Effects of Norepinephrine Transporter Gene Variants on NET Binding in ADHD and Healthy Controls Investigated by PET

Helen L. Sigurdardottir,¹ Georg S. Kranz,¹ Christina Rami-Mark,² Gregory M. James,¹ Thomas Vanicek,¹ Gregor Gryglewski,¹ Alexander Kautzky,¹ Marius Hienert,¹ Tatjana Traub-Weidinger,² Markus Mitterhauser,² Wolfgang Wadsak,² Marcus Hacker,² Dan Rujescu,³ Siegfried Kasper,¹ and Rupert Lanzenberger¹*

¹Department of Psychiatry and Psychotherapy, Medical University of Vienna, Vienna, Austria
²Department of Biomedical Imaging and Image-Guided Therapy, Division of Nuclear Medicine, Medical University of Vienna, Vienna, Austria
³Department of Psychiatry, University of Halle, Halle, Germany

Abstract: Attention deficit hyperactivity disorder (ADHD) is a heterogeneous disorder with a strong genetic component. The norepinephrine transporter (NET) is a key target for ADHD treatment and the NET gene has been of high interest as a possible modulator of ADHD pathophysiology. Therefore, we conducted an imaging genetics study to examine possible effects of single nucleotide polymorphisms (SNPs) within the NET gene on NET nondisplaceable binding potential (BPND) in patients with ADHD and healthy controls (HCs). Twenty adult patients with ADHD and 20 HCs underwent (S,S)-[18F]FMeNER-D₂ positron emission tomography (PET) and were genotyped on a MassARRAY MALDI-TOF platform using the Sequenom iPLEX assay. Linear mixed models analyses revealed a genotype-dependent difference in NET BPND between groups in the thalamus and cerebellum. In the thalamus, a functional promoter SNP (−3081 A/T) and a 5’-untranslated region (5’UTR) SNP (−182 T/C), showed higher binding in ADHD patients compared to HCs depending on the major allele. Furthermore, we detected an effect of genotype in HCs, with major allele carriers having lower binding. In contrast, for two 3’UTR SNPs (*269 T/C, *417 A/T), ADHD subjects had lower binding in the cerebellum compared to HCs depending on the major allele. Additionally, symptoms of hyperactivity and impulsivity correlated with NET BPND in the cerebellum depending on genotype. Symptoms correlated positively with cerebellar NET BPND for the major allele, while symptoms correlated negatively to NET BPND in minor allele carriers. Our findings support the role of genetic influence of the NE system on NET binding to be pertubated in ADHD.

Hum Brain Mapp 37:884–895, 2016.

© 2015 The Authors Human Brain Mapping Published by Wiley Periodicals, Inc.

Contract grant sponsor: the Austrian Science Fund (FWF; R.L.; Project No: 22981)
Conflict of interest: The authors declare no conflict of interest with regards to this paper.
*Correspondence to: Rupert Lanzenberger, Neuroimaging Labs (NIL) – PET & MRI & EEG & Chemical Lab, Department of Psychiatry and Psychotherapy, Medical University of Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria. E-mail: rupert.lanzenberger@meduniwien.ac.at
Received for publication 30 June 2015; Revised 18 November 2015; Accepted 18 November 2015.
DOI: 10.1002/hbm.23071
Published online 17 December 2015 in Wiley Online Library (wileyonlinelibrary.com).

© 2015 The Authors Human Brain Mapping Published by Wiley Periodicals, Inc.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is the most frequent neurodevelopmental disorder diagnosed in children. It is characterized by inattention, hyperactivity, and impulsiveness which frequently leads to severe social, academic, and vocational dysfunction [De La Fuente et al., 2013]. In around 30% of ADHD cases, the symptoms persist through adolescence into adulthood [Barbaresi et al., 2013]. Symptoms differ in adults compared to children, such as hyperactivity decreases while problems with inattention persist [Volkow and Swanson, 2013]. ADHD has a strong genetic component with a heritability estimated to be around 0.77 [Curatolo et al., 2009]. Though the heritability is rather high in ADHD, studies have failed to indicate a single gene responsible for the course of ADHD, suggesting complex polygenetic mechanisms and gene environment interactions to be of importance [Banaschewski et al., 2010].

Norepinephrine (NE) neurotransmission has been hypothesized to be altered in various disorders, such as depression, PTSD, Alzheimer’s disease, and ADHD [Biederman and Spencer, 1999; Gulyas et al., 2010; Klimpek et al., 1997; Pietrzak et al., 2013]. NE has long been discussed to be dysregulated in ADHD since frequently prescribed psychopharmacota such as methylphenidate (MPH) and atomoxetine (ATX) target the dopaminergic and NE systems by increasing the extracellular neurotransmitter levels through inhibition of the respective reuptake transporters [Hannestad et al., 2010; Logan et al., 2007]. MPH and ATX, a selective NE reuptake inhibitor, have been proven clinically effective in improving core symptoms in ADHD [Asherson et al., 2014], although up to 40% of patients being ascribed to stimulant and nonstimulant medication do not respond [Newcorn et al., 2008, 2009]. Recently, guanfacine, an alpha-2 adrenergic receptor agonist, has also been used as an effective treatment option for patients with ADHD [Newcorn et al., 2013]. It is, therefore, likely that alterations in the NE system may predispose to ADHD and thus, the norepinephrine transporter (NET) gene is suspected to play a major role in ADHD pathogenesis. The gene encoding for the NET (SLC6A2) contains certain single nucleotide polymorphisms (SNPs) that have been investigated in pathological conditions [Hahn and Blakely, 2007]. In association and linkage studies, various SNPs have been found to be involved throughout the ADHD population [Kim et al., 2006a; Sengupta et al., 2012]. Results, however, have varied, and there is some contradictory results confounding this theory [Barr et al., 2002; Xu et al., 2005].

As for in vivo brain quantification of the NET, specifically in ADHD, literature is quite scarce until now. In a recently published study, our group demonstrated no differences in NET nondisplaceable binding potential (BP_{ND}) in patients with ADHD compared to healthy controls (HCs) [Vanicek et al., 2014]. It is of high interest to examine whether genetic variants in the NE system have an effect on NET BP_{ND} which could shed light on individual differences in susceptibility to ADHD. Additionally, to the best of our knowledge, no positron emission tomography (PET) study is available so far investigating polymorphisms in the NE system on the NET binding, neither in HCs nor in patients with ADHD.

