



Review

Imaging genetics in ADHD

Sarah Durston*

Department of Child and Adolescent Psychiatry, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht - HP A 01.468, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands

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ABSTRACT

Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent neuropsychiatric disorder, with 5% of school age children affected. Up to 80% of the phenotypic variance can be explained by genetic factors. The intermediate or endophenotype approach allows for mapping of the effect of individual risk genes on neurobiological parameters, such as brain structure, chemistry and, ultimately, function. There are two obvious advantages of applying such an approach to complex disorders: first, these measures are causally closer to genes and gene expression than behavior, meaning that gene effects should be magnified. Second, neuroimaging provides a means to uncover the neurobiological mechanisms by which gene variants impact the brain. To date, only fourteen studies have applied an imaging genetics approach to ADHD. Eight of these used MRI, four SPECT and two EEG. These imaging modalities have afforded us a window on the brain, permitting a glimpse of how genetic changes can affect brain structure, chemistry and function. The studies to date have often focused on two prime candidate genes in the dopamine system, the DRD4 and DAT1 genes. However, the effects of neither are yet fully understood. Imaging genetics in ADHD is in its infancy. While attempts to integrate the findings to date are hinting at how genes may impact various aspects of neural functioning, studies testing clear model-based hypotheses, using multimodal approaches may provide a means to link various windows on the brain.

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Contents

Introduction	832
The intermediate or endophenotype approach	833
Modalities used to date in endophenotype approaches to ADHD.	833
Imaging genetics in ADHD	836
The DRD4 gene	836
The DAT1 gene	836
Other genes.	837
Concluding remarks	837
Acknowledgments	837
References	837

Introduction

This special issue of NeuroImage focuses on imaging genetics in the brain, both in the healthy brain and in disorders. This is relevant as although many psychiatric disorders have well-established heritable bases, psychiatric genetic research has not been able to uncover the genetic causes underlying them. Attention Deficit Hyperactivity Disorder (ADHD) is a prevalent neuropsychiatric disorder, with 5% of school age children receiving the diagnosis, compared to prevalence rates of .5%

for schizophrenia and .5–1.0% for epilepsy. However, its prevalence is not necessarily reflected in the amount of studies published on it: a Pubmed search in July 2009 gave nearly 16000 hits for ADHD, but over 84000 for schizophrenia, and 116000 for epilepsy. ADHD is a heritable disorder, making it a prime candidate for imaging genetics studies. Up to 80% of the phenotypic variance can be explained by additive genetic factors (Faraone et al., 2005), similar to estimates for schizophrenia and some (familial) forms of epilepsy (Cardno and Gottesman, 2000; Kjeldsen et al., 2003). Of the approximately 16000 publications to date that mention ADHD, only 14.5% mention imaging modalities (MRI, EEG, or PET/SPECT). That is similar to the total number of publications on ADHD that mention neuropsychology (13.5%).

* Fax: +31 88 755 5444.

E-mail address: S.Durston@umcutrecht.nl.

The intermediate or endophenotype approach

The intermediate or endophenotype approach is the focus of this special issue and is explained in some detail by Bigos & Weinberger (2010). As such, it is not outlined in detail here. Briefly, the intermediate or endophenotype approach allows for mapping of the effect of individual risk genes on neurobiological parameters, such as brain structure, brain activity or neurochemistry. Criteria for an endophenotype in psychiatry include being continuously quantifiable, stable, closer to the causative agent (e.g., genes and gene expression) than the disorder, being associated with the disorder, being found in unaffected relatives of affected individuals and being grounded in neuroscience (Almasy and Blangero, 2001; Durston et al., 2009; Castellanos and Tannock, 2002; Gottesman and Gould, 2003). There are two obvious advantages of applying such an approach to complex disorders: first, these measures are causally closer to genes and gene expression than behavior. As such, gene effects should be magnified in such phenotypes. For example, MR-based measures of brain structure and functioning have been shown to be under heritable influences both in typical development (Lenroot and Giedd, 2008) and in ADHD (Durston et al., 2004, 2006; Mulder et al., 2008) and, as such, form a strong candidate for investigating gene effects in this disorder. This means that theoretically, smaller samples will be necessary to detect the effects of individual genes, in some cases as few as 40+ subjects (Meyer-Lindenberg and Weinberger, 2006). A second advantage is that neuroimaging phenotypes provide a means to uncover the neurobiological mechanisms by which gene variants can affect behavior.

