jain U, Inkster B, Kennedy JL (2002) A quantitative trait locus analysis of the dopamine transporter gene in adults with ADHD. Neuropsychopharmacology 27:655–662
126. Muglia P, Jain U, Macciardi F, Kennedy JL (2000) Adult attention deficit hyperactivity disorder and the dopamine D4 receptor gene. Am J Med Genet 96:273–277
127. Nguyen T, Bard J, Jin H, Taruscio D, Ward DC, Kennedy JL, Weinshank R, Seeman P, O'Dowd BF (1991) Human dopamine D5 receptor pseudogenes. Gene 109:211–218
128. Nigg JT, Swanson JM, Hinshaw SP (1997) Covert visual spatial attention in boys with attention deficit hyperactivity disorder: lateral effects, methylphenidate response and results for parents.

Neuropsychologia 35: 165–176
129. Noble EP, Ozkaragoz TZ, Ritchie TL, Zhang X, Belin TR, Sparkes RS (1998) D2 and D4 dopamine receptor polymorphisms and personality. Am J Med Genet 81:257–267
130. Ogdie MN, MacPhie IL, Minassian SL, Yang M, Fisher SE, Francks C, Cantor RM, McCracken JT, McGough JJ, Nelson SF, Monaco AP, Smalley SL (2003) A genome wide scan for attention deficit/hyperactivity disorder in an extended sample: suggestive linkage on 17p11. Am J Hum Genet 72: 1268–1279
131. Oh KS, Shin DW, Oh GT, Noh KS (2003) Dopamine transporter genotype influences the attention deficit in Korean boys with ADHD. Yonsei Med J 44:787–792
132. Okuyama Y, Ishiguro H, Nankai M, Shibuya H, Watanabe A, Arinami T (2000) Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait. Mol Psychiatry 5:64–69
133. Ono Y, Manki H, Yoshimura K, Muramatsu T, Mizushima H, Higuchi S, Yagi G, Kanba S, Asai M (1997) Association between dopamine D4 receptor (D4DR) exon III polymorphism and novelty seeking in Japanese subjects. Am J Med Genet 74:501–503
134. Oosterlaan J, Scheres A, Sergeant J (2004) Which executive functioning deficits are associated with AD/HD, ODD/CD and comorbid AD/HD + ODD/CD? MS submitted
135. Paterson AD, Sunohara GA, Kennedy JL (1999) Dopamine D4 receptor gene: novelty or nonsense? Neuropsychopharmacology 21:3–16
136. Payton A, Holmes J, Barrett JH, Hever T, Fitzpatrick H, Trumper AL, Harrington R, McGuffin P, O'Donovan M, Owen M, Ollier W, Worthington J, Thapar A (2001a) Examining for association between candidate gene polymorphisms in the dopamine pathway and attention-deficit hyperactivity disorder: a family-based study. Am J Med Genet 105:464–470
137. Payton A, Holmes J, Barrett JH, Sham P, Harrington R, McGuffin P, Owen M, Ollier W, Worthington J, Thapar A (2001b) Susceptibility genes for a trait measure of attention deficit hyperactivity disorder: a pilot study in a nonclinical sample of twins. Psychiatry Res 105:273–278
138. Pecina S, Cagniard B, Berridge KC, Aldridge JW, Zhuang X (2003) Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. J Neurosci 23: 9395–9402
139. Persson ML, Wasserman D, Geijer T, Frisch A, Rockah R, Michaelovsky E, Apter A, Weizman A, Jonsson EG, Bergman H (2000) Dopamine D4 receptor gene polymorphism and personality traits in healthy volunteers. Eur Arch Psychiatry Clin Neurosci 250:203–206
140. Phillips MS, Lawrence R, Sachidanandam R, Morris AP, Balding DJ, Donaldson MA, Studebaker JF, Ankener WM, Alfisi SV, Kuo FS, Camisa AL, Pazorov V, Scott KE, Carey BJ, Faith J, Katari G, Bhatti HA, Cyr JM, Derohannessian V, Elosua C, Forman AM, Grecco NM, Hock CR, Kuebler JM, Lathrop JA, Mockler MA, Nachtman EP, Restine SL, Varde SA, Hozza MJ, Gelfand CA, Broxholme J, Abecasis GR, Boyce-Jacino MT, Cardon LR (2003) Chromosome-wide distribution of haplotype blocks and the role of recombination hot spots. Nat Genet 33:382–387
141. Plomin R (1999) Genetics and general cognitive ability. Nature 402:C25–C29
142. Pogue-Geile M, Ferrell R, Deka R, Debski T, Manuck S (1998) Human novelty-seeking personality traits and dopamine D4 receptor polymorphisms:

