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Dopamine Transporter (SLC6A3) genotype impacts neurophysiological correlates of cognitive response control in an adult sample of patients with ADHD

Running Title: The impact of SLC6A3 on response control in ADHD

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Abstract

Studies provide ample evidence for a dysfunction in dopaminergic neurotransmission in Attention-Deficit / Hyperactivity Disorder (ADHD). In that respect, a common variable number of tandem repeats (VNTR) polymorphism in the 3' untranslated region (UTR) of the dopamine transporter gene (SLC6A3) has been repeatedly associated with the disorder. Here, we examined the influence of the common 9- and 10-repeat alleles of SLC6A3 on prefrontal brain functioning and cognitive response control in a large sample of adult ADHD patients (n = 161) and healthy controls (n = 109). To this end, we inspected a neurophysiological marker of cognitive response control (NoGo anteriorization, NGA) elicited by means of a Go-NoGo task (continuous performance test, CPT). Within the group of ADHD patients, 9-repeat allele carriers showed a significantly reduced NGA, whereas no influence of SLC6A3 genotype was observed in the control group. In contrast to previous association studies in children, the 9-repeat – not the 10-repeat – allele was associated with functional impairments in our sample of adult ADHD patients. Our findings confirm a significant impact of SLC6A3 genotype on neurophysiological correlates of cognitive response control in ADHD, and indicate that still to-be-identified age-related factors are important variables modulating the impact of genetic factors on endophenotypes.

Keywords: ADHD, Dopamine, DAT, *SLC6A3*, Continuous Performance Test, NoGo-Anteriorisation, CPT, Imaging Genetics

Introduction

Attention-Deficit / Hyperactivity Disorder (ADHD) is one of the most prevalent psychiatric disorders in childhood (Biederman and Faraone, 2005) which – in contrast to previous assumptions – often persists into adolescence and adulthood (Barkley, 1998; Simon *et al*, 2009). A plethora of family, adoption and twin studies demonstrate that heritability plays a major role (see Biederman, 2005; Faraone *et al*, 2005; Faraone and Doyle, 2000 ; Martin *et al*, 2002) as up to 80 percent of the phenotypic variability can be explained by genetic influence (e.g., Faraone and Doyle, 2000; Levy *et al*, 1997). Results from molecular genetic studies support a complex genetic architecture of ADHD, i.e. genetic vulnerability is mediated by a multitude of risk genes with small individual effects (Faraone *et al*, 2005). One of the most extensively studied genes in (childhood) ADHD is SLC6A3 the gene encoding the dopamine transporter (DAT). DAT regulates the reuptake of dopamine from the extracellular space, thereby terminating its synaptic action. A genetic polymorphism identified as a potential risk factor for ADHD is a 40 base pair (bp) variable number of tandem repeats (VNTR) polymorphism within the 3' untranslated region (UTR) of SLC6A3 (Vandenbergh *et al*, 1992). It has two common alleles designated as 9-repeat and 10-repeat which have been suggested to influence SLC6A3 expression and, thereby, dopamine regulation (Mill *et al*, 2005; vanNess *et al*, 2005; Fuke *et al*, 2001; Miller and Madras, 2002; Greenwood and Kelsoe, 2003 Michelhaugh *et al*, 2001). However, these *in vitro* findings concerning the impact of the two allelic variants on SLC6A3 expression remain inconsistent with partly contradicting results.

Meta-analyses of genetic association studies indicate an overrepresentation of the 10-repeat allele in children with ADHD (Faraone *et al*, 2005). However, findings are heterogeneous and may depend on the specific statistical analysis method (Todd *et*

al, 2005; Li *et al*, 2006; Yang *et al*, 2007; Langley *et al*, 2005; Cheuk *et al*, 2006). Furthermore, in adult samples, the 9-repeat allele has been shown to be associated with the disease, suggesting a differential association depending on age, and a role of SLC6A3 in modulating the ADHD phenotype over life time (Franke *et al*, 2008; Franke *et al*, 2010).

These inconsistent findings emphasize the necessity to not only identify potential risk genes for ADHD, but also specific mechanisms by which genetic variants affect the risk for disease. A promising approach might therefore be to relate candidate polymorphisms to specific behavioral, cognitive or neurophysiological phenotypes. These so-called “endophenotypes” are considered to be more directly connected to the relevant underlying psycho- or neuropathology – and thereby etiological factors – than categorical clinical diagnoses. Response inhibition, which plays a key role in many of the neuropsychological models (see Barkley, 1997; Barkley, 1998), has been suggested as a possible endophenotype for ADHD (Sonuga-Barke, 2002; see also Crosbie *et al*, 2008). While brain imaging studies in ADHD confirm functional deficits in areas underlying inhibitory control (e.g., Rubia *et al*, 1999; Smith *et al*, 2006), current concepts additionally consider other aspects of prefrontal response control, e.g. higher-order controlled motor function, attentional control, and mental flexibility (Slaats-Willemse, 2003). Paradigms involving one or more of these processes therefore seem to be particularly suited to study altered cognitive functioning in ADHD and the impact of genetic variants on neural processing.