Of 16000 publications on ADHD, only 52 mention the term 'endophenotype'. Of these, approximately half focus on neuropsychological endophenotypes, whereas only 10 (19%) mention neuroimaging modalities. Fig. 1 shows the number of publications that mention the various modalities, as a percentage of the number of publications on ADHD (nearly 16000) and as a percentage of the number that mentions endophenotypes (52). The order of the modalities on the X-axis reflects their relative cost, as well as how easily available they are. As can be seen, these factors have a large impact on the frequency with which they are applied in the total number of publications, but less so for publications on endophenotypes, suggesting that other factors also play a role here.

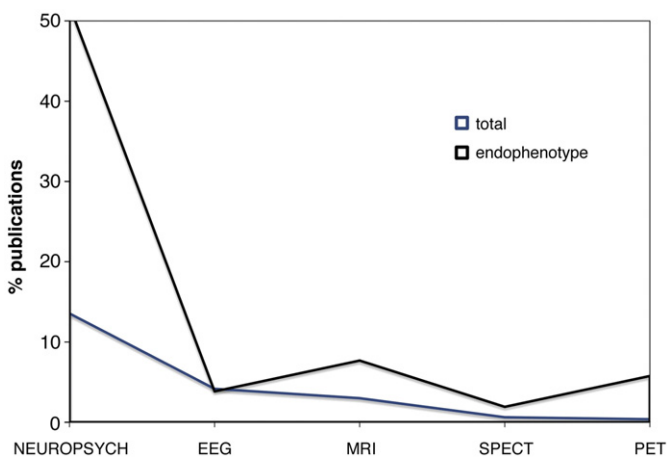


Fig. 1. Publications on ADHD mentioning the modalities on the X-axis as a percentage of the total number of publications (16000; in blue) and of those that mention the term endophenotype (52; in red). The order of the modalities on the X-axis reflects their relative cost and availability. As can be seen, these factors have a large impact on the frequency with which they are mentioned in the total number of publications, but less so for publications on endophenotypes.

Modalities used to date in endophenotype approaches to ADHD

As Fig. 1 illustrates, most studies to date that have applied an endophenotype approach to ADHD have used neuropsychology as an outcome measure. The advantages are obvious: the methods are cheap and readily available. Furthermore, neuropsychological measures have been shown to meet a number of criteria for endophenotypes, including being (1) affected in ADHD; (2) under familial influences in ADHD and (3) relatively stable over time (for review, see Bellgrove et al., 2008; Nigg, 2005; Rommelse et al., 2008). However, while such measures can provide a clue to what brain systems are involved in genetic influences on ADHD, they do not provide a direct measure of the brain. As such, investigators interested in the biology of these effects have applied brain imaging techniques. Brain imaging methods that have been used include *electroencephalography* (EEG) and the derived application *Event-Related Potentials* (ERP), *Magnetic Resonance Imaging* (MRI), *Positron Emission Tomography* (PET) and *Single Photon Emission Computed Tomography* (SPECT). Advantages of EEG are that it is relatively cheap and readily available at many sites, as well as a high temporal resolution. Disadvantages include its limited spatial resolution and the fact that signal can only be detected from certain populations of neurons. Advantages of MRI include its non-invasive nature and versatility, as it can be applied to image numerous aspects of brain structure and function. Disadvantages include the temporal resolution, which is in the second-range for functional MRI. A second disadvantage is the relatively high cost of this technique and high level of expertise required, although facilities are available at many universities and medical centers. Perhaps the most appealing aspect of PET and SPECT is that they can be used to quantitatively assess brain neurochemistry *in vivo*, for example allowing quantitative estimates of striatal dopamine transporter (DAT) availability. Disadvantages of these techniques are that they are relatively expensive and are not available at all locations. Some radioactive ligands have such short half-lives that they need to be produced on-site. As such, their use requires the availability of a cyclotron and radionuclide lab, in addition to the scanners. Furthermore, as these techniques make use of radioactivity they are not necessarily suitable for studies involving children. In some cases, medical-ethical review boards have approved studies involving children with neuropsychiatric disorders, but the use of radioactivity does effectively prohibit the inclusion of a control group. As such, most studies of ADHD using these modalities have focused on the adult form of the disorder. Theoretically, the spatial resolution of SPECT is higher than that of PET, as in PET the positron can travel several millimeters before it is annihilated by an electron, sending out two photons. In SPECT, a single photon is emitted from the location of the binding of the compound, allowing resolution of up to 2 μm in micro-SPECT. However, this requires a special multiple pinhole camera to allow for very precise detection of individual photons and is currently only available for small animal imaging (Beekman and van der Have, 2007). In everyday practice, the resolution of PET is superior to that of SPECT, as the two photons emitted are detected simultaneously, allowing for a better spatial reconstruction of the localization of the annihilation (Webb, 2003). The practical resolution of PET is in the range of 6–10 mm, depending on the tracer used and scan-time. In addition, PET can be used for quantitative analysis. SPECT is cheaper and available at more locations than PET, but current cameras have a spatial resolution of >10 mm. SPECT is usually used in a semi-quantitative approach (Webb, 2003). These techniques require different radiotracers, and as such which one is superior for a given research question depends on the availability of suitable ligands, in addition to technical and financial considerations.

