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Association Among SNAP-25 Gene *Ddel* and *Mnll* Polymorphisms and Hemodynamic Changes During Methylphenidate Use: A Functional Near-Infrared Spectroscopy Study

Özgür Öner¹, Ata Akın², Hasan Herken³, Mehmet Emin Erdal⁴, Koray Çiftçi², Mustafa Ertan Ay⁴, Duygu Bicer⁵, Bedriye Öncü⁶, Ozlem Hekim Bozkurt¹, Kerim Münir⁷, Yankı Yazgan⁵

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Abstract

Objective: To investigate the interaction of treatment-related hemodynamic changes with genotype status for Synaptosomal associated protein 25 (SNAP-25) gene in participants with attention deficit hyperactivity disorder (ADHD) on and off single dose short-acting methylphenidate treatment with functional near-infrared spectroscopy (fNIRS). **Method:** A total of 15 right-handed adults and 16 right-handed children with *DSM-IV* diagnosis of ADHD were evaluated. Ten milligrams of short-acting methylphenidate was administered in a crossover design. **Results:** Participants with SNAP-25 *Ddel* T/T genotype had decreased right deoxyhemoglobin ([HHb]) with treatment. SNAP-25 *Mnll* genotype was also associated with right deoxyhemoglobin ([HbO₂]) and [HHb] changes as well as left [HHb] change. When the combinations of these genotypes were taken into account, the participants with [*Ddel* C/C or T/C and *Mnll* G/G or T/G] genotype had increased right [HHb] with MPH use whereas the participants with [*Ddel* T/T and *Mnll* T/T] or [*Ddel* T/T and *Mnll* G/G or T/G] genotypes had decreased right prefrontal [HHb]. **Conclusions:** These results suggested that SNAP-25 polymorphism might be associated with methylphenidate induced brain hemodynamic changes in ADHD participants. (*J. of Att. Dis.* 2011; 15(8) 628-637)

Keywords

functional near-infrared spectroscopy, ADHD, methylphenidate, SNAP-25

ADHD is one of the most common neurodevelopmental disorders of childhood. There is an increasing convergence of results demonstrating both structural and functional brain differences in participants with ADHD. Structural and functional neuroimaging studies have shown abnormalities of the whole brain, frontal lobes, basal ganglia, corpus callosum, parietal lobes, temporal cortex, and cerebellar vermis (Bush, Valera, & Seidman, 2005; Seidman, Valera, & Makris, 2005).

Numerous genetic alterations have been reported in participants with ADHD (Faraone et al., 2005). Most of the proposed genes are associated with the dopaminergic system. SNAP-25 is a presynaptic plasma membrane protein that plays an important role in the synaptic vesicle membrane docking and fusion (Zhao, Hashida, Takahashi, & Sakaki, 1994). SNAP-25 constitutes complexes with synaptobrevin on synaptic vesicles and syntaxin on plasma membrane that

are related with neuronal exocytosis that is triggered by calcium (Hu et al., 2003). The transmission of SNAP-25 alleles in ADHD has been investigated and these studies reported an increased transmission of *Ddel* allele (Brophy et al., 2002; Kustanovich et al., 2003), of a haplotype (made of allele 1 of

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MnII and allele 2 of the *DdeI*; Barr et al., 2000), and microsatellites in ADHD participants (Mill et al., 2002, 2004). Meta-analysis of these studies showed a significant association of *MnII* and ADHD (OR = 1.19; 95% confidence interval 1.03-1.38; Faraone et al., 2005).

Previous studies reported decreased (Gilbert et al., 2006; Winsberg & Comings, 1999), increased (Roman et al., 2002; Stein et al., 2005), or no effect (Langley et al., 2005; van der Meulen et al., 2005) of DAT1 10 repeat allele on the methylphenidate response. There have been fewer studies with other genes; one study reported that participants with DRD4 7R allele needed higher MPH doses (Hamarman, Fossella, Ulger, Brimacombe, & Dermody, 2004). It has been shown that ADHD participants who were homozygous for *MnII* T allele might have a different response pattern to MPH treatment than participants who were homozygous for the *DdeI* T allele (McGough et al., 2006). Participants who were homozygous for *MnII* G or *DdeI* C alleles were at higher risk for side effects like sleep difficulties and abnormal movements.