Behavioral studies in children report a detrimental effect of the 10-repeat allele on neurocognitive performance in vigilance and attention tasks (Loo *et al*, 2003; Bellgrove *et al*, 2005). However, Barkley and colleagues (2006) conducted a longitudinal study on children, adolescents and young adults with ADHD, and report greater ADHD and externalizing symptoms, as well as family, educational and

occupational impairments at all three follow-up examinations in 9/10- compared to 10/10-repeat allele carriers. Also, 9/10-repeat carriers in the control group were prone to higher impairment on educational, occupational and neurocognitive measures.

Neurophysiological measures might have the advantage of being even more closely linked to neurobiological mechanisms than behavioral measures alone (“Imaging Genetics” approach; Fallgatter *et al*, 1999; Bookheimer *et al*, 2000; Ehlis *et al*, 2007; Egan *et al*, 2001). In the present investigation, we therefore used a topographical ERP parameter (NoGo-Anteriorization / NGA), which has been proposed to reflect mechanisms of prefrontal response control, to examine a group of adult ADHD patients and age-matched healthy controls stratified for SLC6A3 genotype (40-bp VNTR within the 3’ UTR). Subjects conducted a Go-NoGo task (Continuous Performance Test / CPT; Rosvold *et al*, 1956) that involves processes of response inhibition and response execution (higher-order motor and attention control). For this paradigm the NGA has been established as a reliable marker reflecting medial prefrontal functioning (Fallgatter *et al*, 1997; Fallgatter and Strik 1999). In schizophrenic patients the NGA was found to be related to variants of the catechol-o-methyltransferase (COMT) gene (Ehlis *et al*, 2007). Furthermore, in one of our previous publications, the tryptophan hydroxylase (TPH2) gene was found to have a significant impact on the NGA in adult ADHD patients (Baehne *et al*, 2009). In the present study, we hypothesized that SLC6A3 genotype would significantly affect the NGA, particularly in ADHD patients. Because of recent results suggesting a specific role of the SLC6A3 9-repeat allele in adult patients with ADHD (Franke *et al*, 2010), we furthermore assumed that the NGA would be particularly altered in adult ADHD patients carrying at least one 9-repeat allele of the VNTR.

Materials and Methods

Participants

161 adult patients, diagnosed with ADHD (DSM-IV criteria), participated in the study. They were recruited via the in- and outpatient facilities of the Department of Psychiatry, Psychosomatics and Psychotherapy of the University of Wuerzburg. 109 healthy subjects without history of psychiatric and neurological disorders were recruited from the local region. All participants gave written informed consent after complete description of the study. Exclusion criteria were age below 18 and above 60 years, IQ below 90, current psychotropic medication, as well as serious somatic or neurological disorders. A total of 171 participants (94 ADHD patients, 77 controls) were also included in one of our previous publications (Baehne *et al*, 2009). All subjects were of Caucasian origin.

Participants were stratified according to their SLC6A3 3' UTR VNTR genotype into a homozygous 10-repeat allele group (10/10) and a group carrying at least one 9-repeat allele (9/9 or 9/10). The 10/10 group comprised 84 patients and 57 controls, the other genotype group 77 patients (9/9: n = 9; 9/10: n = 68) and 52 controls (9/9: n = 8; 9/10: n = 44). Descriptive statistics are displayed in table 1. Genotype frequencies (9/9 & 9/10 vs. 10/10) did not differ between diagnostic groups ($\chi_1^2 < 0.01$, p = 0.99). 10-repeat allele frequencies were in Hardy-Weinberg equilibrium for both patients ($\chi_1^2 = 1.00$, p = 0.32) and controls ($\chi_1^2 = 0.02$, p = 0.90). Gender ratios did not differ between genotypes (ADHD: $\chi_1^2 = 0.05$, p = 0.82; control group: $\chi_1^2 = 2.59$, p = 0.11). Left-handedness was overrepresented in 9/9 and 9/10 carriers with ADHD ($\chi_1^2 = 6.82$, p = 0.01), whereas no differences were found in the

control group ($\chi_1^2 = 0.26$, $p = 0.61$; see table 1). For IQ scores¹, as assessed with the MWT-B (Lehrl 2005), a measure of crystallized verbal intelligence, a 2 x 2 analysis of variance (ANOVA) showed a significant effect for diagnosis ($F_{1, 256} = 6.10$, $p = 0.01$), with higher scores for the controls, but no significant main effect “genotype” ($F_{1, 256} = 0.57$, $p = 0.45$) and no interaction of diagnosis x genotype ($F_{1, 256} = 0.05$, $p = 0.82$). Neither diagnosis nor genotype had an impact on the distribution of age ($F_{1, 266} < 1.00$, $p > 0.50$). As expected, diagnosis had a significant impact on the German short-version of the Wender Utah Rating Scale (WURS-k) ($F_{1, 260} = 218.73$, $p < 0.01$; for a total of 6 patients, no WURS-k score was available due to single items missing), with ADHD patients scoring higher than control participants, whereas genotype and the interaction of diagnosis x genotype had no significant influence ($F_{1, 260} < 0.25$, $p > 0.6$).