In sum, a limited number of imaging modalities have yet been used for imaging genetics in ADHD. Each has its advantages and disadvantages. All have the capability to offer us a window on the brain, permitting a glimpse of how genetic changes may affect brain structure, chemistry and, ultimately, function.

Table 1

Studies on imaging genetics in ADHD. Legend: BD Bipolar Disorder; car carrier; C-car C-allele carrier; CN caudate nucleus; CPT continuous performance task; EEG ElectroEncephalyGram; ERP event-related potential; G/G G-allele homozygote; GM gray matter; hr hour; med medial; med free medication free; med naïve medication naïve; meds medications; mid middle; MPH methylphenidate; MRI magnetic resonance imaging; NC normal controls; OFC orbitofrontal cortex; PFC prefrontal cortex; PAL pallidum; post posterior; PET positron emission tomography; rCBF regional Cerebral Blood Flow; R repeat; ROI region of interest; SPECT single photon emission computed tomography; sup superior; TB total brain; T Tesla; T-car T-allele carrier; T/TT-allele homozygote; US United States; wk week; yrs years.

Authors	Participants	Modality and methods	Results
Brain structure Castellanos et al. (1998)	41 ADHD (9.7 +− 2.6 yrs; all psychoactive meds) 57 NC (17.6 +− 9.1 yrs) Mixed ethnicity; US study	Structural MRI (1.5 T); semi-automated volumes of TB, cerebellum, PFC; manual volumes of CN, PAL DRD4 VNTR exon 3; 7R-car vs not: 17 ADHD-7R; 22 NC-7R	No genotype effects No group × genotype interactions
Bobb et al. (2005)	163 ADHD (86 M; 9.0 +− 2.2 yrs; meds not reported) 129 NC (74 M; 16.0 +− 8.1 yrs) Mixed ethnicity; US study	Structural MRI (1.5 T); fully automated volumes of TB, lobes, BG, cerebellum DRD1; rs4532 C-car vs not: 64 ADHD C-car and 36 NC C-car; rs265981 T-car vs not: and 62 ADHD T-car; 36 NC T-car NET1; rs998424 C-car vs not: 112 ADHD C-car and 90 NC C-car; rs3785157 T-car vs not): 114 ADHD T-car and 92 NC T-car	No genotype effects. No group × genotype interactions
Durston et al. (2005)	26 ADHD (30 M; 12.1 +− 2.5 yrs; all MPH) 26 unaffected siblings (30 M; 11.6 +− 3.2 yrs) 20 NC (30 M; 10.7 +− 1.9 yrs) Caucasian sample; Dutch study	Structural MRI (1.5 T); automated volume of PFC GM; manual volume of CN DRD4 VNTR exon 3; 4R/4R vs not: 34 4R/4R DAT1 3' VNTR; 10R/10R vs carrier 9R: 40 10R/10R	Main effects: DAT1 on CN: 9R > 10R DRD4 on PFC GM: 4R < car variant alleles
Shaw et al. (2007)	105 ADHD (50 M; 10.1 +− 2.8 yrs; 85 stimulant meds) 103 NC (58 M; 10.0 +− 2.9 yrs) Mixed ethnicity; US study	Structural MRI (1.5 T); longitudinal; Automated cortical thickness DRD4 VNTR exon 3; 7R-car vs not: 43 ADHD 7R and 35 NC 7R	Main effect of diagnosis in OFC, sup/med PFC and post parietal cortex: ADHD < NC Main effect of DRD4-7R in similar regions: ADHD 7R < ADHD not-7R < NC 7R < NC not-7R
Monuteaux et al. (2008)	24 ADHD (12 M; 38.1 +− 10.8 yrs; meds not reported) 19 ADHD and BD (13 M; 35.8 +− 14.1 yrs) 20 NC (13 M; 33.2 +− 10.0 yrs) Mixed ethnicity; US study	sMRI (1.5 T) volumes of sup frontal, mid frontal, ACG, cerebellar cortices DRD4 VNTR exon 3; 7R-car vs not: 6 ADHD; 7 ADHD and BD; 6 NC 7R	Main effect genotype in frontal and cerebellar cortex for ADHD only: 7R-car < not