Thus, the aim was to examine the relationship between the effects of SNPs in the NE system and the NET BP_{ND} in a cohort comprising of ADHD subjects and HCs matched for age and sex. We hypothesized that ADHD subjects carrying either major or minor alleles will have higher binding compared to their healthy matched controls. High binding subcortical regions believed to be principal areas in behavioral and attentional control were selected [Arnsten and Rubia, 2012], whereas cortical regions were dismissed due to the defluorination and bone spill over of the radioligand (S,S)-[18F]FMoNER-D2. Moreover, we hypothesized that the symptoms of hyperactivity and impulsivity would correlate to with NET BP_{ND} in areas related to motoric activity (putamen, cerebellum, midbrain) whilst symptoms of inattention would correlate with NET BP_{ND} in the thalamus.

MATERIALS AND METHODS

Subjects

Twenty adult ADHD patients (age ± SD: 30.8 ± 10.9, 14 males) and 20 HCs (age ± SD: 30.4 ± 10.9, 14 males) were recruited through ADHD outpatient clinic at the Department of Psychiatry and Psychotherapy, Medical University of Vienna, and from the local community via advertisement as previously published elsewhere [Vanicek et al., 2014]. All patients had been free from psychopharmacological treatment for at least 6 months prior to screening visit. During the prescreening, medical examinations including withdrawal of blood samples were performed to ensure physical well being of participants. All participants underwent a multidrug urine test to assess current substance abuse. For inclusion, patients had to have a current ADHD diagnosis as well as a history of childhood ADHD. Five of the 20 patients had their first diagnosis in childhood ADHD. Subjects were interviewed using the Conners’ Adult ADHD Diagnostic Interview for DSM-IV (CAADID, Connors, 1999), Conners’ Adult ADHD Rating Scale Investigator-Screen Version (CAARS-Inv:SV), Conners’
Linkage disequilibrium plot of single nucleotide polymorphisms (SNPs) considered for inclusion. Depicted are 11 genotyped SNPs and the pairwise $R^2$ between them. At the top are the relative positions of the SNPs to one another on the NET gene. Below are the rs numbers for each corresponding SNP and the color scheme shows the strength of the of their $R^2$ value. White $R^2 = 0$, shades of gray $0 < R^2 < 1$ and black $R^2 = 1$.

**Selection of Single Nucleotide Polymorphisms**

Eleven SNPs (Fig. 1) were considered for inclusion, which were selected upon previous association studies [Bobb et al., 2005; Sengupta et al., 2012; Thakur et al., 2012] and functional effect studies [e.g., functional promoter −3081 A/T (rs28386840), where the minor allele (T) has been shown to decrease promoter activity] [Kim et al., 2006a]. Three SNPs were included in genotyping to extend the 3′ flanking region (rs15534, rs40615, rs7188230). Furthermore, three SNPs were chosen to extend the 5′ region of NET (rs2397771, rs168924, rs2242246). Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview/) was used to test whether frequencies were according to Hardy–Weinberg equilibrium. Furthermore, the tag function in Haploview was used to identify SNPs in high-linkage disequilibrium. It refers to the nonrandom association of alleles at different loci, allowing an identification of genetic variations by the information derived from one or more SNPs [Hu et al., 2004; Stram, 2004]. Thus, these tagged SNPs were used for further analysis. For final analysis, the following four SNPs were used as identified using the tag function in Haploview: −3081 A/T (rs28386840) and −182 T/C (rs2242446) ($r^2 = 0.869$), and *269 T/C (rs15534) and *417 A/T (rs40615) ($r^2 = 0.866$). For simplicity’s sake, they will be referred to by their rs number in this article.

**Genotyping**

Procedures were performed as previously described [Baldinger et al., 2014]. In short, 9 ml EthyleneDiamineTetraacetic Acid (EDTA) blood samples were drawn from each subject and DNA was isolated from whole blood using the QiaAmp DNA blood maxi kit (Qiagen, Hilden, Germany). Genotyping was performed using the iPLEX assay on the MassARRAY MALDI-TOF mass spectrometer as described [Oeth et al., 2009]. Allele specific extension products were identified and genotypes allocated by Typer 3.4 Software (Sequenom, San Diego, CA). All applied quality criteria were met [individual call rate >80%, SNP call rate >99%, identity of genotyped of CEU trios (Coriell Institute for Medical research, Camden, NJ) with HapMap database >99%].

**Positron Emission Tomography**

Scans were conducted at the Department of Biomedical and Image-guided Therapy, Division of Nuclear Medicine at the Medical University of Vienna. Each subject underwent a PET (General Electric Medial Systems, Milwaukee, WI) scan using the tracer (S,S)-[18F]FMeNER-D2 synthesized as previously described [Rami-Mark et al., 2013]. (S,S)-[18F]FMeNER-D2 is currently the most suitable radioligand for in vivo NET quantification previously described [Vanicek et al., 2014]. Briefly, fluorine-18-labelled reboxetine analogue allows, due to its long half-life ($t_{1/2} = 109.77$ min) and excellent affinity and selectivity, to reach the specific binding equilibrium within the time-frame of the PET measurement. A 5-min transmission scan using a retracable 68Ge rod sources for tissue attenuation correction was performed prior to the dynamic emission scan acquired in 3-D mode. Data acquisition started 120 min after a bolus i.v. injection of 4.7 MBq/kg body weight (ADHD patients: 393 ± 95 MBq, HC: 384 ± 61 MBq; $P > 0.05$, t-test) of (S,S)-[18F]FMeNER-D2. Mean specific radioactivity of (S,S)-[18F]FMeNER-D2 was 537 ± 383 GBq/µmol (ADHD patients) and 473 ± 218 GBq/µmol (HC) ($P > 0.05$, t-test). Brain radioactivity was measured in a series of six consecutive time frames lasting 10 min each in the interval of 120–180 min after tracer bolus application. Acquired data were reconstructed in volumes consisting of 35 transaxial sections (128 × 128 matrix) using an iterative filtered back projection algorithm (FORE-ITER) with a spatial resolution of 886.
of 4.36-mm full-width at half maximum 1 cm next to the center of the field of view. For coregistration, magnetic resonance (MR) images were acquired from all participants on a 3-Tesla Philips scanner (Achieva) using a 3-D T1 FFE-weighted sequence, yielding 0.88-mm slice thickness and in plane resolution of 0.8 × 0.8 mm [Vanicek et al., 2014].