According to DSM-IV, 99 patients were classified as the combined subtype, 43 as the inattentive subtype and 10 as the hyperactive/impulsive subtype of ADHD. Subtype composition² did not differ significantly between the two genotype groups ($\chi_2^2 = 0.84$, $p = 0.66$). Regarding current psychiatric comorbidities, as assessed with the Structured Clinical Interview for DSM-IV (SCID-I, Wittchen *et al*, 1997), 47% of the patients displayed a comorbid axis I disorder: 19 patients were diagnosed with substance misuse/dependency (8 for the 9/9 & 9/10 genotype vs. 11 for the 10/10 genotype; specific diagnoses: alcohol abuse [F10.1; n=2] or dependency [F10.2; n=2]; cannabinoid abuse [F12.1; n=3] or dependency [F12.2., n=12]), 32 with mood disorders (18 vs. 14; specific diagnoses: bipolar affective disorders [F31.0, F31.8; n=5], depressive episodes [F32.1, F32.8; n=3], recurrent depressive episodes [F33.0, F33.1; n=8], cyclothymia [F34.0; n=2], dysthymia [F34.1; n=7], and other recurrent

¹ For a total of 10 subjects (4 patients / 6 controls) no IQ data were available.

² For 9 patients subtype diagnosis was missing.

mood disorders [F38.1; n=7]) and 25 with neurotic, stress-related and somatoform disorders (14 vs. 11; specific diagnoses: agoraphobia [F40.0; n=1], social phobias [F40.1; n=10], specific phobias [F40.2; n=6]; panic disorder [F41.0; n=1]; generalized anxiety disorder [F41.1; n=2], obsessive compulsive disorder [F42.0; n=1], post-traumatic stress disorder [F43.1; n=2], bulimia nervosa [F50.2; n=1] and unspecified eating disorders [F50.9; n=1]). Regarding smoking status, 62 ADHD patients (38.5%) and 17 healthy controls (15.6%) were daily tobacco smokers ($\chi_1^2 = 16.49$, $p < 0.001$). The study was reviewed and approved by the Ethics Committee of the University of Wuerzburg, the procedures involved were in accordance with the Declaration of Helsinki.

SLC6A3 genotyping

Genomic DNA was extracted from whole blood samples by salt precipitation according to standard protocols. Genotyping of the SLC6A3 3' UTR VNTR was performed by polymerase chain reaction (PCR) and subsequent gel electrophoresis as published previously (Hünnerkopf *et al*, 2007). Further details on protocols are available upon request.

Electrophysiological investigation

The participants performed an OX-version of the Continuous Performance Test (Rosvold *et al*, 1956). The measurements took place in a dimly lit, quiet room. All the letters were presented sequentially on a computer screen (viewing distance: 80 cm) in pseudo-randomized order. The stimuli were approximately 30 mm high and 20 mm

wide, resulting in a visual angle of 2.15° vertically and 1.43° horizontally. Patients and controls were instructed to press a response button only when the letter O was directly followed by the letter X. Speed and accuracy were emphasized equally during explanation of the test. Before the actual CPT, a short training session was performed. The complete stimulus set consisted of 400 letters (114 letters O = primer condition, 57 X following an O = Go-condition, 57 other letters following an O = NoGo-condition, and 172 letters not following an O = distractors) with a stimulus-onset asynchrony (SOA) of 1850 ms and a stimulus presentation time of 200 ms.

During performance of the task, the continuous EEG was recorded from 21 scalp electrodes placed according to the International 10/20-System (Jasper, 1958). Three additional electrodes were attached at the outer canthi of both eyes and below the right eye for registration of eye movements. The technical equipment consisted of a 32-channel DC-amplifier and the data acquisition software "Vision Recorder" (Brain Products, Munich, Germany). The hardware filter was set to a bandpass from 0.1-100 Hz; A/D rate was 1000 Hz. The recording reference was placed between Fz and Cz, the ground electrode between Fpz and Fz. All electrode impedances were kept below 5 kOhm.

Data analysis

Electrophysiological data were processed using the „Vision Analyzer“ software (Brain Products, Munich, Germany). In a first step, data were filtered offline with a bandpass from 0.1-70 Hz, re-referenced to an average reference and corrected for ocular artifacts using the standard algorithm implemented in the software (Gratton and Coles, 1989). After a computerized artifact rejection (only amplitudes < 70 µV in all

EEG-channels within 100 ms before and 700 ms after stimulus presentation were allowed) artifact-free epochs after correct responses were segmented and individually averaged to Go and NoGo event-related potentials (ERPs). Based on the centroid method proposed by Lehmann (Lehmann, 1987), the individual P300 latencies were determined based on the peaks of the global field power (GFP) in a P300 time-window of 275-530 ms post-stimulus. The GFP is a one-number estimator of the electrical field strength in multi-channel EEG recordings and corresponds to the standard deviation of all measured potential values at a given point of time. At this latency, the two-dimensional topography of both the Go and the NoGo condition was analyzed by means of the area centroids of the P300 field maps. The area centroids are amplitude-weighted locations (centers of gravity) of the positive and the negative areas of the brain electrical field after elimination of the spatial DC offset (average reference). The location of each individual centroid was quantified on an anterior – posterior axis by a coordinate system resulting from the planar projection of the electrode array onto a rectangular grid. Centroids could obtain values between 1 (level of electrode position Fpz) and 5 (level of Oz) as illustrated in Figure 1 (locations somewhere in between two electrode positions were expressed by respective decimal numbers). Smaller values of centroid-locations indicate a more anterior localization. Based on our previous studies (e.g., Fallgatter *et al*, 1997), only the anterior-posterior location of the positive centroid during the Go- and NoGo-condition was considered in more detail. The individual NoGo-Anteriorization (NGA) was calculated as the difference between Go- and NoGo-centroid on the anterior-posterior axis.