Brain chemistry			
Cheon et al. (2005)	11 ADHD (9 M; 9.8 + − 1.3 yrs; all med naïve) Pharmacogenetics study: 8 wk MPH treatment Ethnicity not reported; Korean study	I[123I]IPT SPECT (to assess DAT availability) DAT1 3' VNTR; 9R-car vs 10R/10R: 4 10R/10R	Striatal DAT availability: 10R>9R 10R associated with poorer MPH response
Krause et al. (2006)	29 ADHD (19 M; 37.6 + − 10 yrs; all med naïve) Caucasian sample; German study	[(99 m)Tc]TRODAT-1 SPECT (to assess DAT availability) DAT1 3' VNTR; 9R-car vs not: 12 9R	No effect of genotype on striatal DAT availability
Brain function			
Rohde et al. (2003)	8 ADHD (8 M; range 8–12 yrs; all MPH-naïve) Pharmacogenetics study: 4-day MPH treatment Ethnicity not reported; Brazilian study	99mTc-ECD SPECT during CPT (rCBF in 5 ROIs; 3 PFC; 2 BG) DAT1 3' VNTR; 10R/10R vs carrier 9R: 4 10R/10R	PFC and BG: 10R/10R>carrier 9R
Loo et al. (2003)	27 ADHD (18 M; 10.1 + − 1.5 yrs; 48 hr med washout) Pharmacogenetics study: single dose MPH Ethnicity not reported; US study	EEG CPT task during MPH challenge DAT1 3' VNTR; 10R/10R vs carrier 9R: 17 10R/10R	10R/10R: increased parietal/central beta-power, decreased frontal theta, decreased theta/beta ratios Carriers 9R: reverse pattern
Szobot et al. (2005)	34 ADHD (34 M; 11.6 + − 2.5 yrs; all med naïve) Ethnicity not reported; Brazilian study	99mTc-ECD SPECT during CPT (whole-brain rCBF) DRD4 VNTR exon 3; 7R-car vs not: 13 7R DAT1 3' VNTR; 10R/10R vs carrier 9R: 17 10R/10R	rCBF R medial temporal gyrus: carriers both risk alleles>not
Baehne et al. (2009)	122 ADHD (72 M; 34.7 + − 9.6 yrs; all med free but not necessarily naïve) 84 NC (44 M; 34.8 + − 10.3 yrs) Ethnicity not reported; German study	EEG Go/No-go task: No-go anteriorization (marker prefrontal functioning) Tryptophan hydroxylase gene (TPH2); rs4570625 G/G: 76 ADHD; 57 NC G/G and rs11178997 T/T: 107 ADHD; 73 NC T/T	Both ADHD and controls: Homozygotes risk alleles at both TPH2 loci<non-homozygotes
Durston et al. (2008)	10 ADHD (10 M; 14.6 + − 2.6 yrs; 1 med naïve; 24 hr meds washout) 10 unaffected sibs (10 M; 14.8 + − 2.3 yrs) 9 NC (9 M; 15.3 + − 2.1 yrs) Caucasian sample; Dutch study	fMRI (1.5 T) Go/No-go task; whole-brain analysis of genotype DAT1 3' VNTR; 10R/10R vs carrier 9R: 6 ADHD; 5 sibs; 6 NC 10R/10R	Main effect genotype: 9R ↑ activation in CN 9R ↓ activation in vermis Group × genotype interaction: Effect in CN related to ADHD and unaffected siblings – not NC
Brown et al. (2009)	42 ADHD (20 M; 35.2 + − 13.5 yrs; 16 med naïve; 24 hr meds washout) Caucasian sample; US study	fMRI (1.5 T) MSIT (interference) task; ACC ROI and whole-brain analysis of genotype DAT1 3' VNTR; 10R/10R vs carrier 9R: 19 10R/10R	9R ↑ activation in ACC, vermis, PFC
Bédard et al. (in press)	33 ADHD (24 M; 11.1 + − 2.5 yrs; 21 med naïve; 2 wk meds washout) Mixed ethnicity; US study	fMRI (3.0 T) Go/No-go task; whole-brain analysis of genotype DAT1 3' VNTR; 10R/10R vs carrier 9R: 21 10R/10R	9R ↓ activation in striatum, premotor cortex, temporoparietal junction