a twin and genetic association study. Am J Med Genet 81:44–48
143. Powell SB, Paulus MP, Hartman DS, Godel T, Geyer MA (2003) RO-10-5824 is a selective dopamine D4 receptor agonist that increases novel object exploration in C57 mice. Neuropharm-acology 44:473–481
144. Price TS, Simonoff E, Waldman I, Asherson P, Plomin R (2001) Hyperactivity in preschool children is highly heritable. J Am Acad Child Adolesc Psychiatry 40:1362–1364
145. Purcell S, Cherny SS, Hewitt JK, Sham PC (2001) Optimal sib-ship selection for genotyping in quantitative trait locus linkage analysis. Hum Heredity 52: 1–13
146. Qian Q, Wang Y, Li J, Yang L, Wang B, Zhou R (2003) Association studies of dopamine D4 receptor gene and dopamine transporter gene polymorphisms in Han Chinese patients with attention deficit hyperactivity disorder. Beijing Da Xue Xue Bao 35: 412–418
147. Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, Basile VS, Beitchman J, Kennedy JL (2003) The serotonin 5-HT1B receptor gene and attention deficit hyperactivity disorder. Mol Psychiatry 8:98–102
148. Roman T, Schmitz M, Polanczyk G, Eizirik M, Rohde LA, Hutz MH (2001) Attention-deficit hyperactivity disorder: a study of association with both the dopamine transporter gene and the dopamine D4 receptor gene. Am J Med Genet 105:471–478
149. Rutter M, Silberg J (2002) Gene-environment interplay in relation to emotional and behavioral disturbance. Annu Rev Psychol 53:463–490
150. Sander T, Harms H, Dufeu P, Kuhn S, Rommelspacher H, Schmidt LG (1997) Dopamine D4 receptor exon III alleles and variation of novelty seeking in alcoholics. Am J Med Genet 74:483–487
151. Seeger G, Schloss P, Schmidt MH (2001) Functional polymorphism within the promoter of the serotonin transporter gene is associated with severe hyperkinetic disorders. Mol Psychiatry 6:235–238
152. Sham P, Bader JS, Craig I, O'Donovan M, Owen M (2002) DNA Pooling: a tool for large-scale association studies. Nature Reviews. Genetics 3:862–887
153. Sherrington R, Mankoo B, Attwood J, Kalsi G, Curtis D, Buetow K, Povey S, Gurling H (1993) Cloning of the human dopamine D5 receptor gene and identification of a highly polymorphic microsatellite for the DRD5 locus that shows tight linkage to the chromosome 4p reference marker RAF1P1. Genomics 18:423–425
154. Slusarek M, Velling S, Bunk D, Eggers C (2001) Motivational effects on inhibitory control in children with ADHD. J Am Acad Child Adolesc Psychiatry 40:355–363
155. Smalley SL, Bailey JN, Palmer CG, Cantwell DP, McGough JJ, Del'Homme MA, Asarnow JR, Woodward JA, Ramsey C, Nelson SF (1998) Evidence that the dopamine D4 receptor is a susceptibility gene in attention deficit hyperactivity disorder. Mol Psychiatry 3:427–430
156. Smalley SL, Kustanovich V, Minassian SL, Stone JL, Ogdie MN, McGough JJ, McCracken JT, MacPhie IL, Francks C, Fisher SE, Cantor RM, Monaco AP, Nelson SF (2002) Genetic linkage of attention-deficit/hyperactivity disorder on chromosome 16p13, in a region implicated in autism. Am J Hum Genet 71: 959–963
157. Sobell JL, Lind TJ, Sigurdson DC, Zald DH, Snitz BE, Grove WM, Heston LL, Sommer SS (1995) The D5 dopamine receptor gene in schizophrenia: identification of a nonsense change and multiple missense changes but lack of