Statistical analysis

Statistical analyses were performed with the software SPSS 14.0. For the NGA, a 2 x 2 analysis of variance (ANOVA) was conducted, comprising the between subject factors “diagnosis” and “genotype”. Post-hoc analyses were calculated by means of two-tailed t-tests for independent samples for between-group comparisons of the NGA as well as Go- and NoGo-centroids. T-tests were used to compare mean Go reaction times between the two genotype groups. Equality of variances was tested by means of Levene’s test, corrections for unequality were performed when necessary. Since none of the CPT error data were normally distributed according to Kolmogorov-Smirnov’s Z-statistic (p-values < 0.01), Mann-Whitney U-tests were used for between-group comparisons. Except for the Go-centroid and NGA data in the group of ADHD patients with the 9/9 & 9/10 genotype, all electrophysiological parameters in the four groups were normally distributed. As an ANOVA with a sufficient number of participants per cell is relatively robust against such violations, we decided to apply the 2 x 2 ANOVA approach to our data. The significance level was set to $p < 0.05$.

To conduct exploratory analyses on any allele dose effects, the group comprising the 9/9 and 9/10 genotype was split into two separate groups, one containing only 9/9, the other only 9/10 allele carriers. Despite the low number of subjects in the 9/9 group (ADHD: $n = 9$, controls: $n = 8$), we opted for an – at least declarative – analysis of these subgroups. To this end, we applied a trend test, assuming a linear relationship of the three genotypes (9/9, 9/10, 10/10) regarding their impact on the electrophysiological data.

Moreover, to conduct exploratory analyses we applied source localization with sLoreta software (Pascual-Marqui *et al*, 1994; Pascual-Marqui, 2002; Pascual-Marqui *et al*, 2002) to examine the source of the topographical EEG markers.

Results

Behavioral Data

ADHD patients made more commission errors after primers and distractors (Mann-Whitney $U = 6565.0$, $Z = -3.90$, $p < 0.01$), more omission errors ($U = 6610.5$, $Z = -3.72$, $p < 0.01$) and had elongated reaction times in Go-trials ($U = 6500.0$, $Z = -3.61$, $p < 0.01$) compared to healthy controls. In addition, they showed a greater variability in reaction time than the controls ($U = 5980.0$, $Z = -4.44$, $p < 0.01$). Patients and healthy controls did not differ with respect to the number of commission errors in NoGo-trials ($U = 8619.5$, $Z = -0.46$, $p = 0.65$). Within the whole sample, no significant behavioral differences were found between the two genetic groups (10/10 vs. 9/10 & 9/9), except for marginally significant differences in the number of commission errors after primers and distractors ($U = 8115.5$, $Z = -1.70$, $p = 0.09$, 10/10 group with more errors) and in the number of commission errors in NoGo-trials ($U = 8525.0$, $Z = -1.65$, $p = 0.10$, 10/10 with less errors). These trend-like differences could be attributed to corresponding effects in the control group ($U = 1260.0$, $Z = -1.65$, $p = 0.10$ and $U = 1325.5$, $Z = -1.69$, $p = 0.09$, respectively), whereas no genotype differences emerged within the group of ADHD patients ($p > 0.20$). When the behavioral data was controlled for the influence of intelligence, which differed between ADHD patients and controls (see methods), the results remained virtually unchanged. Descriptive statistics of the behavioral data are shown in table 2.

ERP data

Concerning the NoGo-Anteriorization (NGA; i.e., the difference measure resulting from subtraction of the NoGo- from the Go-centroid: $\text{Centroid}_{\text{Go}} - \text{Centroid}_{\text{NoGo}}$), the 2 x 2 ANOVA conducted to test for effects of the factors “diagnosis” (ADHD vs. control group) and “genotype” (9/10 & 9/9 vs. 10/10) revealed a marginally significant effect for “diagnosis” ($F_{1, 266} = 3.53$, $p = 0.06$) and a significant interaction “diagnosis x genotype” ($F_{1, 266} = 6.42$, $p = 0.01$). A tendency of lower NGA values was observed in patients compared to controls (0.36 ± 0.77 vs. 0.50 ± 0.53). The interaction effect of “diagnosis x genotype” was attributable to the fact that the 3’ UTR VNTR had a significant effect on the NGA only within the group of adult ADHD patients (see Figure 2): More precisely, patients with the 9/9 or 9/10 genotype showed a significantly diminished NGA as compared to patients carrying the 10/10 genotype (0.21 ± 0.78 vs. 0.49 ± 0.74 , $t_{159} = 2.34$, $p = 0.02$). In contrast, the NGA was not affected by SLC6A3 genotype in the group of healthy controls (0.58 ± 0.61 vs. 0.44 ± 0.43 , $t_{92} = 1.42$, $p = 0.16$). Directly comparing patients and controls for each of the two genotype groups also revealed a significant reduction of the NGA in ADHD patients only within the group of 9/9 and 9/10 allele carriers (0.21 ± 0.78 vs. 0.58 ± 0.61 , $t_{127} = 2.87$, $p < 0.01$). No significant difference between diagnostic groups was observed in the 10/10 group (0.49 ± 0.74 vs. 0.44 ± 0.43 , $t_{137} = 0.56$, $p = 0.58$). Subgroup analysis assuming a linear relationship of the three genotypes³ revealed an allele-dose effect of the 10-repeat allele in adult ADHD patients ($F_{1, 158} = 5.18$, $p = 0.02$), but no significant result for the control group ($F_{1, 106} = 1.42$, $p = 0.24$). Within the group of ADHD patients, we further tested whether a quadratic trend may explain more variance than the linear one⁴. As this was not the case ($F_{1, 158} = 0.34$, $p = 0.56$), a linear model seems to be the best model for ADHD