Imaging genetics in ADHD

A systematic review of the published literature on imaging genetics in ADHD shows that to date, only fourteen imaging genetics studies have been published in ADHD (see [Table 1](#)). These papers were retrieved through a pubmed search using the search terms “ADHD” and “MRI” “fMRI” “SPECT” “PET” “EEG” or “ERP” and “gene” “DAT1” “DAT” or “DRD4”. The reference lists of retrieved papers (and reviews) were further checked for other publications in this area. Of the fourteen publications to date, eight used MRI, four SPECT and two EEG. Three papers reported on pharmacogenetics studies and focused on treatment response, although here the focus is on their baseline results. Five studies on brain anatomy included medicated subjects or did not report medication status, whereas the two papers on brain chemistry included only medication naïve subjects and the seven studies on brain function either included only medication naïve subjects or required a washout period. All took a theory-driven approach, where they selected candidate genes (for review [Durston et al., 2009](#)). Most focused on the dopamine system. The two most frequently studied and most frequently replicated risk genes for ADHD are the DRD4 and DAT1 genes ([Heiser et al., 2004](#); [Thapar et al., 2005](#); [Waldman and Gizer, 2006](#)), with 31 association studies published for DRD4 (21 positive) and 26 for DAT1 (17 positive; [Durston et al., 2009](#)). Meta-analytic studies have consistently supported the involvement of the DRD4 gene in ADHD ([Faraone et al., 2005](#); [Li et al., 2006](#)), but have been more equivocal in terms of the DAT1 gene, with three negative results from meta-analyses ([Li et al., 2006](#); [Maher et al., 2002](#); [Purper-Ouakil et al., 2005](#)) and two providing only weak support for its involvement ([Faraone et al., 2005](#); [Yang et al., 2007](#)). Of the fourteen imaging genetics studies published in ADHD to date, nine focused on the DAT1 gene and five on the DRD4 gene.

The DRD4 gene

The first studies of genotype effects on brain structure used numerous volumetric outcome measures on MRI and did not find any differences by genotype, likely due to the number of comparisons to be controlled for ([Bobb et al., 2005](#); [Castellanos et al., 1998](#); for review [Durston et al., 2009](#)). More recent studies have shown effects of DRD4 on the volume of the brain area where this gene is preferentially expressed: the cerebral cortex ([Durston et al., 2005](#); [Monuteaux et al., 2008](#); [Shaw et al., 2007](#)). Interestingly, the direction of findings has differed across studies for DRD4, where the original report showed greater volumes for individuals carrying a variant allele, whereas recent reports have shown smaller volumes or cortical thickness for individuals carrying the best-established risk-allele for ADHD, the DRD4-7R ([Monuteaux et al., 2008](#); [Shaw et al., 2007](#)). An important distinction between these studies may be that the original study broke down the sample into two groups based on carriers of any variant allele of the DRD4 (i.e., not the 4R), based on reports that variants have similar biochemical properties to the 7R risk-allele: the 7R-allele is a ‘new’ variant of the gene and is reported to result in a receptor which has a blunted response to dopamine stimulation compared to the ancestral 4R-allele. The 2R-allele, which is derived from a recombination of the 4R and 7R-alleles, has a dopamine response intermediate between the two parent alleles ([Wang et al., 2004](#)). As such, biochemically it is plausible that more dopamine release is necessary to create a similar response in carriers of variant alleles compared to homozygotes for the ancestral allele (4R). This ties in to reports that children with ADHD carrying the 7R-allele may require higher doses of methylphenidate to achieve more robust clinical improvement ([Hamerman et al., 2004](#)). The later imaging genetics studies of DRD4 effects compared carriers of the 7R-allele to individuals without this allele, rather than comparing carriers of a variant to individuals homozygous for the ancestral allele ([Monu-](#)