**Data Preprocessing and Quantification of NET**

As described previously [Vanicek et al., 2014], each time frame of the dynamic PET scan was realigned to the mean of frames with no head motion, which was identified by visual inspection. These summed realigned images were then coregistered to each individual’s MRI scan using a mutual information algorithm in SPM8 (Wellcome Trust Centre for Neuroimaging, London, UK; http://www.fil.ion.ucl.ac.uk/spm/). Parametric images of NET BPND were computed using the caudate as the reference region. The quantification was done as previously described [Arakawa et al., 2008]. Briefly, the ratio method was used to express the BPND as done as previously described [Arakawa et al., 2008] using the caudate as the reference region. The quantification such as the prefrontal cortex (PFC) were not taken into account. These were the thalamus, midbrain with pons (including the locus coeruleus), putamen, and cerebellum. Cortical regions, such as the thalamus, midbrain with pons (including the locus coeruleus), putamen, and cerebellum. Cortical regions, such as the prefrontal cortex (PFC) were not taken into account due to the bone spillover of (S,S)-[18F]FMENR-D2 inherent to the radioligand. NET BPND for each region was extracted from parametric maps from the Hammers Maximum Probability Atlas [Hammers et al., 2003].

**Regions of Interest**

Four regions of interest (ROIs) were selected including NET rich regions [Schou et al., 2005] as well as regions thought to be “core” regions in behavioral control (inattention, impulsivity, hyperactivity) [Arnsten and Rubia, 2012]. These were the thalamus, midbrain with pons (including the locus coeruleus), putamen, and cerebellum. Cortical regions, such as the prefrontal cortex were not taken into account due to the bone spillover of (S,S)-[18F]FMENR-D2 inherent to the radioligand. NET BPND for each region was extracted from parametric maps from the Hammers Maximum Probability Atlas [Hammers et al., 2003].

**Statistical Analysis**

Descriptive statistics were computed and regional NET BPND values were evaluated for normality using the Shapiro–Wilk test. For each analysis, subject were grouped according to their genotype, that is, minor allele carriers versus major allele homozygotes. Genotype frequencies were determined and found to be distributed according to the Hardy–Weinberg equilibrium (P > 0.1).

To examine the effect of genotypes on NET BPND, linear mixed models for each SNP were computed, using the genotype (homozygous major vs. minor allele carriers) and group (ADHD patients vs. HC) as fixed factors and ROI as a repeated factor and the NET BPND as the dependent variable. Possible effects of cofactors (age and sex) were also tested for and were excluded if insignificant.

A separate model for each SNP was computed as follows; linear mixed model with the factors group, ROI, and genotype as the independent variables and the BPND as the dependent variable. For each model, main effects were tested for, and interactions among ROI, group, and genotype were determined. If rendered significant, further analysis included testing for interaction between group and genotype, separated by ROI. Further analysis included post hoc t-tests.

The model prevailing the best fit was the autoregressive 1 (AR(1)). Individual slopes and intercepts were fitted for each ROI. Further analysis included testing for interaction between group and genotype, separated by ROI. Further analysis included post hoc t-tests.

To test whether there was any effect of behavioural subscales on NET BPND depending on genotypes, Pearson’s correlation coefficient was used. All analyses were computed using SPSS version 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp). Each model was corrected for multiple comparisons using the false discovery rate (FDR) at a significance level of x = 0.05 [Benjamini et al., 2001].

**RESULTS**

Demographics and allele counts of study subjects can be seen in Table I. Control and ADHD groups did not differ significantly in terms of age and sex.

| TABLE I. Demographics, psychological tests, past comorbidities, and allele frequencies between patients with ADHD and HCs |
|--------------------------------------------------------------|
| Age | Sex | SNP rs28386840 | SNP rs2242446 | SNP rs15534 | SNP rs40615 | CAARS Total score | CAARS Hyperactive/Impulsive Inattention | Past comorbidities | Depression | Drug abuse |
|----------|-----|---------------|--------------|-------------|-------------|-------------------|-----------------------|-----------------|-------------|-----------|-----------|
| 30.4 ± 10.9 | M/F | A/T | T/C | C/T | T/A | 0.32 ± 0.82* | 0.21 ± 0.63* | 0.11 ± 0.32* | n = 7 | n = 2 |
| 30.8 ± 10.9 | 14/6 | 9/10 | 9/9 | 13/7 | 12/8 | 37.45 ± 8.23* | 19.45 ± 5.89* | 18 ± 4.78* |          |          |

Significant differences between groups are indicated with * at P < 0.001. Genotype frequencies are shown for major/minor allele carriers.
For rs2242446, three-way interaction was detected among ROI, group, and genotype ($F_{2,96} = 103.57$, $P = 0.003$, $P < 0.05$, corrected). Based on different ROIs, the analysis demonstrated an interaction between group and genotype in the thalamus ($F_{10.05} = 33.90$, $P = 0.003$, $P < 0.05$, corrected). Post hoc t-test revealed that ADHD subjects had higher binding for the major allele (T) ($t = -3.0$, $P = 0.008$, $P < 0.05$, corrected) than controls and no difference was detected for minor allele (C) between groups (Table III and Fig. 2b). Which is likely due to difference in HCs between major and minor allele groups, which did not survive corrections ($t = -2.54$, $P = 0.022$, $P < 0.05$, corrected).

A three-way significant interaction was detected among rs15534 genotype, group, and ROIs ($F_{2.75} = 117.52$, $P = 0.004$, $P < 0.05$, corrected). After separating the analysis by each ROI to determine where the difference was, an interaction was detected between rs15534 genotypes and group in the cerebellum ($F_{7.75} = 35.63$, $P = 0.009$, $P < 0.05$, corrected). Post hoc t-test revealed that controls carrying the major allele (C) in rs15534 had higher binding compared to major allele carrying patients ($t = 3.19$, $P = 0.004$, $P < 0.05$, corrected) (Table IV and Fig. 3a). No difference was detected between minor allele (T) groups, and a trend between patients was detected between major and minor allele groups ($t = 2.09$, $P = 0.051$) and between minor and major allele in HCs ($t = 1.80$, $P = 0.088$).