The effect of certain genetic alterations in the brain function in patients with ADHD is not clear. In fact, there is very scarce data in this field. Integrating data that are obtained from different fields of investigation, like genetics and brain imaging are essential to have a better understanding of the pathophysiology of complex disorders like ADHD. It has recently been suggested that SNAP-25 polymorphism might be associated with treatment response to and side effects of MPH (McGough et al., 2006). However, the relationship between SNAP-25 polymorphism and cerebral hemodynamic response to MPH has not been investigated. In this study, authors' aim was to investigate the effects of SNAP-25 genotype on the hemodynamic response evaluated by functional near-infrared spectroscopy (fNIRS) to interference condition in ADHD participants on or off MPH. The authors did not suggest specific hypothesis due to the lack of previous data, therefore, the study was pilot in this sense.

Materials and Method

Participants

Adult ADHD Participants. A total of 15 adult ADHD participants were ascertained from outpatient clinics of two university hospitals. Diagnoses were made according to *Diagnostic and Statistical Manual of Mental Disorders* (4th ed., *DSM-IV*) ADHD criteria. Wender Utah Rating Scale (WURS) was administered, using a cut-off score of 36 or higher, which is 96% sensitive and 96% specific for detecting adult ADHD patients (Ward, Wender, & Reimherr, 1993). Turkish translation of WURS (Oncu, Olmez, & Senturk, 2005) showed adequate validity and reliability

(Cronbach's alpha, .88). To be diagnosed as adult ADHD, an individual must have (a) more than six current inattention or hyperactivity/impulsivity symptoms; (b) WURS score greater than 36 and a cut-off score validated by Ward et al. (1993) and Oncu et al. (2005); (c) onset of symptoms before the age of 7 as confirmed by the parental interviews, whenever possible. The authors screened all participants for current and lifetime psychosis, schizophrenia, major depression, substance dependence (other than smoking), and bipolar affective disorder by the corresponding structural clinical interview for *DSM-IV* diagnosis (SCID-I; First, Spitzer, Gibbon, & Williams, 1996) modules. One ADHD participant had current anxiety disorder (social phobia) and three participants had a lifetime history of major depression. None of them had a history of documented or suggested medical or neurological conditions. None of them had antisocial personality disorder symptoms. Three patients, who were currently on medication, were asked to omit a regular regime of MPH medication for a minimum of 24 hr (at least six half-lives; Gualtieri et al., 1982) before assessment.

Pediatric ADHD Participants. Pediatric participants included 16 children with ADHD. Six of them had other comorbid conditions including anxiety disorder, elimination disorders, depression, and conduct disorder. ADHD, anxiety disorder, conduct disorder, and depression diagnoses were based on *DSM-IV* criteria and made by the first author, using K-SADS-PL semistructured interview (Kaufman et al., 1997). All participants were recruited from the general outpatient clinic of a pediatric hospital. Inclusion criteria for pediatric patients included a diagnosis of ADHD, combined type per *DSM-IV* criteria, being drug-naïve, and age between 7 and 14. Exclusion criteria were the history of neurological disorders or head trauma resulting in unconsciousness longer than 30 min and a positive history of psychotropic drug use. Informed consent was obtained from the parents before enrollment with local ethics committee approval for inclusion in the study. The parents could select to opt out of the study at any time but none of the parents refused to participate or continue. All ADHD participants had unremarkable medical history. All patients were diagnosed for the first time and had never been evaluated for psychiatric disorders or treated with psychopharmacological medicine.

Both pediatric and adult ADHD participants underwent two consecutive fNIRS evaluations with an interval of 24 hr: in the first evaluation half of the participants were evaluated after a fixed dose of 10 mg of short-acting MPH 45 min beforehand and the other half did not take MPH. In the second evaluation the latter half was on MPH while the former was not.

fNIRS Procedures. Experiments were performed using a continuous wave near-infrared spectroscopy device (NIROSCOPE 301) built in Biophotonics Laboratory of

Bogazici University (Akgül, Akin, & Sankur, 2006; Akin et al., 2006). The device is capable of transmitting near-infrared light in two wavelengths (730 nm and 850 nm), which are known to be able to penetrate through the scalp and probe the cerebral cortex (Villringer & Chance, 1997). Calculation of concentration changes in oxygenated and deoxygenated ([HbO₂] and [HHb]) is based on Beer-Lambert law. Employing four light emitting diodes (LEDs) and 10 detectors, the device can sample 16 different volumes in the brain simultaneously. Distance between each source and detector is 2.5 cm, which guarantees a probing depth of approximately 2 cm from the scalp surface LEDs and detectors were placed in a rubber band that was specially designed to fit the forehead. Figure 1 depicts an approximate placement of the head probe on the forehead. The base of the probe is positioned to align with the eyebrows while the midline of the probe also matches the middle of the forehead. This way, detectors 1 through 4 scan the left dorsolateral prefrontal cortex while detectors 13 through 16 scan the right dorsolateral prefrontal cortex (DLPFC). Since Stroop task is known to evoke a higher response bilaterally in DLPFC, we chose to concentrate our analysis to detectors 1-4 and 13-16. [3] Sampling frequency of the device was 1.7 Hz. In this study the authors collected data from left and right prefrontal regions with four detectors for each region (Fp1/F3 and Fp2/F4) and then they averaged the [HbO₂] and [HHb] levels (mol) obtained from these detectors. All four detectors in each side had one LED. [2]