[teaux et al., 2008](#); [Shaw et al., 2007](#)) and reported opposite results to the initial study. This may relate to reports of differences in neuropsychology and outcome between individuals with ADHD that carry the 7R-allele and those that do not: here, some suggest that ADHD 7R-carriers may represent a subgroup of affected individuals with better cognitive functioning and outcome ([Johnson et al., 2008](#); [Manor et al., 2002](#); [Swanson et al., 2000](#); for review, [Kebir et al., 2009](#)). Interestingly, the DRD4-7R was indeed associated with better clinical outcome and cortical thinning in certain regions in the study by [Shaw et al. \(2007\)](#).

The DAT1 gene

Studies of gene effects on brain biochemistry have focused on the DAT1 VNTR in the UTR 3’ region. This gene is preferentially expressed in striatum and striatal dopamine transporters are implicated in ADHD, both in the disorder itself and in treatment using methylphenidate (for review, [Krause, 2008](#); [Plomp et al., in press](#)). An original report with a small number of subjects suggested that the DAT1 genotype that has been most consistently associated with ADHD (homozygosity for the 10R-allele) was associated with more availability of the dopamine transporter in striatum ([Cheon et al., 2005](#)). However, this was not confirmed in a larger study ([Krause et al., 2006](#)).

Overall, SPECT and PET studies of DAT availability in ADHD have suggested changes in striatal levels, regardless of DAT1 genotype. However, the direction of this effect is not clear and both increases and decreases have been reported ([Spencer et al., 2005, 2007](#); [Volkow et al., 2007](#)). Studies of DAT1-gene effects on brain functioning and structure have shown similarly contradictory results: we have reported reduced striatal activity for boys with ADHD and their unaffected sibling who were homozygous for DAT1 10R-allele ([Durston et al., 2008](#)). Furthermore, we found that the caudate nucleus, where the DAT1 gene is preferentially expressed was smaller in subjects homozygous for the 10R-allele ([Durston et al., 2005](#)). Two recent studies using functional MRI to investigate the effects of the DAT1 polymorphism in ADHD also found effects on brain regions previously implicated in ADHD, including striatum ([Bédard et al., in press](#)) and prefrontal cortex ([Brown et al., 2009](#)). However, the complexity of the system is underlined by the fact that although these papers replicate the regions where we also found effects of this polymorphism (vermis in [Brown et al., 2009](#); striatum in [Bédard et al., in press](#)), in both cases the findings are in the opposite direction with decreased activity reported in the vermis and increased activation reported in the striatum for individuals homozygous for the 10R-allele polymorphism ([Brown et al., 2009](#); [Bédard et al., in press](#)). There are differences between studies that may in part account for these opposite results, including differences in developmental stage, gender distribution, medication status of samples, as well as the functional imaging paradigm used (although the latter was identical for [Durston et al., 2008](#); [Bédard et al., in press](#)). For example, the sample included by Brown and colleagues consisted of adult ADHD subjects and recent genetic data have suggested that in adults, it may be 9R-allele rather than the 10R that is associated with ADHD ([Franke et al., 2008](#)), possibly tying in to opposite gene effects in the brain. Furthermore, such seemingly contradictory results suggest that the effects of the dopamine transporter in ADHD may be opposite depending on the existing equilibrium in the system: both hypo- and hyper-catecholamine levels in striatum could conceivably disrupt efficient prefrontal neuromodulation and may therefore relate to ADHD-like symptoms. Increased DAT1 expression could then lead to the exacerbation of symptoms in an already hypo-dopaminergic system ([Waldman et al., 1998](#)), while it is beneficial to ADHD symptoms in the other scenario.