For the SNP rs40615, a three-way interaction was also observed between genotypes, ROI and group ($F_{2.65} = 108.78$, $P = 0.006$, $P < 0.05$, corrected). Further analysis demonstrated an interaction in the cerebellum between genotypes and status ($F_{8.94} = 35.41$, $P = 0.005$, corrected).
Values given are degrees of freedom (df), F values, and P values

**Behavioral Correlation**

To test whether these effects were associated with specific ADHD symptoms, scores from CAARS-Inattentiveness and CAARS hyperactivity/impulsiveness were tested between genotype groups and NET BPND. No correlation of symptoms with NET binding was detected in any region in patient groups separated by SNPs rs28386840 and rs2242446 with any region. Conversely, a significant correlation was detected between the behavioral subscales CAARS hyperactivity/impulsiveness (P < 0.05) and CAARS total score (P < 0.05) with NET BPND in the cerebellum depending on genotype for rs15534 and rs40615. For the major allele in rs15534, CAARS hyperactivity/impulsiveness was positively associated with NET BPND (r = 0.664, P = 0.026). For the minor allele group, the scale was negatively associated with NET BPND (r = −0.729, P = 0.026) (Fig. 4a). For the CAARS total score, in the major allele group the positive correlation was r = 0.772, P = 0.005 (Fig. 5a). No association was detected for the minor allele. For rs40615, differential association between CAARS hyperactivity/impulsiveness was also detected depending on genotype. Depending on the major allele, the positive association detected was r = 0.689 (P = 0.028). On the contrary, the negative association for the minor allele was r = −0.669 (P = 0.034) (Fig. 4b).

**DISCUSSION**

Here, we report the influence of genetic variants within the NE system and its effect on in vivo NET binding using PET and the radioligand (S,S)-[18F]FMeNER-D2. Our results showed significant differences in cerebellar and thalamic NET binding dependent on genotypes between patients with ADHD and HCs. These were largely due to the impact of NET gene polymorphisms on NET BPND in HCs which is not as pronounced in patients with ADHD. Strikingly, in patients with ADHD, a high correlation between specific behavioural symptoms, that is, hyperactivity/impulsivity, and NET BPND in the cerebellum was detected, an effect which was strongly moderated by genotype.

Our results for the functional promoter SNP (rs28386840) deviate from in vitro experiments which found that the minor (T) allele resulted in decreased promoter activity and the major allele (A) in higher expression [Kim et al., 2006a]. We detected high NET binding for the minor (T) allele carriers which, indicating high expression of this allele. However, a possible reason for this opposite effect are changes in gene expression based epigenetic mechanisms. The T allele was found to bind to transcriptional repressors, slug, and scratch, which result in decreased expression [Kim et al., 2006b]. Slug recruits a corepressor, which in turn recruits histone deacetylase (HDAC) [Shirley et al., 2010] resulting in tighter packing.

**TABLE III. Linear mixed model effects summary for the SNP rs2242446**

| Fixed effects           | Model rs2242446 |
|-------------------------|-----------------|
| Intercept               | 9.10 930.46     |
| group                   | 20.97 0.42 0.52 |
| ROI                     | 80.45 200.78    |
| rs2242446               | 32.83 1.61 0.21 |
| group*ROI*rs2242446     | 103.57 2.90 0.003 |
| separated by ROI        |                 |
| Cerebellum group*rs2242446 | 31.50 0.57 0.46 |
| Midbrain group*rs2242446 | 34.66 6.47 0.026 |
| Putamen group*rs2242446 | 32.12 1.41 0.24 |
| Thalamus group*rs2242446 | 33.90 10.05 0.003 |

Values given are degrees of freedom (df), F values, and P values

| Fixed effects           | Model rs15534 |
|-------------------------|--------------|
| Intercept               | 16.92 733.19 |
| group                   | 33.25 0.29 0.59 |
| ROI                     | 88.09 194.08 |
| rs15534                 | 51.62 0.61 0.44 |
| group*ROI*rs15534       | 117.52 2.75 0.004 |
| separated by ROI        |              |
| Cerebellum group*rs15534 | 35.62 7.73 0.009 |
| Midbrain group*rs15534  | 35.62 0.48 0.56 |
| Putamen group*rs15534   | 35.62 2.78 0.89 |
| Thalamus group*rs15534  | 33.90 0.95 0.34 |

Values given are degrees of freedom (df), F values, and P values.
of the DNA of thus lower transcription of the gene. A counteraction of a repressor, such as degradation, inactivation by interaction with other elements, such as small interfering RNAs or HDAC inhibitors, could lead to overexpression and thus result in a reversed effect of the polymorphism in vivo as observed in our findings [Prelich, 2012; Tuschl, 2001]. Further research is needed to determine the functional effect of the major (A) allele. No significant difference was detected between major and minor allele in ADHD subjects indicating that this effect is not pronounced in ADHD. Moreover, Kim et al. [2006b] found the T allele to be overtransmitted in ADHD, and thus concluded it to be a risk allele for ADHD. Even though no SNP reached significance for association to ADHD in our sample, the strongest effect was seen for the SNP rs28386840 with the A as the associative allele. This is compatible with a recent study by Hohmann et al. [2015] which reports homozygotic A allele carriers to have a higher rate of lifetime ADHD diagnosis. Additionally, another study reported higher response times for ADHD subjects carrying the A allele [Kim et al., 2013]. Nonetheless, one has to bear in mind that studies have been inconsistent, possibly due to confounding factors, such as medication history, individual differences, comorbidities, and differences in sample sizes [de Zubizaray et al., 2008; Leo and Cohen, 2003].

The SNP rs2242446, first determined by Zill et al. [2002] also showed this similar binding in the thalamus as for the functional promoter SNP. This SNP is located on the 5' flanking region of the NET and the functional effects of the 5' flanking region is crucial in transcription regulation [Kim et al., 1999; Meyer et al., 1998]. It has been implicated in antidepressant response to milnacipran in depressed subjects. Yoshida et al. [2004] found that major allele (T) carriers responded better to the treatment than the minor (C) allele. The comorbidity of ADHD and depression ranges from 5% to 40%. Symptoms of depression often overlap with those in ADHD, such as distractibility, poor concentration, and impulsivity [Goodman and Thase, 2009; McIntosh et al., 2009]. In addition to the antidepressant effect of milnacipran, it has also been demonstrated to alleviate symptoms of inattention and impulsivity [Hiraide et al., 2013; Kako et al., 2007]. Here, the major allele carrier ADHD group had higher binding than the major allele HC carriers. Thus, the major allele may lend support to the

**Figure 3.**
Differences in NET BPND in the cerebellum between alleles rs15534 (*269 T/C) (a) and rs40615 (*417 A/T) (b). The white bars depict the major alleles while the gray ones depict the minor alleles. Major allele (C) carriers for rs15534 were (n = 13) for HCs and (n = 11) for ADHD subjects. Minor allele (T) carriers for rs15534 were (n = 7) for controls and (n = 9) for ADHD subjects. Major allele (T) carriers for rs40615 were (n = 12) for HCs and (n = 10) for ADHD subjects. Minor allele (A) carriers for rs40615 were (n = 8) for controls and (n = 10) for ADHD subjects. Error bars indicate 95% confidence interval. Difference between groups is marked with an * in which the difference is at P < 0.05 corrected level significance.