Experimental Paradigm. Participants were asked to perform color-word matching Stroop task as described earlier (Schroeter, Zysset, Kupka, Kruggel, & Cramon, 2002). They were presented with two words one written over the other. The top one was written in ink color whereas the below one was in white (over a black background). Participants were asked to judge whether the word written below correctly denotes the color of the upper word or not. If so, participants were to press the left mouse button with their finger and if not to press the right mouse button with their middle finger. Participants were informed to perform the task as quickly and correctly as possible. Experiment consisted of neutral, congruent, and incongruent trials. In the neutral condition upper word consisted of four X's (XXXX) in ink color. In the congruent condition ink color of the upper word and the word itself were the same whereas in incongruent condition they were different. The stimuli were presented in a semiblocked manner. Each block consisted of six trials. Interstimulus interval within the blocks was 4 s, and blocks were placed 20 s apart in time. The stimulus type within a block was homogeneous (but the arrangement of correct and false stimuli might change), that is, a block consisted only of, for instance, congruent trials, which may be correct or false. There were five blocks of each stimulus

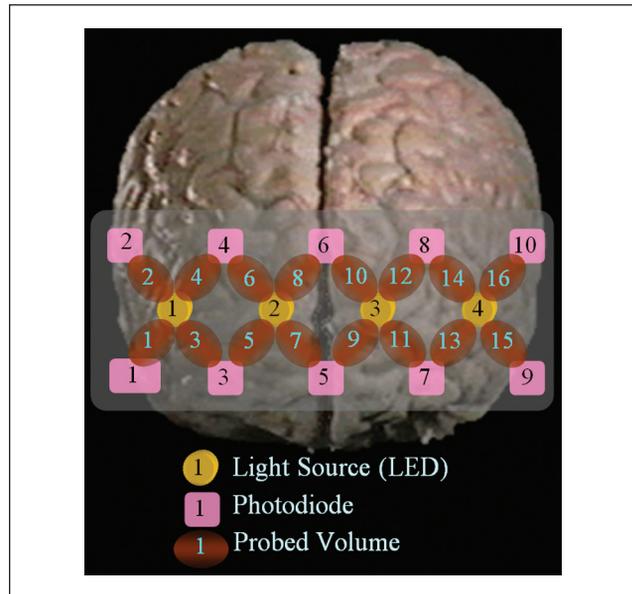


Figure 1. Location of the sources and detectors on the forehead and their approximate probed volumes on the cortex.

type. The stimuli stayed on the screen until the participant responded or disappeared at the end of 3 s in case the participant did not respond. Experiments were performed in a silent, lightly dimmed room. Stimuli were presented via an LCD screen that is 0.5 m away from the participants. Incorrect or absent responses on congruent, incongruent, and neutral trials were classified as errors.

fNIRS Analysis. We employed the well-known SPM (Statistical Parametric Mapping) approach for the analysis of the data (Ciftçi, Sankur, Kahya, & Akin, 2008; Friston, Holmes, Poline, Frith, & Frackowiak, 1995). In the present study the authors have evaluated the effects of MPH on brain hemodynamics during the interference condition. Thus they calculated the difference of [HbO₂] (or [HHb]) levels recorded during incongruent stimuli (IS) and neutral stimuli (NS) (hemodynamic interference: IS ([HbO₂]/[HHb]) – NS ([HbO₂]/[HHb])).

Molecular Analysis

DNA extraction and analysis. With written informed consent, a blood sample was drawn from each individual. Venous blood samples were collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes. DNA was extracted from whole blood by salting out procedure (Miller et al., 1988).

Genotypic analysis of the SNAP-25 gene Ddel and MnlI polymorphisms. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) assays were used to determine SNAP-25 gene (GenBank Accession Number D21267) *Ddel* (rs1051312) and *MnlI* (rs3746544) polymorphisms.

