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

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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

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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