**TABLE V. Linear mixed model effects summary for the SNP rs40615**

| Fixed effects                          | Model rs40615 |
|----------------------------------------|---------------|
| Intercept                              | df           | F value  | P value  |
| group                                  | 14.95        | 749.56   | <.000    |
| ROI                                    | 29.72        | 0.30     | 0.59     |
| rs40615                                 | 86.53        | 200.42   | <.000    |
| group*ROI*rs40615                      | 51.60        | 0.26     | 0.62     |
| Separated by ROI                       | 108.78       | 2.65     | 0.006    |
| Cerebellum group*rs40615               | 35.41        | 8.94     | 0.005    |
| Midbrain group*rs40615                 | 35.94        | 0.08     | 0.78     |
| Putamen group*rs40615                  | 35.79        | 2.05     | 0.16     |
| Thalamus group*rs40615                 | 34.97        | 0.46     | 0.51     |

Values given are degrees of freedom (df), F values, and P values.
use of milnacipran to treat ADHD patients with comorbid depression.

Noticeable, our major novel findings was the inverse effect of genotype on NET BPND between controls and patients for rs15534 and rs40615. Even more intriguingly, we detected correlation of scores on CAARS Hyperactivity/Impulsivity and CAARS total score scales with the NET BPND in the cerebellum for ADHD subject which was strongly modulated by genotype. For both SNPs, in patients carrying the major allele higher NET availability was associated with higher symptom scores. On the contrary, as NET availability decreased, scores increased for the minor allele. From a pharmacological point view, these findings can only explain the higher NET binding found for the minor allele as it reflects lower NE levels. However, further influencing factors remain unclear. These results resemble the classic inverted U-shaped effect as Aston–Jones and associates (1999) established for interaction of LC and NE on task performance. The hypothesis states that ADHD symptoms are due to increased tonic activity in the LC which in turn inhibits the basal activity in the cerebellum and other areas and thus increases motor hyperactivity and impulsivity [Berridge and Waterhouse, 2003; Howells et al., 2012]. Aston–Jones and associates research on monkeys showed that with increased tonic discharge of the LC, phasic activity of LC neurons is decreased. This results in poorer performance on focusing on task stimuli. Moreover, they state that for optimal

| ROI          | rs2836840 | rs2242446 | rs15534 | rs40615 |
|--------------|-----------|-----------|---------|---------|
|              | A/T       | T/C       | C/T     | T/A     |
| Putamen      | 0.16 ± 0.04/0.18 ± 0.04 | 0.16 ± 0.04/0.19 ± 0.05 | 0.18 ± 0.04/0.21 ± 0.03 | 0.19 ± 0.04/0.16 ± 0.02 |
| Midbrain/pons| 0.22 ± 0.08/0.30 ± 0.12 | 0.22 ± 0.08/0.29 ± 0.12 | 0.26 ± 0.10/0.26 ± 0.11 | 0.27 ± 0.10/0.25 ± 0.11 |
| Thalamus     | 0.39 ± 0.07/0.52 ± 0.12 | 0.39 ± 0.07/0.52 ± 0.13 | 0.45 ± 0.13/0.48 ± 0.11 | 0.45 ± 0.13/0.48 ± 0.10 |
| Cerebellum   | 0.20 ± 0.06/0.26 ± 0.06 | 0.20 ± 0.06/0.26 ± 0.07 | 0.24 ± 0.07/0.21 ± 0.06 | 0.25 ± 0.06/0.20 ± 0.06 |

**Figure 4.**

Association between NET BPND in the cerebellum and the CAARS hyperactive/impulsive scale depending on genotype in rs15534 (**269 T/C**) (a) and rs40615 (**417 A/T**) (b). The scatter plot shows the correlation split by major (white circles) and minor (gray circles) alleles in ADHD subjects only. The significance for the SNP rs15534, depending on major allele C was \( P = 0.026 \), and for minor allele T, \( P = 0.026 \). For the SNP rs40615, depending on major allele T, \( P = 0.028 \), for minor allele A, \( P = 0.034 \).
performance, balanced levels of tonic, and phasic activity are needed. If the levels of tonic discharge are too low or too high, attentional performance suffers vastly [Aston-Jones et al., 1999]. Extracellular levels of NE have been demonstrated to follow a positive linear relationship with tonic discharge from the LC [Berridge and Abercrombie, 1999; Florin-Lechner et al., 1996]. With that in mind, for the major allele carriers, the tonic release may be too high as NET binding is lower indicating high levels of extracellular NE. This inverted-U relationship between tonic activity and task performance may explain why we detected this inverse genotype effect. The level of this inverse effect is, therefore, likely determined by the genotype. Along these lines, studies indicate a region specific LC stimulation and LC–NE effect. The LC effect may differ in terms of interaction with receptor subtypes and sensitivity as well as for NE concentration in that region [Berridge and Waterhouse, 2003; Devilbiss et al., 2006]. A study done on healthy rats revealed that tonic stimulation of the LC had differential effects on cortical cells versus cells in the thalamus [Devilbiss and Waterhouse, 2004]. Another study revealed projections to the PFC and the motor cortex to differ [Chandler et al., 2014]. They revealed that neurons projecting from LC to the PFC show more spontaneous activity and are more excitable than those projecting to the motor cortex. The LC might have a differential effect on the thalamus and the cerebellum and this may explain why we detected opposite binding for the major alleles on SNPs located in the 5'UTR versus those in the 3'UTR region.