The oligonucleotide primers used to determine the *DdeI* and *MnII* polymorphisms within the SNAP-25 gene were described previously (Barr et al., 2000). The primers, forward 5'-TTCTCCTCCAAATGCTGTCG-3' and reverse 5'-CCACCGAGGAGAGAAAATG-3' were used to amplify the SNAP-25 gene. PCR was performed in a 25 μ l volume with 100 ng DNA, 100 μ M dNTPs, 20 pmol of each primer, 1.5 mM MgCl₂, 1 \times PCR buffer with (NH₄)₂SO₄ (Fermentas, Vilnius, Lithuania), and 1 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). PCR conditions were 2 min for initial denaturation at 95 $^{\circ}$ C, 35 cycles at 95 $^{\circ}$ C for 45 s for denaturation, 1 min at 58 $^{\circ}$ C for annealing, and 2 min at 72 $^{\circ}$ C for extension, followed by 7 min at 72 $^{\circ}$ C for final extension. After amplification PCR products were digested by restriction endonuclease 10 U *DdeI* (Fermentas, Vilnius, Lithuania) or 10 U *MnII* (Fermentas, Vilnius, Lithuania) for 14 h at 37 $^{\circ}$ C. The genotyping of the SNAP-25 gene *DdeI* or *MnII* polymorphisms was determined by fragment separation at 120 V for 40 to 50 min on a 3.5% Agarose gel containing 0.5 μ g/ml ethidium bromide. A 100-bp marker (50-bp DNA Ladder, Fermentas) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat). For allele 1 (T) of *DdeI* the 261-bp fragment was not cut and for allele 2 (C) the fragment was cut into two fragments of 228 bp and 33 bp. For allele 1 (T) of *MnII* digest, the 261-bp fragment was cut into two fragments of 256 bp and 5 bp but was not cut at the polymorphic restriction site. For allele 2 (G), the 261-bp fragment was cut into 210-, 46-, and 5-bp fragments. The 5-, 33-, and 46-bp fragments were difficult to visualize because of both its small size and comigration with the similar size primer residue; however, detection of this fragment was not critical in determining genotypes. Genotyping was based on independent scoring of the results by two reviewers who were unaware of case/control status.

Data Analysis. The SPM analysis provides an average activation amplitude parameter for each detector for each stimulus type; hence it is a univariate analysis technique. Before the analyses, data were digitally low pass filtered with a cut-off frequency of 0.33 Hz for removing high-frequency noise. The analyses were carried out using the general linear model (Friston et al., 1995). The design matrix of the model consisted of stimulus onset vectors for each type of stimulus that were convolved with the canonical hemodynamic response function. To be able to cope with low-frequency oscillations, discrete cosine transform functions with a minimum period of 120 s (\sim 0.008 Hz) were generated. These functions were added to the design matrix as nuisance variables. Consequently, regression analysis was performed and appropriate contrast vectors were applied

to get the effects of interest. This analysis yields three numbers for each detector representing the average response to neutral, congruent, and incongruent stimuli. The authors have contrasted the IS to NS and set a significance threshold of $p < .05$ to obtain an fNIRS parameter for each detector. Once this interference amplitude parameter is found for each detector and each participant for off MPH, that value is then compared against the same participant's on-MPH parameter. Hence, we basically calculated a "difference measure" of the interference amplitudes between the off and on MPH cases that were then used as the dependent variable in the analysis of, for example, genotype effects in the ANOVAs. A detailed mathematical treatise of this approach and its power over other conventional ones is given in Ciftçi et al. (2008).

Effect of treatment on reaction time (RT) and percent errors (PE) was computed with repeated measures analysis of variance with age and gender as covariates. Analysis of covariance (ANCOVA) with SNAP-25 gene *DdeI* and *MnII* polymorphisms as fixed factors and age and gender as covariates was performed. Significant associations were followed with univariate tests. To compute the effect of various polymorphism combinations the authors regrouped the *MnII* and *DdeI* polymorphisms as T/T homozygous or others (G/G or G/C and C/C or T/C, respectively). This resulted in four groups (1: *DdeI* C/C or T/C and *MnII* G/G or T/G, 2: *DdeI* T/T and *MnII* T/T, 3: *DdeI* C/C or T/C *MnII* T/T, 4: *DdeI* T/T and *MnII* G/G or T/G; Table 1). The authors also analyzed the data using these combined subgroups.

