Psychiatry Research 226 (2015) 425–433

Contents lists available at ScienceDirect

Psychiatry Research

journal homepage: www.elsevier.com/locate/psychres

Association of norepinephrine transporter (*NET, SLC6A2*) genotype with ADHD-related phenotypes: Findings of a longitudinal study from birth to adolescence

Sarah Hohmann [a,1], Erika Hohm [a,1], Jens Treutlein [b], Dorothea Blomeyer [a], Christine Jennen-Steinmetz [c], Martin H. Schmidt [a], Günter Esser [d], Tobias Banaschewski [a], Daniel Brandeis [a,e], Manfred Laucht [a,d,*]

[a] Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany
[b] Molecular Genetics Laboratory, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany
[c] Department of Biostatistics, Central Institute of Mental Health, Medical Faculty Mannheim/Heidelberg University, Mannheim, Germany
[d] Department of Psychology, University of Potsdam, Potsdam, Germany
[e] Department of Child and Adolescent Psychiatry, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Article history:
Received 1 July 2014
Received in revised form
16 December 2014
Accepted 18 December 2014
Available online 27 December 2014

Keywords:
Attention-deficit/hyperactivity disorder
Norepinephrine transporter
Genetic association
Polymorphism
Molecular heterosis
Continuous performance task

ABSTRACT

Variation in the gene encoding for the norepinephrine transporter (*NET, SLC6A2*) has repeatedly been linked with ADHD, although there is some inconsistency regarding the association with specific genes. The variants for which most consistent association has been found are the *NET* variants rs3785157 and rs28386840. Here, we tested for their association with ADHD diagnosis and ADHD-related phenotypes during development in a longitudinal German community sample. Children were followed from age 4 to age 15, using diagnostic interviews to assess ADHD. Between the ages of 8 and 15 years, the Child Behavior Checklist (CBCL) was administered to the primary caregivers. The continuous performance task (CPT) was performed at age 15. Controlling for possible confounders, we found that homozygous carriers of the major A allele of the functional promoter variant rs28386840 displayed a higher rate of ADHD lifetime diagnosis. Moreover, homozygous carriers of the minor T allele of rs3785157 were more likely to develop ADHD and showed higher scores on the CBCL externalizing behavior scales. Additionally, we found that individuals heterozygous for rs3785157 made fewer omission errors in the CPT than homozygotes. This is the first longitudinal study to report associations between specific *NET* variants and ADHD-related phenotypes during the course of development.

1. Introduction

Attention-deficit/hyperactivity disorder (ADHD) is one of the most common child and adolescent psychiatric disorders. It is characterized by pervasive behaviors of inattentiveness, impulsivity and hyperactivity, and affects 3–10% of school-age children (Faraone et al., 2003; Polanczyk et al., 2007). Due to extensive comorbidity, the clinical expression of ADHD is heterogeneous, exhibiting a number of additional symptoms, in particular of the externalizing spectrum. Among the behavioral symptoms, different impairments of neuropsychological functioning in children with ADHD have been found, such as deficits in attention, executive function, motor control, response inhibition, working memory and motivation (de Zeeuw et al., 2012; Fair et al., 2012; Sonuga-Barke et al., 2010). There is ample evidence that ADHD has a strong genetic component, with an estimated heritability of 70–80% (Biederman and Faraone, 2005; Faraone et al., 2005). Intensive research on its molecular genetic basis has indicated that ADHD is caused by multiple genes, each having small effects (Banaschewski et al., 2010; Faraone and Mick, 2010).

Considerable evidence from pharmacological studies suggests the dysregulation of the noradrenergic system to be a key player in ADHD pathophysiology (Albayrak et al., 2008; Brookes et al., 2006; Pliszka, 2005; Wilens, 2006). Neuroimaging and animal studies have provided further support for a role of dopamine (DA) and norepinephrine (NE) in ADHD (Arnsten and Pliszka, 2011; Del Campo et al., 2011). NE is known to be involved in a broad range of

* Corresponding author at: Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, J 5, 68159 Mannheim, Germany. Tel.: +49 621 1703 4903; fax: +49 621 1703 1205.
E-mail address: manfred.laucht@zi-mannheim.de (M. Laucht).
[1] These authors contributed equally to this work and should be considered as co-first authors.

http://dx.doi.org/10.1016/j.psychres.2014.12.029
0165-1781/

neuropsychological functioning, including visual attention, initiation of the adaptive response, sustained attention, learning and memory, executive functions and general alertness (Arnsten, 2006; Bruno et al., 2007; Kostrzewa et al., 2008; Russell, 2007; Sontag et al., 2010). The synaptic neurotransmitter homeostasis of DA and NE in prefrontal areas is modulated by reuptake into the presynaptic neuron via NE transporter (*NET*). While norepinephrine is the main substrate of *NET*, it is also capable of mediating the reuptake of DA in prefrontal areas, underlining the importance of this effector in prefrontal brain activity. Treatment of ADHD targets both the DA and NE equilibration. While methylphenidate and amphetamine increase the synaptic concentration of these neurotransmitters by inhibiting the respective transporters (Seeman and Madras, 1998), atomoxetine predominantly binds to *NET*, blocking its transporter function (Bymaster et al., 2002; Seneca et al., 2006; Wong et al., 1982).

Given this evidence, the gene encoding *NET* has been suggested as a major candidate gene for psychiatric disorders with abnormal NE transmission und metabolism (Bruss et al., 1993; Lasky-Su et al., 2008). The human *NET* gene [solute carrier family 6 (neurotransmitter transporter), member 2 (norepinephrine transporter; *SLC6A2*)] maps to chromosome 16 (16q12.2) and consists of 16 exons (Porzgen et al., 1995), encoding for a protein of 617 amino-acids (Gelernter et al., 1993). So far, reports on potential associations between specific single nucleotide polymorphisms (SNPs) of the *NET* gene and ADHD or related phenotypes have been controversial (Barr et al., 2002; Bobb et al., 2005; Brookes et al., 2006; Cho et al., 2008; Joung et al., 2010; Kim et al., 2006, 2008b; McEvoy et al., 2002; Renner et al., 2011; Xu et al., 2005).

One SNP within *SCL6A2*, which is repeatedly associated with ADHD, is rs3785157 (C⇒T), which is located in the 8th intron of the gene. The minor T allele of this variant was first reported to be preferentially transmitted in ADHD patients by Bobb et al. (2005). While Xu et al. (2005) were unable to confirm this finding, they detected a trend for the rs3785157 C allele to be associated with ADHD. However, further attempts to replicate these results in larger samples failed (Brookes et al., 2006; Kim et al., 2008b). In these studies, another SNP, rs11568324, which was in high linkage disequilibrium (LD) with rs3785157 ($D'=0.96$–1.0), emerged as significantly related to ADHD. The authors speculated that the involvement of opposite alleles of rs3785157 in previous studies might be explained as allele reversal phenomenon resulting from a correlation (by LD) of the investigated variant with the causal variant (rs11568324) (Kim et al., 2008b). A study by Ilott et al. (2010) showed a moderate, but nominally significant association of ADHD with rs3785157 at ages 2 and 3, while a link with rs11568324 only emerged at age 2. Recently, the group of Sengupta et al. (2012) identified several haplotype blocks within the *NET* gene, which were differently associated with ADHD depending on sex and comorbidity. While the first haplotype block (including the promoter region with rs28386840) was reported to be linked to ADHD along with internalizing problems of the child behavior checklist (CBCL) in girls, the second (including rs3785157) and third haplotype blocks were found to be related to ADHD symptoms together with CBCL aggression and attention problems in boys.