Another explanation involves the location of these SNPs. They are located in the 5'UTR and 3'UTR regions which have been demonstrated to play an important part in translation, stability and localization of the mRNA. The SNPs rs2836840 and rs2242446 are located within the promoter regions while rs40615 and rs15534 are located downstream at the termination codon. The NET may be deregulated by changes in gene expression, mRNA translation or stability, post-translational modifications such as phosphorylation, protein trafficking, cytoskeleton interaction, and oligomerization [Chatterjee and Pal, 2009]. Substrates involved in the aforementioned processes have also shown to have a regulatory effect on the NET. An injection of the enzyme inhibitor \( \alpha\)-methyl-\( p\)-tyrosine (AMPT), resulted in around 50% reduced levels of NE as well as lower mRNA levels in the brainstem indicating a compensatory mechanism for reduced extracellular NE levels [Xiao et al., 1995]. Furthermore, activation of protein kinase C (PKC) has also been shown to regulate the NET. Activation of PKC is believed to result in redistribution of surface NET as radioligand binding demonstrated a reduction in \( B_{\text{MAX}} \) to NET without any change to \( K_{D} \) [Apparsundaram et al., 1998]. Different location of the SNPs and function may explain why we only detected differences for one allele and why we detected opposite binding on major alleles between SNPs in the 5'UTR and the SNPs in the 3'UTR.

**Limitations and Future Directions**

A limitation of this study is that with (S,S)-\([^{18}F]\)MeNER-D2 cortical areas cannot be properly assessed due to spill over from suspected bone uptake. Therefore, genetic influence in the neocortex could not be examined. Studying the cortex, specifically the frontal cortex due to its vast role in cognitive and behavioral control would be very intriguing to test whether and what effects polymorphisms in the NET gene would have on the binding. Another limitation is that we did not include the CAARS inconsistency index and, therefore, we could not assess whether there was any inconsistencies or irregularities in...
responses within subjects, nor could we analyze that in respect to our data.

The evidence presented in this article gives rise to a role for genetic influence of the NE system and alterations in NE signalling as a part of the pathophysiology contributing to ADHD and thus strengthening the hypothesis of imbalances in NE system in the neurobiological mechanism of ADHD. However, we did not detect any differences in the midbrain and the putamen. We can only hypothesize about the possible reasons for these distinct effects. Our results may suggest that the NET genotype effects modulate the NET availability in a region specific manner. Conversely, we cannot exclude other possibilities, such as other influential genetic or nongenetic factors that interact with these regions. In addition, we did not detect any association of any SNP to ADHD, which is probably due to insufficient power of this sample to assess these subtle effects. Due to the heterogeneous nature of ADHD further research requires larger samples for the validation and for the establishment of potential endophenotypes in ADHD. Replication in vivo and in vitro studies is needed to establish if NET genotypic influence could serve as an endophenotype for NE neurotransmission. Moreover, SNP's within the NET may also be very important in relation to the dopaminergic system. The NET is also responsible for reuptake of dopamine in cortical regions [Morón et al., 2002], and therefore, SNPs could possibly affect the availability of dopamine within the cortex. Future studies could explore the possibility whether there are any effects of the NET on the dopaminergic system.

To conclude, this is the first imaging genetic study showing significant differences in NET BPND in patients with ADHD compared to HCs, depending on their genotype. We find genotypic difference in the thalamus between major and minor alleles for a functional promoter SNP in HCs only. The inverse effect of genotype which was detected in the cerebellum indicates genetic influence of NET on the binding in the cerebellum to differ between groups of ADHD subjects and HCs. The results are compatible with the theory that NE follows an inverted-U-shaped curve. Its effect on differential association of behavioral scales with binding further demonstrates a functional and neuropsychological activity to be imbalanced in ADHD.

ACKNOWLEDGMENTS

We are grateful to Nora D. Volkow, MD (National Institute on Alcohol Abuse and Alcoholism, National Institutes of Health, Bethesda, Maryland, USA) for scientific support in planning the study. We thank Anna Höfflich, MD, Pia Baldinger, MD, PhD, Marie Spies, MD, Mara Stamenkovic, MD, Anastasios Konstantinidis, MD, Alexandra Kutzelnigg, MD, Diana Meshkat, MD, Jan Losak, MD (Department of Psychiatry and Psychotherapy, Medical University of Vienna), and Claudia Klier, MD and Brigitte Hackenberg, MD (Department of Child and Adolescence Medicine, Medical University of Vienna), and Ralf Gößler, MD (Department of Child and Adolescence Psychiatry, Neurological Centre Rosenhügel, Vienna, Austria) for medical support, and Georgios Karanikas, MD, Lucas Nics, MSc, PhD, Daniela Häusler, MSc, PhD, and Cecile Philippe, MSc, PhD (Department of Biomedical Imaging and Image-guided Therapy, Division of Nuclear Medicine, Medical University of Vienna), Markus Savli, PhD and Andreas Hahn, MSc, PhD (Department of Psychiatry and Psychotherapy, Medical University of Vienna) for technical support. Administrative support was provided by Marian Cotten (Department of Psychiatry and Psychotherapy, Medical University of Vienna).

REFERENCES

Apparsundaram S, Schroeter S, Giuvanetti E, Blakely RD (1998): Acute regulation of norepinephrine transport: II. PKC-modulated surface expression of human norepinephrine transporter protein. J Pharmacol Exp Ther 287:744–751.

Aragawa R, Okumura M, Ito H, Seki C, Takahashi H, Takano H, Nakao R, Suzuki K, Okubo Y, Halldin C, Suhrara T (2008): Quantitative analysis of norepinephrine transporter in the human brain using PET with (S,S)–18F-FMeNER-D2. J Nuclear Med 49:1270–1276.

Arntsén AF, Rubia K (2012): Neurobiological circuits regulating attention, cognitive control, motivation, and emotion: Disruptions in neurodevelopmental psychiatric disorders. J Am Acad Child Adolesc Psychiatry 51:356–367.

Asherson P, Buşce C, Saylor K, Tanaka Y, Deberdt W, Upadhyaya H (2014): Efficacy of atomoxetine in adults with attention deficit hyperactivity disorder: An integrated analysis of the complete database of multicenter placebo-controlled trials. J Psychopharmacol 28:837–846.

Aston-Jones G, Rajkowski J, Cohen J (1999): Role of locus coerulescens in attention and behavioral flexibility. Biol Psychiatry 46: 1309–1320.

Baldinger P, Hahn A, Mitterhauser M, Kranz GS, Friedl M, Wadsak W, Kraus C, Ungerbock J, Hartmann A, Giegling I, Rujescu D, Kasper S, Lanzenberger R (2014): Impact of COMT genotype on serotonin-1A receptor binding investigated with PET. Brain Struct Funct 219:2017–2028.

Banachowsk T, Becker K, Scherag S, Franke B, Coghil D (2010): Molecular genetics of attention-deficit/hyperactivity disorder: An overview. Eur Child Adolesc Psychiatry 19:237–257.