Results

Demographic Variables

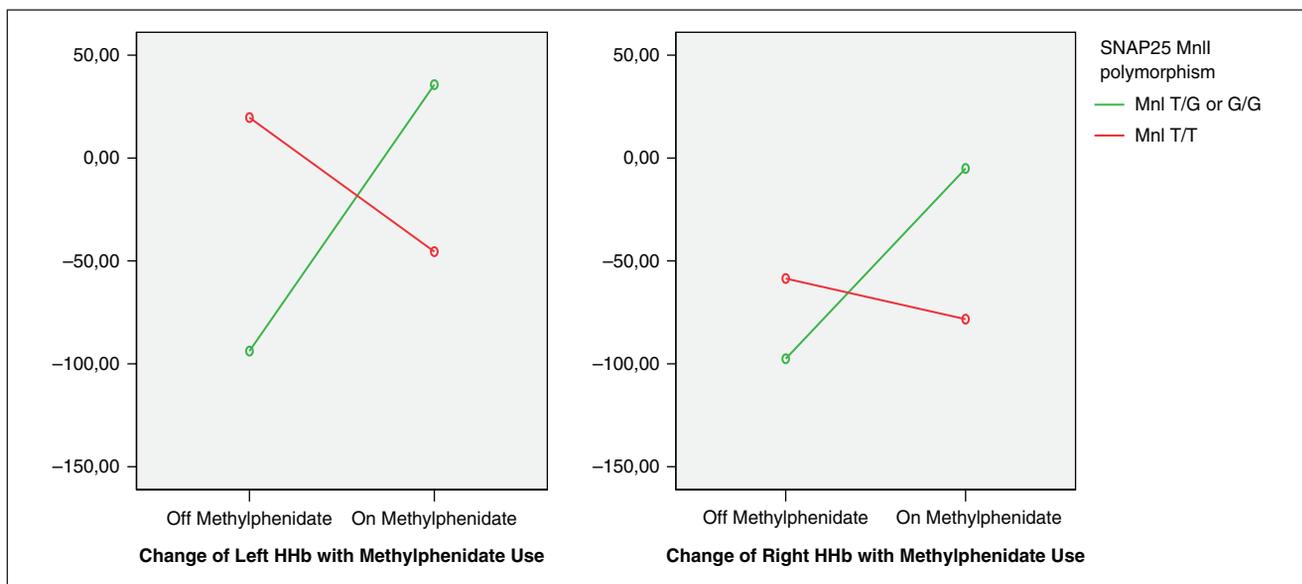
A total of 15 adult, right-handed participants with ADHD (8 men, 7 women; aged between 18 and 43, M : 26.1, SD : 7.7) and 16 right-handed pediatric ADHD participants (13 men, 3 women; aged between 7 and 14, M : 9.7, SD : 4.5) were included in the study. Gender distribution of the pediatric and adult ADHD participants was not significantly different ($\chi^2 = 2.7$; p : NS). The frequency of SNAP-25 gene *DdeI* and *MnII* polymorphisms and the combined groups is summarized in Table 1. Age and gender distribution were not different among the *DdeI*, *MnII*, and the combined polymorphism groups ($F = 0.04$ - 2.2 ; $p > .12$; $\chi^2 = 0.06$ - 0.60 , $p > .44$).

Behavioral Results

Main treatment effect was nonsignificant for PE (pretreatment: M : 5.6%, SD : 7.7%; posttreatment: M : 1.9% SD : 7.3%; $F(1, 28) = .26$, $p = .61$) and RT (pretreatment: M : 386.6 ms, SD : 206.9 ms; posttreatment: M : 348.1 ms, SD : 226.3 ms; $F(1, 28) = .23$, $p = .63$). Change of RT and

Table 1. Mean \pm Standard Deviation of Age, Right and Left (HbO₂) and (HHb) Levels of Patients With SNAP-25 *Ddel* and *MnII* Genotypes