Another *NET* SNP of particular interest is a variant in the promoter region of the gene (rs28386840, A⇒T) which was first described by Kim et al. (2006). For this variant, a functional impact on transporter expression by decreasing promoter function has been reported. These authors found an association of the rs28386840 minor T allele with ADHD and were able to replicate their findings in a second sample (Kim et al., 2008a). While the group of Joung et al. (2010) confirmed the original results in their study population, other investigators failed to replicate them in independent samples of German and Korean origin (Cho et al., 2008; Renner et al., 2011). Despite these conflicting reports, rs28386840 has recently been suggested to influence response to methylphenidate medication (Kim et al., 2010). Furthermore, the T allele of this SNP has been found to be associated with a higher elevation of heart rate after medication with osmotic controlled-release oral delivery system (OROS) methylphenidate (Cho et al., 2012). These results might best be explained by a diminished cell-surface expression of the *NET* protein (due to decreased promoter function), resulting in a more global transporter inhibition as induced by stimulant medication. Moreover, ADHD patients carrying the T allele of rs28386840 have been reported to show a significant reduction in commission errors in the continuous performance task (CPT) when medicated with methylphenidate (Park et al., 2012). In summary, rs28386840 seems to exert an impact on transporter function (most likely via influencing the expression of the protein) thereby affecting the drug action of stimulant medication, although evidence of its association with ADHD remains controversial.

As the current psychiatric nosology is based on historically defined clinical categories rather than on a scientific foundation, the diagnosis of ADHD might not constitute an adequate entity for studying genetic associations. One possible approach to solve this problem is to study intermediate phenotypes (reviewed by Meyer-Lindenberg and Weinberger, 2006), which are heritable traits on the pathogenesis path from genetic predisposition to psychopathology. These intermediate phenotypes, also called endophenotypes, are thought to be associated with more basic and proximal etiological processes, and should therefore be more amenable to genetic investigation. The CPT (Continuous Performance Task) is a widely used neuropsychological test that assesses both sustained attention and inhibitory control under certain conditions. As the measures derived from CPT have been found to be distributed continuously and to be predictive of the diagnosis of ADHD (Frazier et al., 2004; Hervey et al., 2004; Kollins et al., 2008), CPT performance has been suggested to possibly constitute an endophenotype of ADHD (Kollins et al., 2008) according to the criteria outlined by Castellanos and Tannock (2002). To date, the search for the genetic basis of CPT performance has focused on dopaminergic genes (Kim et al., 2009; Loo et al., 2003; Manor et al., 2004). However, given the role of the noradrenergic system in executive functioning, it is equally important to unravel the contribution of noradrenergic genes. Findings so far have been inconsistent. While Kollins et al. (2008) reported an association of CPT reaction time variability with *SLC6A2* variant rs3785155, and Song et al. (2011) found an association of *SLC6A2* variant rs5569 with commission errors, Cho et al. (2008) were unable to replicate these results. Instead, they demonstrated a trend for higher response time variability in children carrying the minor T allele of rs28386840.

To our knowledge, no study so far has addressed the association of *NET* genotypes with ADHD and externalizing behavior problems using data from a longitudinal study. Given the inconsistent results regarding the link between different SNPs of the *NET* gene and ADHD, the present study aims to extend current knowledge by examining the association of two *NET* SNPs most frequently studied with regard to ADHD, namely the only known functional variant rs28386840 and rs3785157, with ADHD-related phenotypes. Specifically, the association of these SNPs with ADHD diagnosis, CBCL externalizing behavior problems and neurocognitive performance in children aged 4 to 15 years was addressed.

2. Methods

2.1. Participants

Participants in this investigation are from the Mannheim Study of Children at Risk, an ongoing epidemiological cohort study of the outcome of early risk factors from infancy into adulthood. Detailed information on this study has been published

previously (e.g. Laucht et al., 1997). A total of 384 children born between 1986 and 1988 of predominantly (> 99.0%) European descent were recruited consecutively according to a two-factorial design intended to enrich and control the risk status in terms of obstetric and psychosocial risks of the sample. For description of the risk factors see Tables 1 and 2. Infants were recruited from two obstetric and six children's hospitals of the Rhine-Neckar Region of Germany. To control for potential confounding effects, only firstborn children with singleton births and German-speaking parents were included in the study. Likewise, children with severe physical handicaps, obvious genetic defects, or metabolic diseases were excluded. After the initial examination at birth, subsequent assessments were conducted at regular intervals throughout development, currently in young adulthood. Of the initial sample of 384 children, 18 (4.7%) were excluded because of severe handicaps (IQ < 70 and/or neurological disorder), 26 (6.7%) were dropouts, and 13 (3.4%) refused to participate in blood sampling. The current investigation included 327 adolescents (155 males, 172 females) who participated in the 15-year assessment and for whom data on *NET* genotype were available. Missing data resulted in different sample sizes for different variables. The study was approved by the ethics committee of the University of Heidelberg and written informed consent was obtained from all participants.

2.2. Psychological assessment

2.2.1. ADHD diagnosis

The Mannheim Parent Interview (MEI) was conducted to assess psychiatric diagnoses in children aged 4 to 11 years (Esser et al., 1989). The MEI is a standardized structured interview adapted from Rutter's parent interviews (Cox and Rutter, 1985), modified to include all symptoms related to major DSM-IV diagnoses. Trained clinical psychologists and child psychiatrists interviewed the parents regarding their child's behavior problems with respect to a broad range of symptoms. Additionally, several symptoms (e.g. hyperactivity and impulsivity) were observed and rated during child examination. Once a child reached school age, the parent interview was supplemented with a child interview. Behavior problems were rated according to severity on a 3-point scale (0=not present, 1=moderately present, 2=severely present). The MEI has been shown to be a sensitive measure of child disturbance (Baving et al., 1999; El Faddagh et al., 2004; Esser et al., 1990; Laucht et al., 2001). Psychiatric diagnoses for adolescents at age 15 years were derived from the Schedule for Affective Disorders and Schizophrenia in School Age Children K-SADS-PL (Ambrosini, 2000). The K-SADS is a widely used structured diagnostic interview conducted independently with parents and adolescents, for which a considerable body of reliability and validity data has been published.

Each participant was assigned all DSM diagnoses for which he or she met the diagnostic criteria. Information from different sources was combined by the logical operator OR. All diagnoses were dichotomous, indicating the presence or absence of a disorder during a specified time period (i.e. six months prior to each assessment). In the present investigation, lifetime diagnoses for ADHD were used, indicating the presence or absence of the disorder during the period of time between 4.5 and 15 years of age. 53 children were diagnosed with ADHD (16.2%). Consistent with epidemiological knowledge, marked gender differences were observed: While lifetime prevalence in boys averaged 10.4%, the respective rate in girls was only 5.8%.

2.2.2. CBCL and YSR

To assess behavior problems during childhood and adolescence, the Child Behavior Checklist (CBCL, Achenbach, 1991a), was completed by the parents when the children were 8, 11 and 15 years old, and the Youth Self-Report Form (YSR, Achenbach, 1991b) was administered to the 15-year-olds. The CBCL is a clinical questionnaire for the assessment of a broad range of problems in young individuals aged 4–18 years as reported by their parents or primary caregivers. It provides scores for two broadband scales (externalizing and internalizing problems) and 8 syndrome scales (withdrawal, somatic complaints, anxious/depressed, social problems, thought problems, attention problems, delinquent behavior and aggressive behavior) as well as for a total problems scale. The YSR is a self-report questionnaire for adolescents aged 12–18 years and was modeled on the CBCL. In the present investigation, z-standardized CBCL scores of the externalizing behavior and attention problem scales were summed up across assessments (8, 11 and 15 years) to create CBCL lifetime scores. In addition, the scores of the externalizing behavior and the attention problems scales of the YSR were z-transformed.

2.2.3. Continuous performance test (CPT)

At age 15 years, adolescents performed a computerized A–X version of the continuous performance test (CPT; constructed by doubling the number of trials of a common previous multicenter version Brandeis et al., 2002; Stanislaw and Todorov, 1999). 800 black-colored capital letters were presented on a white background in the center of the computer screen for 150 ms. The stimulus onset asynchrony (SOA) between the different letters was 1600 ms. Whenever an 'A' was followed by an 'X' (50% probability), participants had to respond with a fast right-hand button press with their index finger on the response pad. The 'A' was followed by an 'X' 80 times and by another letter 80 times. Additionally, single distractor letters were presented. An 'X' without a preceding 'A' occurred 80 times. Another nine letters of the alphabet ('B', 'C', 'D', 'E', 'F', 'G', 'H', 'J', 'L') were employed as distractors. The distractor 'H' occurred 160 times (frequent distractor). The distractors 'B', 'C', 'D', 'E', 'F', 'G', 'J', and 'L' appeared 40 times each. Five outcome measures were derived from CPT performance for the present analyses: number of omission errors (failure to detect target-Xs 200–1350 ms after stimulus onset, 80 possible), hit reaction time (RT, in ms after target-X onset) and hit reaction time standard error (RTSD, variability of hit reaction times). In addition, two nonparametric measures from signal detection theory (SDT) were formed, one for perceptual sensitivity (D') and one for response criterion setting (C, response bias).