Other genes

Two studies focused on genes in other monoamine or catecholamine systems (Baehne et al., 2009; Bobb et al., 2005). Bobb and colleagues investigated association with ADHD of twelve candidate genes. They found significant association of the Dopamine-1 Receptor (DRD1) and norepinephrine transporter (NET1) with ADHD. They followed up these genes by investigating their effects on brain anatomy, but found no association between either and regional measures of brain volume. More recently, Baehne and colleagues investigated two polymorphisms of the tryptophan hydroxylase gene (TPH2). This gene is involved in the synthesis of serotonin, another catecholamine that has been implicated in ADHD. They hypothesized that as serotonin is known to modulate prefrontal cortex, TPH2 genotype would impact upon an EEG measure of prefrontal function known to be associated with ADHD, the so-called No-Go Anteriorization (NGA). Indeed, they showed this to be the case.

In sum, imaging genetics studies in ADHD have often focused on two prime candidate genes in the dopamine system. By combining findings from studies using various methods, we are beginning to learn how polymorphisms in these genes may impact the brain at the levels of structure, chemistry and function. However, the effect of neither gene is yet fully understood.

Concluding remarks

Imaging genetics in ADHD is in its infancy. To date, only fourteen studies have used neuroimaging methods to assess the effect of ADHD risk genes on the brain. However, this is of pivotal importance if we want to address how genetic risk can impact a biological system and ultimately result in ADHD. Genetic variations that affect gene expression in the brain can affect brain function. Imaging approaches permit us to visualize these changes *in vivo*. This is essentially the simple approach that we have been applying in imaging genetics studies in ADHD to date. However, this approach is clearly too simple to explain the full complexity of the system: Changes in one area of the system are likely to have downstream effects that are hard to predict based on simple models of gene expression. This is further complicated by the fact that ADHD is a developmental disorder and, although genetic variations are present throughout development, their impact may change over time, depending on developmental stage and earlier effects. As such, different (or even opposite) effects may be found (and are being reported) from samples of different ages. The contradictory findings described in this paper may further be related to the fact that ADHD is associated with several genes of small effect (Faraone et al., 2005). Here, certain combinations of genes affecting the system simultaneously may be necessary to result in ADHD symptoms. However, to date studies have focused on one or two genes at a time and have not yet considered possible interactive effects. Limited power has also meant that such confounds as co-morbid disorders, medication history and sample composition have received little consideration. Clearly, these issues can be addressed in future studies. However, given the complexity and the risk of false positive findings it seems crucial to work with clear model-based hypotheses. Developing animal models that parallel the human polymorphisms as was recently done for the BDNF gene (Soliman et al., 2010) is one way to develop clear predictions of the effects of gene variants on the brain. Added value may also be expected from multimodal approaches that can provide a means to link the various windows on the brain provided by imaging techniques. In addition, developments in genetics are likely to widen the imaging genetics field: the recent interest in copy number variations (CNVs) in autism and schizophrenia is likely to also spark interest in these genetic mutations in other disorders and raises an additional class of genetic variants that

can be investigated using imaging genetics approaches. Here also it will be important to develop testable hypotheses based on the predicted effect of CNVs on gene expression in the brain.

The focus of this paper (and indeed, this issue) is on applying the endophenotype approach to studying gene effects on the brain. However, it can equally be applied to studying non-genetic causative agents (for review, Plomp et al., *in press*). For example, four studies have now addressed gene–environment interactions in the effect of maternal, prenatal smoking on ADHD: two reported that children homozygous for the DAT1 10R-allele and exposed to prenatal smoking were at increased risk for hyperactivity–impulsivity (Kahn et al., 2003; Becker et al., 2008), whereas two others did not replicate these findings (Brookes et al., 2006; Neuman et al., 2007). Rather, one found an interaction between prenatal smoking and the DAT1 9R-allele and/or the DRD4 7R-allele (Neuman et al., 2007). This suggests that there may be different biological (genetic) mechanisms at play in mediating the effect of maternal behavior on the ADHD-phenotype. Intermediate phenotype approaches using imaging measure can be applied to investigate the biological mechanisms by which prenatal risk factors interact with genotype. As such, intermediate phenotypes have potential applications beyond imaging genetics.

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