³ This test examines linear relationships by testing the contrast $[-1 \ 0 \ +1]$ weighting individual values according to the number of 10-repeat alleles.

⁴ This test examines quadratic linear relationships by testing the contrast $[+1 \ -2 \ +1]$.

patients (as can be seen in Figure 3). As the diagnostic groups differed in their verbal intelligence scores we conducted an analysis of covariance (ANCOVA) with the same factors mentioned above, while adding the standardized covariate “intelligence”. Results remained virtually unchanged, except for the factor diagnosis that now reached statistical significance ($F_{1, 273} = 4.21, p = 0.04$), with smaller values of the NGA in ADHD patients compared to controls (see Figure 2).

To further elucidate the origin of the NGA effects in our study, we performed an additional analysis on the positions of the Go and NoGo centroids, both of which contribute to the difference measure of the NGA ($NGA = \text{Centroid}_{Go} - \text{Centroid}_{NoGo}$; see above). This analysis revealed that only the Go centroid was significantly affected by the between-subject factors “diagnosis” and “genotype”. More precisely, a significant main effect of the factor “diagnosis” ($F_{1, 266} = 9.03; p < 0.01$) could be explained by a more anterior position of the Go centroid in ADHD patients (mean Go centroid: 3.50 ± 0.74) as compared to healthy controls (mean Go centroid: 3.74 ± 0.49). Moreover, a significant interaction “diagnosis x genotype” occurred ($F_{1, 266} = 4.25, p < 0.05$), indicating that only within the group of ADHD patients carriers of the 9/9 or 9/10 genotype tended to have a more anterior location of the Go centroid (3.40 ± 0.87) than carriers of the 10/10 genotype ($3.60 \pm 0.57; t_{129} = 1.76, p < 0.1$). In accordance with the results for the NGA reported above, patients and controls furthermore differed only within the group of 9/9 and 9/10 allele carriers, with a more anterior location of the Go centroid in ADHD patients (3.40 ± 0.87) as compared to healthy controls ($3.80 \pm 0.50; t_{124} = 3.35, p < 0.01$). For 10/10 allele carriers, no significant impact of the factor “diagnosis” was observed ($t_{139} = 0.82, p = 0.41$).

Explorative analyses with sLoreta software were applied to examine sources of the topographical ERP findings. The source localization indicated that in all genetic

subgroups the NGA could be localized to the anterior cingulate cortex. Furthermore, for this contrast (NoGo – Go), healthy subjects with the 9/9 or 9/10 genotype showed a significantly higher recruitment of cingulate cortices compared to ADHD patients of the same genotype (Figure 4, upper panel). However, for the contrast of the two genotypes within the group of ADHD patients we did not find significant source differences, despite the NGA findings reported above. As our topographic results were mainly driven by the Go centroid, an additional sLORETA analysis was conducted to compare sources for the Go condition within our group of ADHD patients: Here, a marginally significant difference between genetic groups was found that located a difference source in the left inferior parietal cortex, with higher activation in the 10/10 group (Figure 4, lower panel).

Discussion

The present EEG study was conducted to further elucidate the influence of the common and functionally relevant 40 bp 3' UTR VNTR in the dopamine transporter gene SLC6A3 on neurophysiological functioning in adult patients with ADHD. To this end, a large sample of ADHD patients was compared to a healthy control sample regarding their neural responses to a common behavioral inhibition task (Continuous Performance Test / CPT). The “endophenotype approach” in psychiatry (Almasy and Blangero 2001) assumes that neuropsychological and other functional concepts (e.g., behavioral inhibition, attentional control, mental flexibility; see Slaats-Willemse, 2003) are more closely related to etiological factors (e.g., genes) than categorical clinical diagnoses. Research on functional endophenotypes might therefore aid a more thorough understanding of a disorder, thereby improving current therapeutic options.