2.2.4. Covariates

A psychosocial adversity score was determined by counting the presence of 11 adverse family factors, encompassing characteristics according to an "enriched" family adversity index as proposed by Rutter and Quinton (1997) of the parents, the partnership, and the family environment during a period of one year before the child was born. An obstetric risk score was obtained by determining the presence of nine adverse conditions during pregnancy, delivery, and the postnatal period. Information was derived from a standardized parent interview conducted at the three-month assessment, and from maternal obstetric and infant neonatal records, respectively.

At age 11 intelligence was measured using the Culture Fair Test CFT 20 (Cattell, 1960).

Table 1
Definition of early life psychosocial adversity items.

	Item	Definition
1	Low educational level of a parent	Parent without completed school education or without skilled job training
2	Overcrowding	More than 1.0 person per room or size of housing $\leq 50\ m^2$
3	Parental psychiatric disorder	Moderate to severe disorder according to DSM-III-R criteria (interviewer rating, kappa=0.98)
4	History of parental broken home or delinquency	Institutional care of a parent/more than two changes of parental figures until the age of 18 or history of parental delinquency
5	Marital discord	Low quality of partnership in two out of three areas (harmony, communication, emotional warmth) (interviewer rating, kappa=1.00)
6	Early parenthood	Age of a parent ≤ 18 years at child birth or relationship between parents lasting less than 6 months at time of conception
7	One-parent family	At child birth
8	Unwanted pregnancy	An abortion was seriously considered
9	Poor social integration and support of parents	Lack of friends and lack of help in child care (interviewer rating, kappa=0.71)
10	Severe chronic difficulties	Affecting a parent lasting more than one year (interviewer rating, kappa=0.93)
11	Poor coping skills of a parent	Inadequate coping with stressful events of the past year (interviewer rating, kappa=0.67)

Risk groups.
Non-risk: No item fulfilled.
Moderate-risk: One or two items fulfilled.
High-risk: More than two items fulfilled.

Table 2
Definition of obstetric risk items.

	Item	Definition
1	Normal birthweight	2500–4200 g
2	Normal gestational age	38–42 weeks
3	No signs of aphyxia	pH $\geq$ 7.20, lactid acid $\leq$ 3.5 mmol/L, and CTG-Fischer score $\geq$ 8
4	No surgical delivery	Except elective
5	EPH-gestosis	Toxaemia of pregnancy with oedema, proteinuria, and hypertonia
6	Pre-term birth	Gestational age $\leq$ 37 weeks
7	Pre-term labor	Tocolytic treatment or cerclage
8	Very low birthweight	$<$ 1500 g
9	Clear case of asphyxia	pH $\leq$ 7.10 or lactid acid $\geq$ 8.00 mmol/L or CTG-score $\leq$ 4 with special care treatment for $\geq$ 7 days
10	Neonatal complications	Seizures or respiratory therapy (mechanical ventilation) or sepsis

Risk groups.
Non-risk: All of items1–4 and none of 5–10.
Moderate-risk: One out of items 5–7 and none of 8–10.
High-risk: One out of items 8–10.

2.3. Genotyping

DNA was extracted from whole blood or saliva using the QIAamp (Qiagen, Chatsworth, California) kit. Applied Biosystems (Applied Biosystems, Darmstadt, Germany) genotyping assay for *SLC6A2* rs3785157 was ID C__27481947_10. For *SLC6A2* rs28386840, Applied Biosystems primer and probe sequences were: primer forward: 5′-CCCCACCAGGGCTGAG-3′, primer reverse: 5′-GGAAGGAAACCAGGA-GAAAGTAGAT-3′, VIC-labeled probe: 5′-CACCAGTTTCCCC-3′, FAM-labeled probe: 5′-CACCTGTTTCCCC-3′. Genotyping was performed on an Applied Biosystems 7900HT Fast Real-Time PCR System in accordance with the manufacturer's instructions. Genotype frequencies were 38 (TT), 141 (TC) and 148 (CC) for rs3785157, and 168 (AA), 128 (AT), and 31 (TT) for rs28386840, respectively. Haplotype frequencies estimated by the Expectation–Maximization algorithm were as follows: C–A 44.5%, C–T 22.3%, T–A 26.5%, T–T 6.7%. Distribution of genotypes was in accordance with the Hardy–Weinberg equilibrium (rs3785157: $p=0.62$; rs28386840: $p=0.36$).

2.4. Data analysis

To test differences between *NET* genotype groups in ADHD diagnosis and in basic characteristics variables, χ^2-tests or analyses of variance were performed, as appropriate. Logistic regression analyses were conducted to examine the effect of *NET* genotypes on ADHD lifetime diagnosis, controlling for possible confounders. Genotypes were analyzed as factors with three levels. To explore the effects of *NET* genotypes on CBCL and YSR externalizing behavior and attention problems as well as on CPT performance, analyses of covariance (ANCOVA) were used, followed by post hoc Least Significant Difference tests (LSD) where appropriate. To investigate the effects of *NET* genotypes on CPT number of omission errors, generalized linear models were used assuming a negative binomial distribution of the target variable. All analyses included sex, psychosocial risk score, obstetric risk score, and IQ as covariates. Significant genotype effects were followed by post hoc comparisons to determine which genotype groups differed. Associations between CPT measures and behavioral phenotypes were examined using Pearson correlations.

3. Results

3.1. ADHD diagnosis

Table 3 presents descriptive data for sample characteristics and ADHD lifetime diagnosis, separately for each *NET* SNP genotype and in the total sample. Rs3785157 and rs28386840 genotypes did not significantly differ in terms of sex, obstetric risk score, psychosocial risk score and IQ. However, significant differences between genotype groups emerged with regard to ADHD diagnosis, indicating higher rates of ADHD in homozygous carriers of the minor rs3785157 T allele and the major rs28386840 A allele. This finding was confirmed by logistic regression models testing for the association of rs3785157 and rs28386840 with ADHD lifetime diagnosis while controlling for possible confounders ($\chi^2=7.82$, $df=2$, $p=0.020$ and $\chi^2=6.71$, $df=2$, $p=0.035$, respectively). With regard to rs3785157, post hoc analyses indicated that carriers of the TT genotype exhibited significantly higher rates of ADHD compared to carriers of the other genotypes (TT > CC: $p=0.006$; TT > TC: $p=0.018$). Differences between rs28386840 genotype groups failed to reach statistical significance.

3.2. CBCL and YSR

In Table 4, upper section, adjusted mean CBCL and YSR subscale scores (and SEs) are listed, depending on *NET* genotypes. With regard to rs3785157, analyses of covariance revealed a significant effect of genotype on the externalizing behavior scale of the CBCL, but not of the YSR. Post hoc analyses indicated that carriers of the TT genotype had significantly higher CBCL externalizing scores than carriers of the other variants. In contrast, the differences between rs3785157 genotype groups with regard to the attention problem scale failed to reach statistical significance for the CBCL, while they did become significant for the YSR, with CC carriers reporting fewer attention problems than those with the TC genotype. In the case of rs28386840, no significant effects of genotype were obtained on either the CBCL or YSR scores.