Group	N (%)	Age (Years)	Left HbO ₂ (mol)	Right HbO ₂ (mol)	Left HHb (mol)	Right HHb (mol)
<i>Ddel</i> T/T	16 (51.6)	18.3 \pm 9.9	181.5 \pm 254.8	-32.8 \pm 140.3	6.2 \pm 161.6	41.0 \pm 108.7
<i>Ddel</i> T/C and C/C	15 (48.4)	17.3 \pm 8.9	95.3 \pm 287.1	20.0 \pm 182.4	-96.5 \pm 363.3	-130.4 \pm 144.8
<i>MnII</i> T/T	14 (45.2)	20.1 \pm 8.4	139.2 \pm 286.9	-94.9 \pm 153.8	65.1 \pm 273.1	19.4 \pm 157.0
<i>MnII</i> T/G and <i>MnII</i> G/G	17 (54.8)	16.0 \pm 9.8	140.2 \pm 263.9	57.6 \pm 141.0	-129.5 \pm 264.3	-92.5 \pm 132.7
<i>Ddel</i> C/C or T/C and <i>MnII</i> G/G or T/G	9 (29.0)	16.2 \pm 10.9	148.0 \pm 355.9	65.5 \pm 174.3	-184.6 \pm 330.4	-179.2 \pm 118.8
<i>Ddel</i> T/T and <i>MnII</i> T/T	8 (25.8)	20.1 \pm 10.7	231.4 \pm 345.2	-141.7 \pm 107.5	90.3 \pm 119.7	76.9 \pm 137.1
<i>Ddel</i> C/C or T/C <i>MnII</i> T/T	6 (19.4)	19.0 \pm 4.7	16.4 \pm 124.9	-48.2 \pm 187.6	35.8 \pm 399.8	-57.2 \pm 159.5
<i>Ddel</i> T/T and <i>MnII</i> G/G or T/G	8 (25.8)	15.8 \pm 8.9	131.5 \pm 119.3	48.8 \pm 102.7	-67.4 \pm 163.2	5.1 \pm 59.8

**Figure 2.** Change of left and right HHb with methylphenidate. Effect of SNAP-25 *MnII* polymorphism. Y-axis: HHb during Stroop neutral stimuli minus HHb during Stroop incongruent stimuli.

PE with treatment was also not significantly associated with genotype.

fNIRS Results

ANCOVA results indicated that SNAP-25 *MnII* genotype was significantly associated with change of right prefrontal [HbO₂] ($F[1, 28] = 6.8, p = .015$) and left prefrontal [HHb] ($F[1, 28] = 5.1, p = .033$) with MPH treatment (Figure 2). Mean left prefrontal [HHb] increased during MPH use in participants with *MnII* T/G or G/G genotypes whereas it decreased in participants with T/T genotype. The reverse pattern is true for right prefrontal [HbO₂]; it increased in the T/T and decreased in the T/G or G/G group.

SNAP-25 *Ddel* polymorphism was significantly associated with change of right prefrontal [HHb] with MPH

use ($F[1, 30] = 13.1, p < .001$; Figure 3). Right [HHb] level increased with MPH treatment in the *Ddel* C/C or T/C group and decreased in the T/T group. There were no significant associations with other hemodynamic variables.

When both *Ddel* and *MnII* phenotypes were taken into account, we found that genotype status was significantly associated with right [HHb] change ($F[3, 30] = 6.2, p = .003$). Pairwise comparisons revealed that participants with [*Ddel* C/C or T/C and *MnII* G/G or T/G] genotype had significantly different right [HHb] change when compared with participants with [*Ddel* T/T and *MnII* T/T] or [*Ddel* T/T and *MnII* G/G or T/G] genotypes ($p = .005$ and $<.001$, respectively; Figure 4). While mean right HHb increased with MPH use in the former group, it decreased in the latter groups (Table 1).