Behavioral data showed that ADHD patients performed worse than healthy controls as indicated by the number of commission errors after primers and distractors, number of omission errors, reaction time in Go-trials and the variability of the reaction time, which is in line with previous findings (e.g., Epstein *et al*, 1998; Leth-Steensen *et al*, 2000). These findings imply that ADHD patients are less sensitive to stimulus features, i.e. to the difference between targets and non-targets, than their normal counterparts (for a meta-analysis see Losier *et al*, 1996). From this point our behavioral data are well in line with the existing literature on CPT performance in ADHD patients. Regarding the genetic analyses, no significant association of SLC6A3 genotype with behavioral data was found. This is in line with the results obtained by Barkley *et al* (2006), who implemented a similar task, but does not replicate findings by Bellgrove *et al* (2005) and Loo *et al* (2003), who report an

influence of the 3' UTR VNTR on behavioral measures in a Go-NoGo task, especially on response variability. In these previous studies, homozygous 10-repeat allele carriers exhibited poorer performance and showed greater response time variability than children with at least one 9-repeat allele. Some methodological differences between studies might account for these diverging findings (e.g., differences in mean age [children vs. adults] and sample size, as well as task specifications).

In the ERP data we found that ADHD patients showed a tendency towards smaller values of the NGA (difference Go- minus NoGo-centroid) than healthy control subjects ($p = 0.06$, in the ANCOVA $p = 0.04$; see Figure 2). This finding is in line with known deficits of inhibitory control and frontal lobe function in ADHD (see Introduction). It also replicates previous reports of altered ERP measures of prefrontal response control in children suffering from ADHD (Fallgatter *et al*, 2004) and a diminished NGA in adult patients with a suspected ADHD during childhood (Fallgatter *et al*, 2005). In the latter study, the diminished NGA was mainly caused by a more anterior centroid in the Go-condition (Fallgatter *et al*, 2005); this is also in line with our results, as patients showed a significantly more anterior location of the Go-centroid as compared to the control group (see also Baehne *et al*, 2008).

The significant interaction of the factors “diagnosis” and “genotype” furthermore indicates that the investigated VNTR had a differential impact on the NGA depending on the participants' diagnosis (ADHD patients vs. healthy controls). In the control sample, SLC6A3 genotype had no significant impact, neither on the NGA itself nor on the position of the Go- and NoGo-centroid. In the ADHD group, however, we found a significant relationship between the genotypes and NGA magnitude. This differential impact of the VNTR in patients and controls indicates that ADHD patients are particularly sensitive to genetic variants influencing dopaminergic transmission, possibly due to a generally altered dopaminergic state. In line with the finding of a

relatively reduced NGA in 9-repeat allele carriers of the ADHD group, a significant difference between patients and controls was only found in the 9/9 & 9/10 genotype, with a significantly reduced mean NGA in the group of ADHD patients (see Figure 2). sLORETA source localization analyses indicated an origin of this effect in the anterior cingulate cortex, that showed significantly reduced NoGo-related activation in patients compared to controls within the subgroup of 9-repeat allele carriers. This finding fits well with previous data on reduced cingulate functioning in children and adults with a current or suspected previous ADHD diagnosis (Fallgatter *et al*, 2004, 2005).

While SLC6A3 is highly expressed in striatal areas, it is found in only low quantities in prefrontal and cingulate cortices (Sesack *et al*, 1998; Lewis *et al*, 2001). With respect to our finding of an impact of SLC6A3 on cortical activation within the group of ADHD patients (particularly within the inferior parietal cortex; cf. sLORETA results), SLC6A3 has nevertheless been assumed to play a critical role in regulating cortical signal-to-noise ratio in two ways. First, it appears to have a direct effect via its influence on prefrontal pyramidal neurons through regulation of DA volume transmission on surrounding GABA inhibitory neurons. Second, it influences cortical signal-to-noise ratio indirectly via effects in the striatum, which regulates activity within the cortico-striato-thalamo-cortical pathway (Newman and Grace, 1999; also cf. Bertolino *et al*, 2006). Due to the involvement of striatal areas in motor regulation and response control, a particular impact of SLC6A3 on the Go-ERP (and related cortical sources) appears particularly plausible.

Our finding of a differential impact of SLC6A3 genotype on the NGA has to be discussed in the light of genotypic consequences on a molecular, cellular and neural network level. The impact of SLC6A3 has been extensively studied both *in vitro* and *in vivo*. However, *in vitro* studies (Mill *et al*, 2005; vanNess *et al*, 2005; Fuke *et al*,

2001; Miller and Madras, 2002; Greenwood and Kelsoe, 2003 Michelhaugh *et al*, 2001) have not yet been able to answer conclusively to what extent the SLC6A3 variants quantitatively affect SLC6A3 expression and DAT density, respectively. Single photon emission computer tomography (SPECT) studies addressing this issue *in vivo* also led to partly inconclusive findings (Martinez *et al*, 2001; Heinz *et al*, 2000). Heinz *et al* (2000) reported a 22% reduction of DAT protein in the putamen of 9/10 compared to 10/10 genotype carriers, whereas Martinez *et al* (2001) found no differences in striatal radiotracer ([¹²³I]β-CIT) binding. Two SPECT studies (van Dyck *et al*, 2005; van de Giessen *et al*, 2009) addressed this issue by investigating larger samples of healthy adult subjects. Both consistently showed that carriers of at least one 9-repeat allele exhibited increased striatal DAT availability. Interestingly, patients suffering from ADHD have also been assumed to exhibit a striatal hypodopaminergic state due to heightened striatal DAT availability (Krause *et al*, 2000; see also Spencer *et al*, 2005). In line with these findings, Winsberg and Comings (1999) found that the 9/10 genotype was associated with a better response to methylphenidate (MPH) – which binds to DAT and inhibits DA transport – in children with ADHD.