3.3. CPT performance

Table 4, lower section, presents the adjusted means (and SEs) for the CPT measures stratified by *NET* genotypes. There was a significant effect of rs3785157 on the number of omission errors, with individuals carrying the heterozygous TC genotype making fewer errors than carriers of both homozygous CC and TT genotypes (at trend level). In addition, a significant effect of rs3785157 on CPT sensitivity was observed, indicating that TC carriers exhibited higher sensitivity than those with the CC genotype. There were no further statistically significant group differences in CPT scores according to rs3785157 and rs28386840 genotypes. Correlation analyses revealed moderate but significant associations between the neurocognitive functioning measures and the behavioral ADHD phenotypes in the expected direction (see Table 5).

4. Discussion

The norepinephrine transporter is thought to play a crucial role in monoaminergic neurotransmission throughout the central nervous system. Thus, genetic variation of the *NET* gene has been widely discussed to be involved in the pathogenesis of several psychiatric disorders, such as ADHD, major depression (Kim et al., 2014), and panic disorder (Buttenschon et al., 2011). In the current study, we provided evidence that two variants of the gene encoding for *NET* were associated with ADHD lifetime diagnosis

Table 3
ADHD diagnosis and sample characteristics by *NET* genotypes: means and SD (in parenthesis) unless otherwise indicated.

	rs3785157				***rs28386840***				**Total**
	CC $n=148$	**TC** $n=141$	**TT** $n=38$	***p***	**AA** $n=168$	**AT** $n=128$	**TT** $n=31$	***p***	$n=327$
ADHD diagnosis[a] *n (%)*	20 (13.5)	21 (14.9)	12 (31.6)	0.023[b]	36 (21.4)	14 (10.9)	3 (9.7)	0.031[c]	53 (16.2)
Sex *n male (%)*	71 (48.0)	63 (44.7)	21 (55.3)	0.502	80 (47.6)	59 (46.1)	16 (51.6)	0.856	155 (47.4)
Obstetric risk score *(range 0–4)*	1.23 (1.08)	0.99 (1.01)	0.97 (1.00)	0.115	1.04 (1.02)	1.13 (1.07)	1.32 (1.05)	0.347	1.10 (1.04)
Psychosocial risk score *(range 0–7)*	1.91 (1.97)	2.05 (2.20)	1.58 (1.75)	0.446	2.03 (2.15)	1.88 (1.93)	1.61 (1.99)	0.541	1.93 (2.05)
IQ at age 11 years	102.7 (15.4)	103.0 (14.2)	102.8 (13.9)	0.986	102.7 (14.7)	103.5 (15.2)	100.7 (12.4)	0.645	102.8 (14.7)

[a] Lifetime diagnosis.
[b] $\chi^2=7.58$, $df=2$.
[c] $\chi^2=6.96$, $df=2$.

Table 4
CBCL, YSR and CPT measures by *NET* rs3785157 genotype: adjusted means and SE (in parenthesis).

	rs3785157 genotype							**Total**
	CC	**TC**	**TT**	***F*/χ^2 (*df*)**	***p***	**Post hoc**	***p***	
CBCL[a]	$n=132$	$n=131$	$n=32$					$n=295$
Externalizing behavior[b]	−0.146 (0.209)	−0.344 (0.209)	0.883 (0.423)	3.40 (2, 288)	0.035	TC < TT	0.010	0.131 (0.171)
						CC < TT	0.030	
						TC < CC	0.504	
Attention problems[b]	−0.227 (0.197)	−0.205 (0.198)	0.536 (0.400)	1.58 (2, 288)	0.209	–		0.035 (0.162)
YSR[c]	$n=148$	$n=141$	$n=38$					$n=327$
Extern. behavior[d]	0.076 (0.082)	−0.074 (0.084)	0.073 (0.161)	0.90 (2, 320)	0.407	–		0.025 (0.066)
Attention problems[d]	−0.152 (0.080)	0.124 (0.082)	0.191 (0.159)	3.63 (2, 320)	0.028	CC < TC	0.017	0.054 (0.065)
						CC < TT	0.055	
						TC < TT	0.708	
CPT[e]	$n=123$	$n=127$	$n=34$					$n=284$
Omission errors[f]	3.53 (0.363)	2.16 (0.234)	3.25 (0.646)	11.26 (2)	0.004	TC < CC	0.001	3.05 (3.971)
						TC < TT	0.073	
						TT < CC	0.708	
Mean hit reaction time[g]	0.349 (0.005)	0.358 (0.005)	0.340 (0.010)	1.62 (2, 277)	0.201	–		0.349 (0.004)
SD hit reaction time	0.093 (0.003)	0.091 (0.003)	0.092 (0.005)	0.14 (2, 277)	0.872	–		0.092 (0.002)
Sensitivity	4.37 (0.06)	4.61 (0.06)	4.39 (0.12)	3.79 (2, 277)	0.024	TC > CC	0.009	4.46 (0.050)
						TC > TT	0.110	
						TT > CC	0.905	
Response bias	0.303 (0.012)	0.281 (0.012)	0.275 (0.023)	1.10 (2, 277)	0.335	–		0.286 (0.010)

All means adjusted for sex, obstetric risk, psychosocial risk and IQ.
[a] Child Behavior Checklist.
[b] *z*-Standardized raw scores summed up over three assessments (8, 11 and 15 years).
[c] Youth Self-Report.
[d] *z*-standardized raw scores of the 15-year assessment.
[e] Continuous performance test.
[f] Complementary to hits.
[g] In seconds.

Table 5
Pearson correlations between CPT measures and behavioral ADHD phenotypes.

	Omission errors	***Mean hit reaction time***	***SD hit reaction time***	***Sensitivity***	***Response bias***
CBCL					
Externalizing behavior	0.143*	0.053	0.228**	−0.182**	−0.010
Attention problems	0.167**	0.078	0.239**	−0.237**	−0.036
YSR					
Externalizing behavior	0.162**	−0.109	0.133*	−0.165**	0.057
Attention problems	0.147*	−0.035	0.148*	−0.137*	0.081

* $p<0.05$.
** $p<0.01$.

and related phenotypes often associated with ADHD, such as externalizing behavior problems and CPT performance. Specifically, we demonstrated that individuals homozygous for the minor T allele of rs3785157 were significantly more likely to be diagnosed with ADHD and scored significantly higher on CBCL externalizing behavior problems and YSR attention problems between the ages of 8 and 15 years than carriers of other alleles. Moreover, there was a significant association between rs28386840 and ADHD lifetime diagnosis, with higher rates in homozygous carriers of the major A allele.

The findings with regard to rs3785157 and ADHD diagnosis are in line with those of Bobb et al. (2005), who reported the T allele to be preferentially transmitted in ADHD trios as compared to controls. Most recently, Hawi et al. (2013) showed an increased transmission of the T allele just missing significance after permutation adjustment. However, several previous studies failed to replicate these findings (Brookes et al., 2006; Kim et al., 2008b), with one group detecting a trend for an association with ADHD, albeit for the C allele (Xu et al., 2005). A possible explanation for these conflicting results could be that rs3785157 does not represent a causal variant but might rather be related with it through high linkage disequilibrium (Kim et al., 2008b). Our finding that T homozygotes displayed more externalizing behavior problems corresponds well with the results of Sengupta et al. (2012), who identified 3 haplotype blocks within *NET* which were differentially related to comorbid symptoms depending on gender. Rs3785157 was located in the second haplotype block, along with other polymorphisms which were found to be associated with higher rates of externalizing and aggressive behavior in boys.

The current finding of higher ADHD rates in homozygous carriers of the major A allele of rs28386840 is in contrast to previous reports by Kim et al. (2006, 2008a), indicating the minor T allele of this polymorphism to be a "risk-inducing" allele for ADHD. As the T allele of this functional SNP, which is located in the promoter region of the gene, seems to cause alterations in gene expression, it might lead to reduced cell surface density of *NET* transporter proteins, thereby inducing higher tonic dopamine and norepinephrine distribution, especially in frontal brain regions. The functional relevance of this variant has been further corroborated by recent findings of its impact on response to stimulant treatment (Kim et al., 2010) and susceptibility to adverse events (Cho et al., 2012). These effects could be attributed to reduced *NET* density generated by this polymorphism, as the *NET*-blocking efficiency of stimulants may be more marked when transporter levels are low. In contrast, the A allele of rs28386840 was found to result in higher expression levels of *NET*. It has previously been reported (Kim et al., 2006, 2008a) that this variant might induce a higher stability in monoaminergic neurotransmission, thereby acting as "protective" against a diagnosis of ADHD. However, it has to be considered that these initial findings could not be reproduced in several other samples (Cho et al., 2008; Renner et al., 2011). Moreover, contrary to our expectations, we were unable to detect an association of rs28386840 with the CBCL or the YSR behavior problems. Based on Sengupta et al. (2012), we would have hypothesized that rs28386840 would be linked with CBCL attention and internalizing behavior problems in females, as this variant is situated in the first haplotype block. However, in our sample, no significant associations of rs28386840 with ADHD and CBCL internalizing behavior problems in girls were obtained (data not shown).