Barbaresi WJ, Colligan RC, Weaver AL, Voigt RG, Killian JM, Katusic SK (2013): Mortality, ADHD, and psychosocial adversity in adults with childhood ADHD: A prospective study. Pediatrics 131:637–644.

Barr CL, Kroft J, Feng Y, Wigg K, Roberts W, Malone M, Ickowicz A, Schachar R, Tannock R, Kennedy JL (2002): The norepinephrine transporter gene and attention-deficit hyperactivity disorder. Am J Med Genetics 114:255–259.

Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I (2001): Controlling the false discovery rate in behavior genetics research. Behav Brain Res 125:270–284.

Berridge CW, Abercrombie ED (1999): Relationship between locus coeruleus discharge rates and rates of norepinephrine release within neocortex as assessed by in vivo microdialysis. Neuroscience 93:1263–1270.
Berridge CW, Waterhouse BD (2003): The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84.

Biederman J, Spencer T (1999): Attention-deficit/hyperactivity disorder (ADHD) as a noradrenergic disorder. Biol Psychiatry 46: 1234–1242.

Bobb AJ, Addington AM, Sidransky E, Gornick MC, Lerch JP, Greenstein DK, Clasen LS, Sharp WS, Inoff-Genain G, Vrieze FWD, Arcos-Burgos M, Straub RE, Hardy JA, Castellanos FX, Rapoport JL (2005): Support for association between ADHD and two candidate genes: NET1 and DRD1. Am J Med Genetics B 134B:67–72.

Chandler DJ, Gao WJ, Waterhouse BD (2014): Heterogeneous organization of the locus coeruleus projections to prefrontal and motor cortices. Proc Natl Acad Sci USA 111:6816–6821.

Chatterjee S, Pal JK (2009): Role of 5’- and 3’-untranslated regions of mRNAs in human diseases. Biol Cell 101:251–262.

Curatolo P, Paloscia C, D’Agati E, Moavero R, Pasini A (2009): The neurobiology of attention deficit/hyperactivity disorder. Eur J Paediatr Neurol 13:299–304.

De La Fuente A, Xia S, Branch C, Li X (2013): A review of attention-deficit/hyperactivity disorder from the perspective of brain networks. Front Hum Neurosci 7:192.

de Zubicaray GI, Chiang MC, McMahon KL, Shattuck DW, Toga AW, Martin NG, Wright MJ, Thompson PM (2008): Meeting the Challenges of Neuroimaging Genetics. Brain Imaging Behav 2:258–263.

Devilbiss DM, Waterhouse BD (2004): The effects of tonic locus ceruleus output on sensory-evoked responses of ventral posterior medial thalamic and barrel field cortical neurons in the awake rat. J Neurosci 24:10773–10785.

Devilbiss DM, Page ME, Waterhouse BD (2006): Locus ceruleus regulates sensory encoding by neurons and networks in waking animals. J Neurosci 26:9860–9872.

Florin-Lechner SM, Druhan JP, Aston-Jones G, Valentino RJ (2009): Enhanced norepinephrine release in prefrontal cortex with burst stimulation of the locus coeruleus. Brain Res 742: 89–97.

Goodman DW, Thase ME (2009): Recognizing ADHD in adults with comorbid mood disorders: Implications for identification and management. Postgrad Med 121:20–30.

Gulyas B, Brockschneider D, Nag S, Pavlova E, Kasa P, Belicza Z, Legradi A, Gulya K, Thiele A, Dyrks T, Halldin C (2010): The norepinephrine transporter (NET) radioligand (S,S)-[18F]MeNER-D2 shows significant decreases in NET density in the human brain in Alzheimer’s disease: A post-mortem autoradiographic study. Neurochem Int 56:789–798.

Hahn MK, Blakely RD (2007): The functional impact of SLC6 transporter genetic variation. Annu Rev Pharmacol Toxicol 47: 401–441.

Hammers A, Allom R, Koepp MJ, Free SL, Myers R, Lemieux L, Mitchell TN, Brooks DJ, Duncan JS (2003): Three-dimensional maximum probability atlas of the human brain, with particular reference to the temporal lobe. Hum Brain Mapp 19:224–247.

Hannestad J, Gallezot JD, Planeta-Wilson B, Lin SF, Williams WA, van Dyck CH, Malison RT, Carson RE, Ding YS (2010): Clinically relevant doses of methylphenidate significantly occupy norepinephrine transporters in humans in vivo. Biol Psychiatry 68:854–860.

Hiraide S, Ueno K, Yamaguchi T, Matsumoto M, Yanagawa Y, Yoshioaka M, Togashi H (2013): Behavioural effects of monoamine reuptake inhibitors on symptomatic domains in an animal model of attention-deficit/hyperactivity disorder. Pharmacol Biochem Behav 105:89–97.

Holmung S, Hohm E, Treutlein J, Blomeyer D, Jennen-Steinmetz C, Schmidt MH, Esser G, Banaschewski T, Brandeis D, Laucht M (2015): Association of norepinephrine transporter (NET, SLC6A2) genotype with ADHD-related phenotypes: Findings of a longitudinal study from birth to adolescence. Psychiatry Res 226:425–433.

Howells FM, Stein DJ, Russell VA (2012): Synergistic tonic and phasic activity of the locus coeruleus norepinephrine (LC-NE) arousal system is required for optimal attentional performance. Metab Brain Dis 27:267–274.

Hu XL, Schrodi SJ, Ross DA, Cargill M (2004): Selecting tagging SNPs for association studies using power calculations from genotype data. Hum Hered 57:156–170.

Kato Y, Niwa Y, Toyomaki A, Yamanaka H, Kitagawa N, Denda K, Koyama T (2007): A case of adult attention-deficit/hyperactivity disorder alleviated by milnacipran. Prog Neuropsychopharmacol Biol Psychiatry 31:772–775.

Kim CH, Hahn MK, Joung Y, Anderson SL, Steele AH, Mazi-Re-Robinson MS, Gizer I, Teicher MH, Cohen BM, Robertson D, Waldman ID, Blakely RD, Kim KS (2006a): A polymorphism in the norepinephrine transporter gene alters promoter activity and is associated with attention-deficit hyperactivity disorder. Proc Natl Acad Sci USA 103:19164–19169.

Kim CH, Kim HS, Cubells JF, Kim KS (1999): A previously undescribed intron and extensive 5’ upstream sequence, but not Phox2a-mediated transactivation, are necessary for high level cell type-specific expression of the human norepinephrine transporter gene. J Biol Chem 274:8507–8518.