Considering all these findings, we therefore favor the hypothesis of a relatively decreased DAT density in 10/10 allele carriers, despite inconsistent *in vitro* and *in vivo* findings. This would imply a relatively higher synaptic DA bioavailability in SLC6A3 10-repeat allele homozygotes as compared to 9-repeat allele carriers. The pattern of a reduced NGA in 9/9 & 9/10 allele carriers within our group of ADHD patients would also be in line with the assumption of an increased DAT density and decreased DA availability in carriers of at least one 9-allele. Here, the SLC6A3 genotype-dependent relative increase of DA availability may underlie the “endophenotypic rescue” within ADHD patients with 10/10 genotype.

Our results are in accordance with longitudinal data provided by Barkley and colleagues, who found that – compared to 10/10 allele carriers – the 9/10 genotype was associated with more severe ADHD symptomatology and more unfavorable social and occupational outcomes. Interestingly, the authors furthermore report that some of their outcome measures were also impacted unfavorably by the 9/10 genotype within their healthy control group (Barkley *et al*, 2006; see Introduction). As this follow-up study comprised many different behavioral, psychological and neuropsychological measures, one can conclude that the genotypic influence goes far beyond the categorical diagnosis of ADHD (as reported in association studies). Most interestingly, the seemingly adverse effects of the 9-repeat allele tended to become greater with increasing age of the subjects (with small effect sizes in children and moderate ones in adolescents). This finding strongly emphasizes the influence of genetics in the development of the individual and indicates that genetic effects may vary considerably throughout life time.

In summary, our findings are in accordance with studies favoring the 9-repeat allele as a risk allele for ADHD in adults (van Dyck *et al*, 2005; Franke *et al*, 2008; Franke *et al*, 2010), but not with studies favoring the 10-repeat allele that seems to be the risk allele for ADHD in children (Chen *et al*, 2003; Cook *et al*, 1995; Curran *et al*, 2001). Here the 9-allele was associated with abnormalities in cognitive response control and related cortical activation in ADHD patients. Moreover, neurophysiological differences between patients and controls were only apparent in one genotype group (9/9 & 9/10), indicating the usefulness of combined approaches involving both the concept of endophenotypes (e.g., behavioral inhibition) and imaging genetics (e.g., influence of *SLC6A3*). One factor possibly accounting for a differential association of *SLC6A3* (and other genes) with ADHD or aspects of its symptomatology in children

and adults might be changes in the requirements on the dopaminergic system throughout life. Spencer and colleagues, for example, found that the dopamine transporter density decreases during life with a numerically steeper slope for patients than control subjects (Spencer *et al*, 2005). From that point of view it is also plausible that in later phases of life the genetic impact on dopaminergic activity might differ qualitatively and quantitatively from the impact in childhood. Thus, our endophenotypic association of the 9/9 & 9/10 genotype with a reduced NGA value in adults might not be contradictory to the association studies in children, but may depend on the subjects' age. However, although age might be the driving force, we cannot rule out influences of other factors that systematically differ between children and adult ADHD samples, i.e. most notably the gender ratio and more extensive comorbidity rates in adult patients (Kessler *et al*, 2006). To further elucidate the development of ADHD throughout life longitudinal studies may provide helpful evidence concerning genetic influences, but are scarce up to now.

Limitations of our study include, although comparable with epidemiological studies (Kessler *et al*, 2006), a relatively high incidence of psychiatric (axis I-) comorbidities in the investigated ADHD sample (47%), which might have led to a respective distortion of the results. Second, the gender ratio in our study differs from studies of children. Although the NGA seems to be independent of the gender in healthy subjects (Fallgatter *et al*, 1999), it may explain some effects. Third, smoking behavior significantly differed between diagnostic groups (see methods section), however, not between SLC6A3 genotype groups ($\chi_1^2 = 0.76$, $p = 0.38$). Therefore, general group comparisons for neurophysiological findings might have been affected by differences in daily nicotine consumed, whereas the reported genetic findings should not be biased by this factor. Regarding the sample size, it would be desirable to investigate

even larger samples to increase the reliability of the drawn conclusions. Therefore, multi center studies should be conducted to increase the sample size.

Disclosure / Conflict of Interest

The authors declare no conflict of interest.

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Tables

Table 1. Sample characteristics (standard deviation in parentheses).

	ADHD patients			controls		
	9/9 & 9/10 (n = 77)	10/10 (n = 84)	all (n = 161)	9/9 & 9/10 (n = 52)	10/10 (n = 57)	all (n = 109)
age	35.22 (9.46)	36.27 (10.26)	35.77 (9.87)	35.81 (10.10)	35.72 (10.57)	35.76 (10.30)
IQ	113.51 (11.30)	114.37 (13.08)	113.96 (12.22)	117.10 (13.36)	118.65 (12.67)	117.93 (12.96)
handedness	65 / 12	81 / 3	146 / 15	48 / 4	51 / 6	99/10
female (%)	51.2	48.8	50.0	44.2	59.6	52.3
WURS-k score	35.14 (14.87)	36.58 (12.42)	35.89 (13.62)	13.69 (9.04)	12.68 (8.42)	13.16 (8.69)

Table 2. Descriptive statistics of behavioural data (standard deviation in parentheses).