Furthermore, it has to be taken into account that externalizing behavior problems often co-occur with ADHD (Angold et al., 1999; Biederman et al., 1991), but cannot be considered as ADHD specific or ADHD-related traits. High scores on the CBCL externalizing behavior scale may, however, point to comorbid disorders and phenotypic dimensions, such as oppositional defiant disorder (ODD), conduct disorder (CD) or irritability, the overlap of which being largely explained by shared genetic factors (Dick et al., 2005; Knopik et al., 2014; Kuja-Halkola et al., 2014; Tuvblad et al., 2009). Also, recent studies have emphasized the importance of pleiotropic effects of genetic risk variants of psychiatric disorders (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013).

In addition to investigating associations of *NET* polymorphisms with behavioral phenotypes, we examined the impact of these variants on CPT performance, which we used in terms of a neuropsychological endophenotype. However, we were unable to replicate the findings of Kollins et al. (2008) who reported an association between a specific SNP of the *NET* gene and reaction time variability during CPT performance. While no effect of rs28386840 genotype on CPT performance was observed, results revealed a heterozygous effect of rs3785157, indicating a higher number of CPT omission errors in both C and T homozygotes (the latter at trend level). This result may be explained by the principle of molecular heterosis, defined as a condition in which individuals heterozygous for a specific genotype tend to show a significantly greater or lesser effect for a quantitative trait than both homozygous allele carriers. Comings and MacMurray (2000) proposed different explanations for molecular heterosis, one of which relies on an inverted U-shaped response curve in which either too little or too much gene expression is deleterious. This kind of inverted U-shape curve has been repeatedly related to several gene variants impacting on dopaminergic neurotransmission (e.g. COMT Egan et al., 2001; Mattay et al., 2003; Meyer-Lindenberg et al., 2005) and might possibly also be applicable for the *NET* variants examined here. Another explanation for molecular heterosis suggested by these authors proposed a hidden stratification of the sample due to an independent third factor. In this context, homozygosity of each variant is associated with the highest phenotype scores in different sets of subjects. For instance, it has been shown that ADHD symptoms in homozygous carriers of both allelic variants of a polymorphism located in monoaminooxidase A (MAOA) gene, encoding for an enzyme involved in dopaminergic neurotransmission, are associated with different neural mechanisms as measured by functional magnetic resonance imaging (fMRI, Nymberg et al., 2013). As we did not record functional measurements such as EEG or fMRI during CPT performance, we are unable to disentangle the effects of *NET* genotype on the brain circuits involved.

Several limitations of this study need to be discussed. First, we focused our analysis on only two specific SNPs in the *NET* gene, and might, therefore, have missed possible associations of other *NET* variants with ADHD. As findings from genome-wide association analyses (Mick and Faraone, 2008) supported the hypothesis that the reported associations of rs3785157 with ADHD might rather result from its linkage disequilibrium with another SNP (rs11568324), it would have made sense to also include this variant in our investigation. However, the literature concerning associations of different *NET* genotypes with ADHD remains controversial. Second, our study sample was relatively small in size, which is known to have an impact on power and false discovery rate (Duncan and Keller, 2011). In particular, the numbers of homozygous carriers of the minor alleles of both SNPs were small. Therefore, replications in larger samples with more rs3785157 T and rs28386840 T homozygotes would be desirable. Third, as our study sample is enriched with children born at risk, it might not be possible to generalize the results to a general population sample. However, both obstetric and psychosocial risks were included as covariates in the analyses, adjusting for a possible distorting effect of sample composition. Fourth, compared to the worldwide overall prevalence of ADHD, which is about 5.3 % (Polanczyk et al., 2007), with the German rate being in the same order (Schlack et al., 2014), the rate of ADHD in the prevailing study is significantly increased. However, given the fact that our study sample included individuals at risk and that prospective measurement was used, the high rate is in line with recent findings suggesting increased lifetime prevalence rates by prospective vs. retrospective ascertainment (Moffitt et al., 2010). Fifth, the results could have been biased by population stratification. However, ethnic heterogeneity is unlikely in the study sample, since almost all participants were of German descent and were drawn from the same well-circumscribed geographical area. Moreover, reports have challenged the notion that ethnic admixture leads to significant bias in genetic epidemiological studies

(Hutchison et al., 2004). Finally, it has to be discussed whether CPT performance itself represents an adequate endophenotype of ADHD (as already discussed by Kollins et al., 2008) or whether brain activity during CPT performance (e.g. event related potentials within EEG or BOLD response in fMRI) might instead constitute the actual endophenotype. By using such measures, it might have been possible to discover genotype effects on preparation processes or inhibitory control, which cannot be captured by monitoring task performance. However, the fact that both *NET* variants examined here showed association with ADHD, while rs28386840 was unrelated to CPT challenges the assumption that CPT performance might represent an adequate endophenotype of the categorical diagnosis of ADHD. This consideration is in line with evolving evidence that ADHD is a rather heterogeneous disorder consisting of several subtypes with specific patterns of neuropsychological deficits (e.g. Nigg, 2005; Sonuga-Barke, 2005). Particular neuropsychological measures such as CPT performance might apply only to a subset of individuals with ADHD.

To summarize, this is the first time that associations have been reported between two specific *NET* variants, ADHD and ADHD-related phenotypes over the course of development in a longitudinal community sample. While we were able to establish associations of rs3785157 and rs28386840 with ADHD diagnosis, only the former was associated with higher scores on the externalizing behavior scales of the CBCL. The heterozygous effect of rs3785157 might be most likely explained by the principle of molecular heterosis. However, the findings reported here require replication in larger samples. Moreover, the recording of more direct measures of brain activity such as fMRI or EEG during CPT performance could contribute to a better understanding of genetic effects on the specific brain circuits underlying ADHD.

Conflict of interest

T. Banaschewski served in an advisory or consultancy role for Hexal Pharma, Lilly, Medice, Novartis, Otsuka, Oxford outcomes, PCM scientific, Shire and Viforpharma. He received conference attendance support and conference support or received speaker's fee by Lilly, Medice, Novartis and Shire. He is/has been involved in clinical trials conducted by Lilly, Shire and Viforpharma. Dr. Hohmann received a speaker's fee from Janssen-Cilag. The present work is unrelated to the above grants and relationships. All other authors declare no conflicts of interest.

Contributors

E. Hohm, S. Hohmann and D. Blomeyer analyzed the data and wrote the manuscript. E. Hohm and D. Blomeyer conducted the clinical interviews. J. Treutlein did the genotyping. M. Laucht, M.H. Schmidt, G. Esser, T. Banaschewski, D. Brandeis and C. Jennen contributed to the study design and the data analysis or interpretation. All authors contributed significantly to and have approved the final manuscript.

Acknowledgement

We thank E. Reichert and S. Heinzel who assisted with the data acquisition. This study was supported by grants from the German Research Foundation (DFG; Part of the Special Research Program SFB 258 "Indicators and Risk Models of the Genesis and Course of Mental Disorders") and the Federal Ministry for Education and Research (Grant no. 01EB0110) as part of the Baden-Wuerttemberg Consortium for Addiction Research and the National Genome Research Network (Grant no. 01GS0117).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.psychres.2014.12.029.