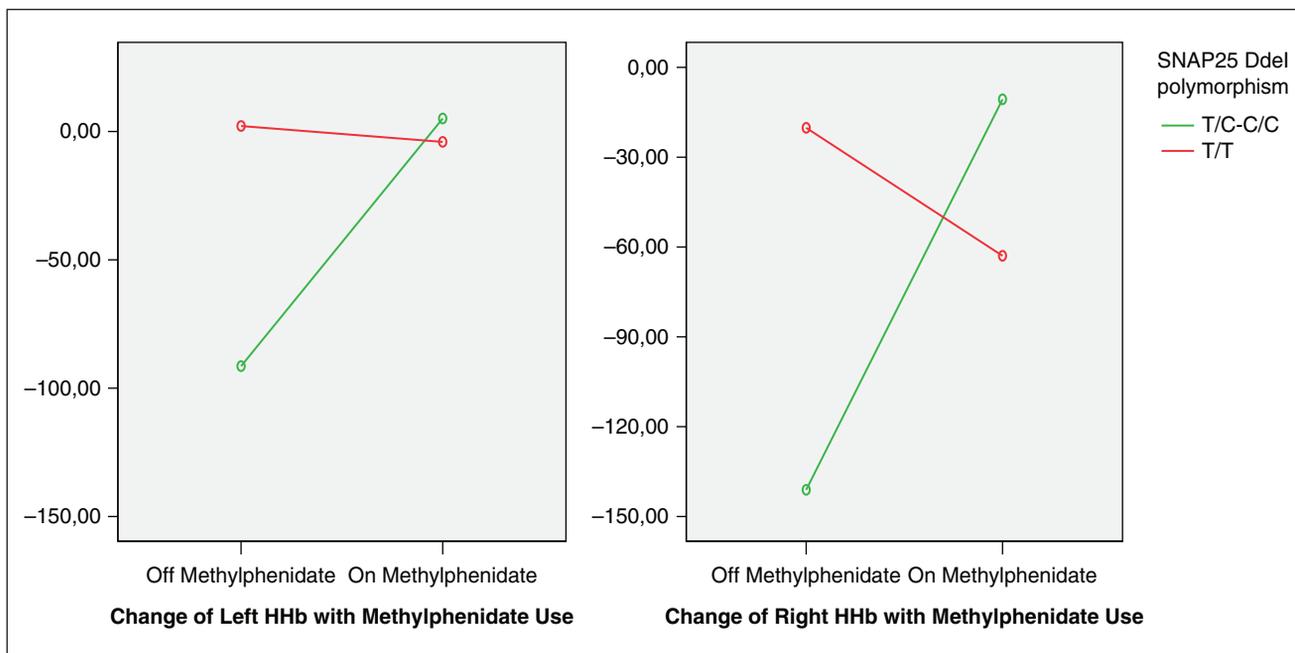


Figure 3. Change of left and right HHb with methylphenidate. Effect of SNAP-25 *Ddel* polymorphism. Y-axis: HHb during Stroop neutral stimuli minus HHb during Stroop incongruent stimuli.

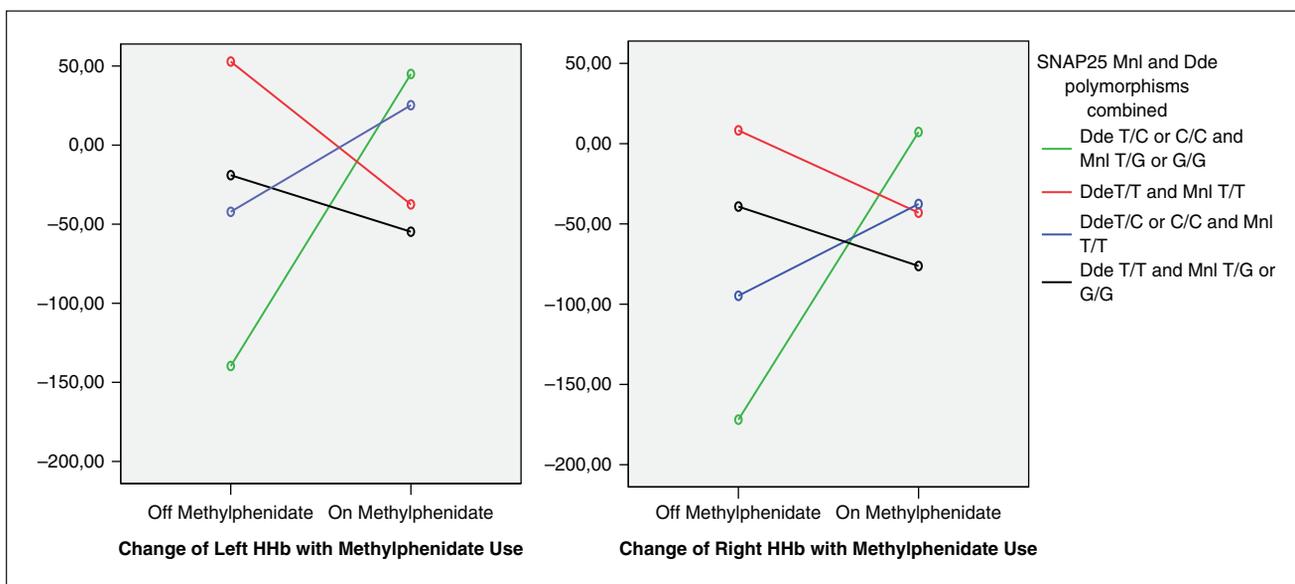


Figure 4. Change of left and right HHb with methylphenidate. Effect of SNAP-25 *MnlI* and *DdeI* polymorphisms combined. Y-axis: HHb during Stroop neutral stimuli minus HHb during Stroop incongruent stimuli.