association with disease. Hum Mol Genet 4:507–514
158. Spencer TJ, Biederman J, Wilens TE, Faraone SV (2002) Overview and neurobiology of attention-deficit/ hyperactivity disorder. J Clin Psychiatry 63 (Suppl 12):3–9
159. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K (2002) Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71: 877–892
160. Stevenson J, Pennington BF, Gilger JW, DeFries JC, Gillis JJ (1993) Hyperactivity and spelling disability: testing for shared genetic aetiology. J Child Psychol Psychiatry and Allied Disciplines 34:1137–1152
161. Strobel A, Lesch KP, Hohenberger K, Jatzke S, Gutzeit HO, Anacker K, Brocke B (2002) No association between dopamine D4 receptor gene exon III and –521C/T polymorphism and novelty seeking. Mol Psychiatry 7:537–538
162. Strobel A, Spinath FM, Angleitner A, Riemann R, Lesch KP (2003) Lack of association between polymorphisms of the dopamine D4 receptor gene and personality. Neuropsychobiology 47: 52–56
163. Sunohara GA, Roberts W, Malone M, Schachar RJ, Tannock R, Basile VS, Wigal T, Wigal SB, Schuck S, Moriarty J, Swanson JM, Kennedy JL, Barr CL (2000) Linkage of the dopamine D4 receptor gene and attention-deficit/ hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 39:1537–1542
164. Swanson J, Oosterlaan J, Murias M, Schuck S, Flodman P, Spence MA, Wasdell M, Ding Y, Chi HC, Smith M, Mann M, Carlson C, Kennedy JL, Sergeant JA, Leung P, Zhang YP, Sadeh A, Chen C, Whalen CK, Babb KA, Moyzis R, Posner MI (2000) Attention deficit/hyperactivity disorder children with a 7-repeat allele of the dopamine receptor D4 gene have extreme behavior but normal performance on critical neuropsychological tests of attention. Proc Natl Acad Sci USA 97:4754–4759
165. Swanson JM, Sunohara GA, Kennedy JL, Regino R, Fineberg E, Wigal T, Lerner M, Williams L, LaHoste GJ, Wigal S (1998) Association of the dopamine receptor D4 (DRD4) gene with a refined phenotype of attention deficit hyperactivity disorder (ADHD): a family-based approach. Mol Psychiatry 3:38–41
166. Tahir E, Yazgan Y, Cirakoglu B, Ozbay F, Waldman I, Asherson PJ (2000) Association and linkage of DRD4 and DRD5 with attention deficit hyperactivity disorder (ADHD) in a sample of Turkish children. Mol Psychiatry 5: 396–404
167. Taylor E (1999) Developmental neuropsychopathology of attention deficit and impulsiveness. Dev Psychopathol 11:607–628
168. Thapar A, Harrington R, McGuffin P (2001) Examining the comorbidity of ADHD-related behaviours and conduct problems using a twin study design. Br J Psychiatry 179:224–229
169. Thapar A, Holmes J, Poulton K, Harrington R (1999) Genetic basis of attention deficit and hyperactivity. Br J Psychiatry 174:105–111
170. Todd RD, Jong YJ, Lobos EA, Reich W, Heath AC, Neuman RJ (2001a) No association of the dopamine trans-

porter gene 3' VNTR polymorph-ism with ADHD subtypes in a population sample of twins. Am J Med Genet 105: 745–748

171. Todd RD, Lobos EA, Sun LW, Neuman RJ (2003) Mutational analysis of the nicotinic acetylcholine receptor alpha 4 subunit gene in attention deficit/hyperactivity disorder: evidence for association of an intronic polymorphism with attention problems. Mol Psychiatry 8:103–108
172. Todd RD, Neuman RJ, Lobos EA, Jong YJ, Reich W, Heath AC (2001b) Lack of association of dopamine D4 receptor gene polymorphisms with ADHD subtypes in a population sample of twins. Am J Med Genet 105:432–438
173. Todd RD, Rasmussen ER, Neuman RJ, Reich W, Hudziak JJ, Bucholz KK, Madden PA, Heath A (2001c) Familiality and heritability of subtypes of attention deficit hyperactivity disorder in a popul-ation sample of adolescent female twins. Am J Psychiatry 158: 1891–1898
174. Van der Meere J, Stemerdink N, Gunning B (1995) Effects of presentation rate of stimuli on response inhibition in ADHD children with and without tics. Percept Mot Skills 81:259–262
175. van Dyck CH, Quinlan DM, Cretella LM, Staley JK, Malison RT, Baldwin RM, Seibyl JP, Innis RB (2002) Unaltered dopamine transporter availability in adult attention deficit hyperactivity disorder. Am J Psychiatry 159: 309–312
176. Waldman ID, Rowe DC, Abramowitz A, Kozel ST, Mohr JH, Sherman SL, Cleveland HH, Sanders ML, Gard JM, Stever C (1998) Association and linkage of the dopamine transporter gene and attention-deficit hyperactivity disorder in children: heterogeneity owing to diagnostic subtype and severity. Am J Hum Genet 63: 1767–1776
177. West A, Langley K, Hamshere ML, Kent L, Craddock N, Owen MJ, O'Donovan M, Thapar A (2002) Evidence to suggest biased phenotypes in children with Attention Deficit Hyperactivity Disorder from completely ascertained trios. Mol Psychiatry 7: 962–966
178. Willcutt EG, Pennington BF, DeFries JC (2000) Twin study of the etiology of comorbidity between reading disability and attention-deficit/hyperactivity dis-order. Am J Med Genet 96:293–301
179. Willcutt EG, Pennington BF, Smith SD, Cardon LR, Gayan J, Knopik VS, Olson RK, DeFries JC (2002) Quantitative trait locus for reading disability on chromosome 6p is pleiotropic for attention-deficit/hyperactivity disorder. Am J Med Genet 114:260–268
180. Wilson MC (2000) Coloboma mouse mutant as an animal model of hyperkinesis and attention deficit hyperactivity disorder. Neurosci Biobehav Rev 24:51–57
181. Zhuang X, Oosting RS, Jones SR, Gainetdinov RR, Miller GW, Caron MG, Hen R (2001) Hyperactivity and impaired response habituation in hyperdopaminergic mice. Proc Natl Acad Sci USA 98:1982–1987
182. Zoroglu SS, Erdal ME, Alasehirli B, Erdal N, Sivasli E, Tutkun H, Savas HA, Herken H (2002) Significance of serotonin transporter gene 5-HTTLPR and variable number of tandem repeat polymorphism in attention deficit hyperactivity disorder. Neuropsycho-biology 45:176–181