References

Achenbach, T.M., 1991a. Manual for the Child Behavior Checklist/4–18 and 1991 Profile. University of Vermont, Department of Psychiatry, Burlington.
Achenbach, T.M., 1991b. Manual for the Youth Self-Report and 1991 Profile. University of Vermont, Department of Psychiatry, Burlington.
Albayrak, O., Friedel, S., Schimmelmann, B.G., Hinney, A., Hebebrand, J., 2008. Genetic aspects in attention-deficit/hyperactivity disorder. Journal of Neural Transmission 115, 305–315.
Ambrosini, P.J., 2000. Historical development and present status of the schedule for affective disorders and schizophrenia for school-age children (K-SADS). Journal of the American Academy of Child and Adolescent Psychiatry 39, 49–58.
Angold, A., Costello, E.J., Erkanli, A., 1999. Comorbidity. Journal of Child Psychology and Psychiatry 40, 57–87.
Arnsten, A.F., 2006. Fundamentals of attention-deficit/hyperactivity disorder: circuits and pathways. The Journal of Clinical Psychiatry 67 (Suppl 8), 7–12.
Arnsten, A.F., Pliszka, S.R., 2011. Catecholamine influences on prefrontal cortical function: relevance to treatment of attention deficit/hyperactivity disorder and related disorders. Pharmacology, Biochemistry & Behavior 99, 211–216.
Banaschewski, T., Becker, K., Scherag, S., Franke, B., Coghill, D., 2010. Molecular genetics of attention-deficit/hyperactivity disorder: an overview. European Child and Adolescent Psychiatry 19, 237–257.
Barr, C.L., Kroft, J., Feng, Y., Wigg, K., Roberts, W., Malone, M., Ickowicz, A., Schachar, R., Tannock, R., Kennedy, J.L., 2002. The norepinephrine transporter gene and attention-deficit hyperactivity disorder. American Journal of Medical Genetics 114, 255–259.
Baving, L., Laucht, M., Schmidt, M.H., 1999. Atypical frontal brain activation in ADHD: preschool and elementary school boys and girls. Journal of the American Academy of Child and Adolescent Psychiatry 38, 1363–1371.
Biederman, J., Faraone, S.V., 2005. Attention-deficit hyperactivity disorder. Lancet 366, 237–248.
Biederman, J., Newcorn, J., Sprich, S., 1991. Comorbidity of attention deficit hyperactivity disorder with conduct, depressive, anxiety, and other disorders. American Journal of Psychiatry 148, 564–577.
Bobb, A.J., Addington, A.M., Sidransky, E., Gornick, M.C., Lerch, J.P., Greenstein, D.K., Clasen, L.S., Sharp, W.S., Inoff-Germain, G., Wavrant-De Vrieze, F., Arcos-Burgos, M., Straub, R.E., Hardy, J.A., Castellanos, F.X., Rapoport, J.L., 2005. Support for association between ADHD and two candidate genes: NET1 and DRD1. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 134B, 67–72.
Brandeis, D., Banaschewski, T., Baving, L., Georgiewa, P., Blanz, B., Warnke, A., Steinhausen, H.C., Rothenberger, A., Scheuerpflug, P., 2002. Multicenter P300 brain mapping of impaired attention to cues in hyperkinetic children. Journal of the American Academy of Child and Adolescent Psychiatry 41, 990–998.
Brookes, K., Xu, X., Chen, W., Zhou, K., Neale, B., Lowe, N., Anney, R., Franke, B., Gill, M., Ebstein, R., Buitelaar, J., Sham, P., Campbell, D., Knight, J., Andreou, P., Altink, M., Arnold, R., Boer, F., Buschgens, C., Butler, L., Christiansen, H., Feldman, L., Fleischman, K., Fliers, E., Howe-Forbes, R., Goldfarb, A., Heise, A., Gabriels, I., Korn-Lubetzki, I., Johansson, L., Marco, R., Medad, S., Minderaa, R., Mulas, F., Muller, U., Mulligan, A., Rabin, K., Rommelse, N., Sethna, V., Sorohan, J., Uebel, H., Psychogiou, L., Weeks, A., Barrett, R., Craig, I., Banaschewski, T., Sonuga-Barke, E., Eisenberg, J., Kuntsi, J., Manor, I., McGuffin, P., Miranda, A., Oades, R.D., Plomin, R., Roeyers, H., Rothenberger, A., Sergeant, J., Steinhausen, H.C., Taylor, E., Thompson, M., Faraone, S.V., Asherson, P., 2006. The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Molecular Psychiatry, 11; pp. 934–953.
Bruno, K.J., Freet, C.S., Twining, R.C., Egami, K., Grigson, P.S., Hess, E.J., 2007. Abnormal latent inhibition and impulsivity in coloboma mice, a model of ADHD. Neurobiology of Disease 25, 206–216.
Bruss, M., Kunz, J., Lingen, B., Bonisch, H., 1993. Chromosomal mapping of the human gene for the tricyclic antidepressant-sensitive noradrenaline transporter. Human Genetics 91, 278–280.
Buttenschon, H.N., Kristensen, A.S., Buch, H.N., Andersen, J.H., Bonde, J.P., Grynderup, M., Hansen, A.M., Kolstad, H., Kaergaard, A., Kaerlev, L., Mikkelsen, S., Thomsen, J.F., Koefoed, P., Erhardt, A., Woldbye, D.P., Borglum, A.D., Mors, O., 2011. The norepinephrine transporter gene is a candidate gene for panic disorder. Journal of Neural Transmission 118, 969–976.
Bymaster, F.P., Katner, J.S., Nelson, D.L., Hemrick-Luecke, S.K., Threlkeld, P.G., Heiligenstein, J.H., Morin, S.M., Gehlert, D.R., Perry, K.W., 2002. Atomoxetine increases extracellular levels of norepinephrine and dopamine in prefrontal cortex of rat: a potential mechanism for efficacy in attention deficit/hyperactivity disorder (official publication of the American College of Neuropsychopharmacology). Neuropsychopharmacology 27, 699–711.