Kim BN, Kim JW, Cummins TD, Bellgrove MA, Hawi Z, Hong SB, Yang YH, Kim HJ, Shin MS, Cho SC, Kim JC, Song JW, Shin YM, Chang US, Han DH (2013): Norepinephrine genes predict response time variability and methylphenidate-induced changes in neuropsychological function in attention deficit hyperactivity disorder. J Clin Psychopharmacol 33:356–362.

Klimke V, Stockmeier C, Overholser J, Meltzer HY, Kalka S, Dilley G, Ordway GA (1997): Reduced levels of norepinephrine transporters in the locus coeruleus in major depression. J Neurosci 17:4851–4858.

Leo J, Cohen D (2003): Broken brains or flawed studies? A critical review of ADHD neuroimaging research. J Mind Behav 24:29–55.

Logan J, Wang GJ, Telang F, Fowler JS, Alesio D, Zabroski J, Jayne M, Hubbard B, Carter P, Shea C, Xu Y, Muench L, Schlyer D, Learned-Coughlin S, Cosson V, Volkow ND, Ding YS (2007): Imaging the norepinephrine transporter in humans with (S,S)-[11C]O-methyl reboxetine and PET: problems and progress. Nuclear Med Biol 34:667–679.

McIntosh D, Tempel A, Binder C, Levitt A, Faludi A, Rosenbluth M (2006): Functional characterization of the human norepinephrine transporter promoter. J Neurosci 17:4851–4858.

Meyer J, Wiedemann P, Okladnova O, Bruss M, Staab T, Stober G, Riederer P, Bonisch H, Lesch KP (1998): Cloning and functional characterization of the human norepinephrine transporter gene promoter. J Neural Transm 105:1341–1350.

Morón JA, Brockington A, Wise RA, Rocha BA, Hope BT (2002): Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: Evidence from knock-out mouse lines. J Neurosci 22:389–395.

Newcorn JH, Kratochvil CJ, Allen AJ, Casad CT, Ruff DD, Moore RJ, Michelson D, Atwood Methylphenidate Comparative...
Study Group (2008): Atomoxetine and osmotically released methylphenidate for the treatment of attention deficit hyperactivity disorder: Acute comparison and differential response. Am J Psychiatry 165:721–730.

Newcorn JH, Sutton VK, Weiss MD, Sumner CR (2009): Clinical responses to atomoxetine in attention-deficit/hyperactivity disorder: The integrated data exploratory analysis (IDEA) study. J Am Acad Child Adolesc Psychiatry, 48:511–518.

Newcorn JH, Stein MA, Childress AC, Youcha S, White C, Enright G, Rubin J (2013): Randomized, double-blind trial of guanfacine extended release in children with attention-deficit/hyperactivity disorder: Morning or evening administration. J Am Acad Child Adolesc Psychiatry 52:921–930.

Pietrzak RH, Gallozzi JD, Ding YS, Henry S, Potenza MN, Southwick SM, Krystal JH, Carson RE, Neumeister A (2013): Association of posttraumatic stress disorder with reduced in vivo norepinephrine transporter availability in the locus coeruleus. JAMA Psychiatry 70:1199–1205.

Prelich G (2012): Gene overexpression: Uses, mechanisms, and interpretation. Genetics 190:841–854.

Rami-Mark C, Zhang MR, Mitterhauser M, Lanzenberger R, Hacker M, Wadsak W (2013): [(18)F]FMENER-D2: Reliable fully-automated synthesis for visualization of the norepinephrine transporter. Nuclear Med Biol 40:1049–1054.

Schou M, Halldin C, Pike VW, Mozley PD, Dobson D, Innis RB, Farde L, Hall H (2005): Post-mortem human brain autoradiography of the norepinephrine transporter using (S,S)-[18F]FMENER-D2. Eur Neuropsychopharmacol 15:517–520.

Sengupta SM, Grizenko N, Thakur GA, Bellingham J, DeGuzman R, Robinson S, TerStepanian M, Poloska A, Shaheen SM, Fortier ME, Choudhry Z, Joober R (2012): Differential association between the norepinephrine transporter gene and ADHD: Role of sex and subtype. J Psychiatry Neurosci 37:129–137.

Shirley SH, Hudson LG, He J, Kusewitt DF (2010): The skinny on slug. Mol Carcinog 49:851–861.

Stram DO (2004): Tag SNP selection for association studies. Genet Epidemiol 27:365–374.

Thakur GA, Sengupta SM, Grizenko N, Choudhry Z, Joober R (2012): Comprehensive phenotype/genotype analyses of the norepinephrine transporter gene (SLC6A2) in ADHD: Relation to maternal smoking during pregnancy. PloS One, 7:e49616

Tuscher T (2001): RNA interference and small interfering RNAs. ChemBiochem 2:239–245.

Vanicek T, Spies M, Rami-Mark C, Savli M, Hoflich A, Kranz GS, Hahn A, Kutzelnigg A, Traub-Weidinger T, Mitterhauser M, Wadsak W, Hacker M, Volkow ND, Kasper S, Lanzenberger R (2014): The norepinephrine transporter in attention-deficit/hyperactivity disorder investigated with positron emission tomography. JAMA Psychiatry 71:1340–1349.

Volkow ND, Swanson JM (2013): Clinical practice: Adult attention deficit-hyperactivity disorder. N Engl J Med 369:1935–1944.

Xiao Q, Yao Y, Tejani-Butt SM (1995): Acute administration of alpha-methyl-para-tyrosine alters levels of norepinephrine transporter mRNA in the rat brainstem. Brain Res Mol Brain Res 30:389–392.

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. Am J Med Genetics Part B Neuropsychiatric Genetics 134B:115–118.

Yoshida K, Takahashi H, Hibuchi H, Kamata M, Ito K, Sato K, Naito S, Shimizu T, Itoh K, Inoue K, Suzuki T, Nemeroff CB (2004): Prediction of antidepressant response to milnacipran by norepinephrine transporter gene polymorphisms. Am J Psychiatry 161:1575–1580.

Zill P, Engel R, Baghai TC, Juckel G, Frodl T, Muller-Siecheneder F, Zwanzger P, Schule C, Minov C, Behrens S, Rupprecht R, Hegler U, Moller HJ, Bondy B (2002): Identification of a naturally occurring polymorphism in the promoter region of the norepinephrine transporter and analysis in major depression. Neuropsychopharmacology 26:489–493.