	ADHD patients			controls		
	9/9 & 9/10 (n = 77)	10/10 (n = 84)	all (n = 161)	9/9 & 9/10 (n = 52)	10/10 (n = 57)	all (n = 109)
Commission errors type 1	.84 (1.15)	1.30 (2.00)	1.08 (1.66)	.38 (.89)	.53 (.76)	.46 (.82)
Commission errors type 2	.22 (.79)	.21 (.45)	.17 (.64)	.21 (.50)	.07 (.26)	.14 (.40)
Omission errors	1.96 (2.92)	1.98 (2.91)	1.97 (2.91)	1.00 (1.86)	.77 (1.34)	.88 (1.60)
Reaction times	510.77 (129.70)	508.89 (123.95)	509.79 (126.34)	452.68 (114.70)	467.11 (108.33)	460.23 (111.14)
s.d., Go	122.92 (59.56)	113.68 (48.93)	118.10 (54.30)	86.36 (40.37)	94.11 (48.73)	90.41 (44.90)

Titles and Legends to Figures

Figure 1

Schematic illustration of the quantification of the NGA as the geometrical distance between Go and Nogo centroid in the anterior-posterior direction. Grand average curves of Go (Pz; black line) and Nogo (Cz; red line) trials for the group of healthy controls homozygous for the 10-repeat allele. Heads illustrate the distribution of the positive brain electrical field at the respective peak of the P300.

Figure 2

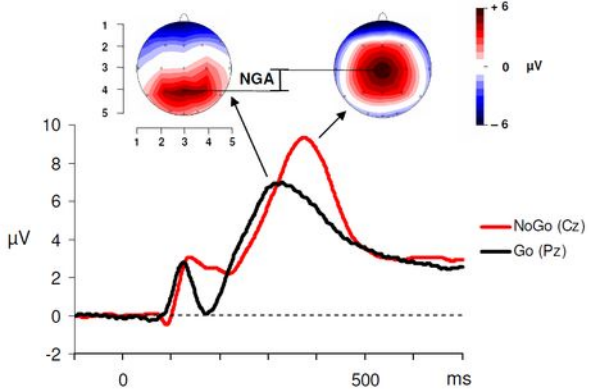
Mean values of the Nogo-Anteriorization (NGA) for ADHD patients and healthy controls with either 0 or 1 10-repeat allele (white columns) or 2 10-repeat alleles of the DAT gene (gray columns). Error bars represent standard errors of the mean.

Figure 3

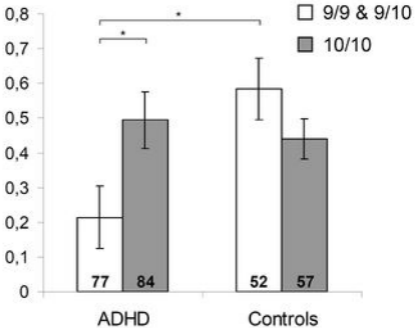
Trend tests to analyze possible gene-dose effects. Mean values of the Nogo-Anteriorization (NGA) for ADHD patients (upper panel) and healthy controls (lower panel) stratified according to number of 10-repeat alleles (0, 1, 2). Error bars represent standard errors of the mean.

Figure 4

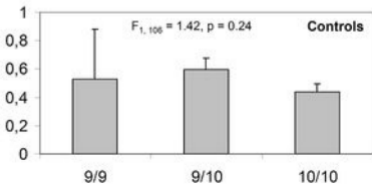
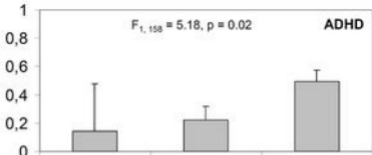
Explorative source localization with sLoreta. The upper panel shows the contrast controls vs. ADHD patients for the NGA (NoGo-Go) within the 9/9 & 9/10 genotype group, the lower panel the contrast 10/10 vs. 9/9 & 9/10 genotype group for the Go centroid within the patients.

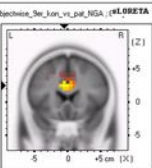
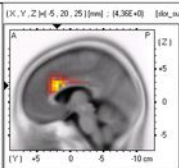
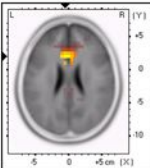


NoGo-Anteriorization (NGA)

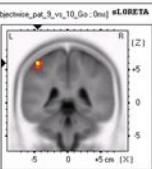
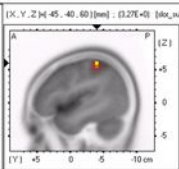
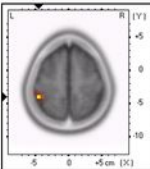


NoGo-Anteriorization (NGA)





[X, Y, Z] = [-5, 20, 25] [mm] : (4.36E+0) [dox_subjective_Ser_kon_vs_pat_NGA] ; (sLORETA)



[X, Y, Z] = [-45, -40, 60] [mm] : (3.27E+0) [dox_subjective_pat_9_vs_10_Go : 0ms] ; (sLORETA)