Castellanos, F.X., Tannock, R., 2002. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. Nature Reviews Neuroscience 3, 617–628.
Cattell, R.B., 1960. Culture Fair Intelligence Test, Scale 2 (Handbook), third ed. IPAT, Champaign, IL.
Cho, S.C., Kim, B.N., Cummins, T.D., Kim, J.W., Bellgrove, M.A., 2012. Norepinephrine transporter -3081(A/T) and alpha-2A-adrenergic receptor MspI polymorphisms are associated with cardiovascular side effects of OROS-methylphenidate treatment. Journal of Psychopharmacology 26, 380–389.
Cho, S.C., Kim, J.W., Kim, B.N., Hwang, J.W., Park, M., Kim, S.A., Cho, D.Y., Yoo, H.J., Chung, U.S., Son, J.W., Park, T.W., 2008. No evidence of an association between norepinephrine transporter gene polymorphisms and attention deficit hyperactivity disorder: a family-based and case-control association study in a Korean sample. Neuropsychobiology 57, 131–138.
Comings, D.E., MacMurray, J.P., 2000. Molecular heterosis: a review. Molecular Genetics and Metabolism 71, 19–31.
Cox, A., Rutter, M., 1985. Diagnostic appraisal and interviewing. In: Rutter, M., Hersov, L. (Eds.), Child and Adolescent Psychiatry. Modern Approaches, second ed. Blackwell, Oxford, pp. 233–284.
Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013. Identification of risk loci with shared effects on five major psychiatric disorders: a genome-wide analysis. Lancet 381, 1371–1379.
de Zeeuw, P., Weusten, J., van Dijk, S., van Belle, J., Durston, S., 2012. Deficits in cognitive control, timing and reward sensitivity appear to be dissociable in ADHD. PLoS One 7, e51416.
Del Campo, N., Chamberlain, S.R., Sahakian, B.J., Robbins, T.W., 2011. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biological Psychiatry 69, e145–e157.
Dick, D.M., Viken, R.J., Kaprio, J., Pulkkinen, L., Rose, R.J., 2005. Understanding the covariation among childhood externalizing symptoms: genetic and environmental influences on conduct disorder, attention deficit hyperactivity disorder, and oppositional defiant disorder symptoms. Journal of Abnormal Child Psychology 33, 219–229.
Duncan, L.E., Keller, M.C., 2011. A critical review of the first 10 years of candidate gene-by-environment interaction research in psychiatry. American Journal of Psychiatry 168, 1041–1049.
Egan, M.F., Goldberg, T.E., Kolachana, B.S., Callicott, J.H., Mazzanti, C.M., Straub, R.E., Goldman, D., Weinberger, D.R., 2001. Effect of COMT Val108/158 Met genotype on frontal lobe function and risk for schizophrenia. Proceedings of the National Academy of Sciences of the United States of America 98, 6917–6922.
El Faddagh, M., Laucht, M., Maras, A., Vohringer, L., Schmidt, M.H., 2004. Association of dopamine D4 receptor (DRD4) gene with attention-deficit/hyperactivity disorder (ADHD) in a high-risk community sample: a longitudinal study from birth to 11 years of age. Journal of Neural Transmission 111, 883–889.
Esser, G., Blanz, B., Geisel, B., Laucht, M., 1989. Mannheim Parent Interview—Structured Interview For Child Psychiatric Disorders. Beltz, Weinheim.
Esser, G., Laucht, M., Schmidt, M., Loffler, W., Reiser, A., Stohr, R.M., Weindrich, D., Weinel, H., 1990. Behaviour problems and developmental status of 3-month-old infants in relation to organic and psychosocial risks. European Archives of Psychiatry and Neurological Sciences 239, 384–390.
Fair, D.A., Bathula, D., Nikolas, M.A., Nigg, J.T., 2012. Distinct neuropsychological subgroups in typically developing youth inform heterogeneity in children with ADHD. Proceedings of the National Academy of Sciences of the United States of America 109, 6769–6774.
Faraone, S.V., Mick, E., 2010. Molecular genetics of attention deficit hyperactivity disorder. The Psychiatric Clinics of North America 33, 159–180.
Faraone, S.V., Perlis, R.H., Doyle, A.E., Smoller, J.W., Goralnick, J.J., Holmgren, M.A., Sklar, P., 2005. Molecular genetics of attention-deficit/hyperactivity disorder. Biological Psychiatry 57, 1313–1323.
Faraone, S.V., Sergeant, J., Gillberg, C., Biederman, J., 2003. The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2, 104–113.
Frazier, T.W., Demaree, H.A., Youngstrom, E.A., 2004. Meta-analysis of intellectual and neuropsychological test performance in attention-deficit/hyperactivity disorder. Neuropsychology 18, 543–555.
Gelernter, J., Kruger, S., Pakstis, A.J., Pacholczyk, T., Sparkes, R.S., Kidd, K.K., Amara, S., 1993. Assignment of the norepinephrine transporter protein (NET1) locus to chromosome 16. Genomics 18, 690–692.
Hawi, Z., Matthews, N., Barry, E., Kirley, A., Wagner, J., Wallace, R.H., Heussler, H.S., Vance, A., Gill, M., Bellgrove, M.A., 2013. A high density linkage disequilibrium mapping in 14 noradrenergic genes: evidence of association between SLC6A2, ADRA1B and ADHD. Psychopharmacology (Berl) 225, 895–902.
Hervey, A.S., Epstein, J.N., Curry, J.F., 2004. Neuropsychology of adults with attention-deficit/hyperactivity disorder: a meta-analytic review. Neuropsychology 18, 485–503.
Hutchison, K.E., Stallings, M., McGeary, J., Bryan, A., 2004. Population stratification in the candidate gene study: fatal threat or red herring? Psychological Bulletin 130, 66–79.
Ilott, N.E., Saudino, K.J., Asherson, P., 2010. Genetic influences on attention deficit hyperactivity disorder symptoms from age 2 to 3: a quantitative and molecular genetic investigation. BMC Psychiatry 10, 102.
Joung, Y., Kim, C.H., Moon, J., Jang, W.S., Yang, J., Shin, D., Lee, S., Kim, K.S., 2010. Association studies of -3081(A/T) polymorphism of norepinephrine transporter gene with attention deficit/hyperactivity disorder in Korean population. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 153B, 691–694.
Kim, B., Koo, M.S., Jun, J.Y., Park, I.H., Oh, D.Y., Cheon, K.A., 2009. Association between Dopamine D4 receptor gene polymorphism and scores on a continuous performance test in korean children with attention deficit hyperactivity disorder. Psychiatry Investigation 6, 216–221.
Kim, B.N., Kim, J.W., Hong, S.B., Cho, S.C., Shin, M.S., Yoo, H.J., 2010. Possible association of norepinephrine transporter -3081(A/T) polymorphism with methylphenidate response in attention deficit hyperactivity disorder. Behavioral and Brain Functions 6, 57.
Kim, C.H., Hahn, M.K., Joung, Y., Anderson, S.L., Steele, A.H., Mazei-Robinson, M.S., Gizer, I., Teicher, M.H., Cohen, B.M., Robertson, D., Waldman, I.D., Blakely, R.D., Kim, K.S., 2006. A polymorphism in the norepinephrine transporter gene alters promoter activity and is associated with attention-deficit hyperactivity disorder. Proceedings of the National Academy of Sciences of the United States of America 103, 19164–19169.
Kim, C.H., Waldman, I.D., Blakely, R.D., Kim, K.S., 2008a. Functional gene variation in the human norepinephrine transporter: association with attention deficit hyperactivity disorder. Annals of the New York Academy of Sciences 1129, 256–260.
Kim, J.W., Biederman, J., McGrath, C.L., Doyle, A.E., Mick, E., Fagerness, J., Purcell, S., Smoller, J.W., Sklar, P., Faraone, S.V., 2008b. Further evidence of association between two NET single-nucleotide polymorphisms with ADHD. Molecular Psychiatry 13, 624–630.
Kim, Y.K., Hwang, J.A., Lee, H.J., Yoon, H.K., Ko, Y.H., Lee, B.H., Jung, H.Y., Hahn, S.W., Na, K.S., 2014. Association between norepinephrine transporter gene (SLC6A2) polymorphisms and suicide in patients with major depressive disorder. Journal of Affective Disorders 158, 127–132.
Knopik, V.S., Bidwell, L.C., Flessner, C., Nugent, N., Swenson, L., Bucholz, K.K., Madden, P.A., Heath, A.C., 2014. DSM-IV defined conduct disorder and oppositional defiant disorder: an investigation of shared liability in female twins. Psychological Medicine 44, 1053–1064.
Kollins, S.H., Anastopoulos, A.D., Lachiewicz, A.M., FitzGerald, D., Morrissey-Kane, E., Garrett, M.E., Keatts, S.L., Ashley-Koch, A.E., 2008. SNPs in dopamine D2 receptor gene (DRD2) and norepinephrine transporter gene (NET) are associated with continuous performance task (CPT) phenotypes in ADHD children and their families. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 147B, 1580–1588.
Kostrzewa, R.M., Kostrzewa, J.P., Kostrzewa, R.A., Nowak, P., Brus, R., 2008. Pharmacological models of ADHD. Journal of Neural Transmission 115, 287–298.
Kuja-Halkola, R., Lichtenstein, P., D'Onofrio, B.M., Larsson, H., 2014. Codevelopment of ADHD and externalizing behavior from childhood to adulthood. Journal of Child Psychology and Psychiatry, http://dx.doi.org/10.1111/jcpp.12340.
Lasky-Su, J., Neale, B.M., Franke, B., Anney, R.J., Zhou, K., Maller, J.B., Vasquez, A.A., Chen, W., Asherson, P., Buitelaar, J., Banaschewski, T., Ebstein, R., Gill, M., Miranda, A., Mulas, F., Oades, R.D., Roeyers, H., Rothenberger, A., Sergeant, J., Sonuga-Barke, E., Steinhausen, H.C., Taylor, E., Daly, M., Laird, N., Lange, C., Faraone, S.V., 2008. Genome-wide association scan of quantitative traits for attention deficit hyperactivity disorder identifies novel associations and confirms candidate gene associations. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 147B, 1345–1354.
Laucht, M., Esser, G., Schmidt, M.H., 1997. Developmental outcome of infants born with biological and psychosocial risks. Journal of Child Psychology and Psychiatry 38, 843–853.
Laucht, M., Esser, G., Schmidt, M.H., 2001. Differential development of infants at risk for psychopathology: the moderating role of early maternal responsivity. Developmental Medicine and Child Neurology 43, 292–300.
Loo, S.K., Specter, E., Smolen, A., Hopfer, C., Teale, P.D., Reite, M.L., 2003. Functional effects of the DAT1 polymorphism on EEG measures in ADHD. Journal of the American Academy of Child and Adolescent Psychiatry 42, 986–993.
Manor, I., Corbex, M., Eisenberg, J., Gritsenkso, I., Bachner-Melman, R., Tyano, S., Ebstein, R.P., 2004. Association of the dopamine D5 receptor with attention deficit hyperactivity disorder (ADHD) and scores on a continuous performance test (TOVA). American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 127B, 73–77.
Mattay, V.S., Goldberg, T.E., Fera, F., Hariri, A.R., Tessitore, A., Egan, M.F., Kolachana, B., Callicott, J.H., Weinberger, D.R., 2003. Catechol *O*-methyltransferase val158-met genotype and individual variation in the brain response to amphetamine. Proceedings of the National Academy of Sciences of the United States of America 100, 6186–6191.
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. American Journal of Medical Genetics 114, 665–666.
Meyer-Lindenberg, A., Kohn, P.D., Kolachana, B., Kippenhan, S., McInerney-Leo, A., Nussbaum, R., Weinberger, D.R., Berman, K.F., 2005. Midbrain dopamine and prefrontal function in humans: interaction and modulation by COMT genotype. Nature Reviews Neuroscience 8, 594–596.
Meyer-Lindenberg, A., Weinberger, D.R., 2006. Intermediate phenotypes and genetic mechanisms of psychiatric disorders. Nature Reviews Neuroscience 7, 818–827.
Mick, E., Faraone, S.V., 2008. Genetics of attention deficit hyperactivity disorder. Child and Adolescent Psychiatric Clinics of North America 17, 261–284 (vii-viii).
Moffitt, T.E., Caspi, A., Taylor, A., Kokaua, J., Milne, B.J., Polanczyk, G., Poulton, R., 2010. How common are common mental disorders? Evidence that lifetime prevalence rates are doubled by prospective versus retrospective ascertainment. Psychological Medicine 40, 899–909.

Nigg, J.T., 2005. Neuropsychologic theory and findings in attention-deficit/hyperactivity disorder: the state of the field and salient challenges for the coming decade. Biological Psychiatry 57, 1424–1435.
Nymberg, C., Jia, T., Ruggeri, B., Schumann, G., 2013. Analytical strategies for large imaging genetic datasets: experiences from the IMAGEN study. Annals of the New York Academy of Sciences 1282, 92–106.
Park, S., Kim, J.W., Yang, Y.H., Hong, S.B., Park, M.H., Kim, B.N., Shin, M.S., Yoo, H.J., Cho, S.C., 2012. Possible effect of norepinephrine transporter polymorphisms on methylphenidate-induced changes in neuropsychological function in attention-deficit hyperactivity disorder. Behavioral and Brain Functions: BBF 8, 22.
Pliszka, S.R., 2005. The neuropsychopharmacology of attention-deficit/hyperactivity disorder. Biological Psychiatry 57, 1385–1390.
Polanczyk, G., de Lima, M.S., Horta, B.L., Biederman, J., Rohde, L.A., 2007. The worldwide prevalence of ADHD: a systematic review and metaregression analysis. The American Journal of Psychiatry 164, 942–948.
Porzgen, P., Bonisch, H., Bruss, M., 1995. Molecular cloning and organization of the coding region of the human norepinephrine transporter gene. Biochemical and Biophysical Research Communications 215, 1145–1150.
Renner, T.J., Nguyen, T.T., Romanos, M., Walitza, S., Roser, C., Reif, A., Schafer, H., Warnke, A., Gerlach, M., Lesch, K.P., 2011. No evidence for association between a functional promoter variant of the Norepinephrine Transporter gene SLC6A2 and ADHD in a family-based sample. Attention Deficit and Hyperactivity Disorders 3, 285–289.
Russell, V.A., 2007. Neurobiology of animal models of attention-deficit hyperactivity disorder. Journal of Neuroscience Methods 161, 185–198.
Rutter, M., Quinton, D., 1977. Psychiatric disorder - ecological factors and concepts of causation. In: McGurk, M. (ed). Ecological factors in human development. North Holland: Amsterdam. pp 173-187.
Schlack, R., Mauz, E., Hebebrand, J., Holling, H., Ki, G.G.S.S.G., 2014. [Has the prevalence of parent-reported diagnosis of attention deficit hyperactivity disorder (ADHD) in Germany increased between 2003–2006 and 2009–2012? Results of the KiGGS-study: first follow-up (KiGGS Wave 1)]. Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 57, 820–829.
Seeman, P., Madras, B.K., 1998. Anti-hyperactivity medication: methylphenidate and amphetamine. Molecular Psychiatry 3, 386–396.
Seneca, N., Gulyas, B., Varrone, A., Schou, M., Airaksinen, A., Tauscher, J., Vandenhende, F., Kielbasa, W., Farde, L., Innis, R.B., Halldin, C., 2006. Atomoxetine occupies the norepinephrine transporter in a dose-dependent fashion: a PET study in nonhuman primate brain using (*S*,*S*)-[18F]FMeNER-D2. Psychopharmacology (Berl) 188, 119–127.
Sengupta, S.M., Grizenko, N., Thakur, G.A., Bellingham, J., DeGuzman, R., Robinson, S., TerStepanian, M., Poloskia, A., Shaheen, S.M., Fortier, M.E., Choudhry, Z., Joober, R., 2012. Differential association between the norepinephrine transporter gene and ADHD: role of sex and subtype. Journal of Psychiatry & Neuroscience 37, 129–137.
Song, D.H., Jhung, K., Song, J., Cheon, K.A., 2011. The 1287 G/A polymorphism of the norepinephrine transporter gene (NET) is involved in commission errors in Korean children with attention deficit hyperactivity disorder. Behavioral and Brain Functions 7, 12.
Sontag, T.A., Tucha, O., Walitza, S., Lange, K.W., 2010. Animal models of attention deficit/hyperactivity disorder (ADHD): a critical review. Attention Deficit and Hyperactivity Disorders 2, 1–20.
Sonuga-Barke, E.J., 2005. Causal models of attention-deficit/hyperactivity disorder: from common simple deficits to multiple developmental pathways. Biological Psychiatry 57, 1231–1238.
Sonuga-Barke, E.J., Bitsakou, P., Thompson, M., 2010. Beyond the dual pathway model: evidence for the dissociation of timing, inhibitory, and delay-related impairments in attention-deficit/hyperactivity disorder. Journal of the American Academy of Child and Adolescent Psychiatry 49, 345–355.
Stanislaw, H., Todorov, N., 1999. Calculation of signal detection theory measures. Behavior Research Methods, Instruments, & Computers 31, 137–149.
Tuvblad, C., Zheng, M., Raine, A., Baker, L.A., 2009. A common genetic factor explains the covariation among ADHD ODD and CD symptoms in 9–10 year old boys and girls. Journal of Abnormal Child Psychology 37, 153–167.
Wilens, T.E., 2006. Mechanism of action of agents used in attention-deficit/hyperactivity disorder. The Journal of Clinical Psychiatry 67 (Suppl 8), 32–38.
Wong, D.T., Threlkeld, P.G., Best, K.L., Bymaster, F.P., 1982. A new inhibitor of norepinephrine uptake devoid of affinity for receptors in rat brain. The Journal of Pharmacology and Experimental Therapeutics 222, 61–65.
Xu, X., Knight, J., Brookes, K., Mill, J., Sham, P., Craig, I., Taylor, E., Asherson, P., 2005. DNA pooling analysis of 21 norepinephrine transporter gene SNPs with attention deficit hyperactivity disorder: no evidence for association. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics 134B, 115–118.