Pairwise comparisons revealed that participants with [*DdeI* T/T and *MnlI* T/T] genotype had significantly different right [HbO₂] change when compared with participants with [*DdeI* C/C or T/C and *MnlI* G/G or T/G] or [*DdeI* T/T

and *MnlI* G/G or T/G] genotypes ($p = .02$ and $.03$, respectively; Table 1).

There were no significant interaction of age and gender for any of the variables studied.

Discussion

fNIRS is a unique method to evaluate the hemodynamic response to cognitive and other stimuli in a continuous way by measuring the changes in oxygenated hemoglobin and deoxyhemoglobin levels in particularly prefrontal cortex (Villringer & Chance, 1997), although it has been used extensively in other brain regions including motor, parietal, and temporal cortices. The most significant advantages of this method include lack of radiation, portable nature of the device, relative easiness, and low cost. The most significant disadvantage is its low spatial resolution and cerebral penetration depth. Previous studies have shown that increased (HbO₂) levels during cognitive tasks could be measured by fNIRS (Akgül et al., 2006; Ciftçi et al., 2008; Fallgatter & Strik, 1998; Matsuo, Watanabe, Onodera, Kato, & Kato, 2004; Schroeter et al., 2002; [4] Toronov et al., 2001). Although fNIRS has the mentioned advantages, this method has not been widely used in ADHD research. One of the few studies comparing ADHD participants with healthy controls reported that ADHD participants did not show the extended hemodynamic response in left prefrontal region during Trail Making Test, a test that measures mental tracking and visual attention performance, like the normal controls did (Weber, Lutschg, & Fahrenstich, 2005). In another study the same group reported lower left-sided increase of oxygenation in the on-MPH condition than the off-MPH condition (Weber et al., 2007). In a very recent study Ehllis, Bähne, Jacob, Herrmann, and Fallgatter (2008) reported reduced task-related increases in a working memory task in adults with ADHD, suggesting prefrontal dysfunction in these participants.

Our results showed that SNAP-25 polymorphisms were significantly associated with right (HbO₂) and bilateral (HHb) changes with MPH use. Participants with SNAP-25 *DdeI* T/T genotype had decreased right (HHb) when on MPH while right (HHb) levels increased with participants with other *DdeI* genotypes. SNAP-25 *MnII* genotype was also associated with right (HbO₂) change as well as left (HHb) change. When the combinations of these genotypes were taken into account, we found that the participants with *DdeI* T/T and *MnII* T/T genotype had decreased right (HHb) with MPH use while right (HHb) increased in the participants with *DdeI* T/C C/C and *MnII* T/G G/G genotypes. This was in line with Barr and associates' (2000) finding that combination of *DdeI* and *MnII* alleles might be important.

Animal models of ADHD include the coloboma mouse mutant, which is the result of a deletion in SNAP-25 gene (Hess, Collins, & Wilson, 1996). Deletion in SNAP-25 gene might be related with impaired dopamine and serotonin release in dorsal (but not ventral) striatum, which has important connections with the prefrontal cortex, the brain region implicated in executive functioning

(Brophy et al., 2002; Raber et al., 1997). It has been shown that ADHD participants homozygous for *MnII* T allele might have a different response pattern to MPH treatment than participants who were homozygous for the *DdeI* T allele (McGough et al., 2006). *MnII* T homozygosity significantly and favorably affected change of parent and teacher ratings with MPH treatment and *DdeI* T homozygosity had an additional but smaller negative effect. Since the changes in SNAP-25 gene expression may lead to alterations in neurotransmitter release (Wilson, 2000), the hyperactivity of the coloboma mouse responds to dextroamphetamine better than MPH. The result of the former pharmacogenomics study indicates that SNAP-25 gene polymorphisms may also be associated with treatment response and side effects of MPH treatment (McGough et al., 2006).

It can be speculated that our finding of association between SNAP-25 genotype and prefrontal hemodynamic changes with MPH might be related with different neurotransmitter release patterns of participants with different polymorphisms. We found that *MnII* T homozygous participants did not show the brain hemodynamics variability with MPH use. Although we did not collect data on treatment response, it can be speculated that this lack of hemodynamic change with MPH treatment may be related with less favorable treatment response reported by other researchers (McGough et al., 2006). On the other hand, participants with the combination of *MnII* and *DdeI* T homozygosity showed significantly different patterns of right prefrontal (HHb) change with MPH when compared with participants with *DdeI* T/C C/C and *MnII* T/G G/G genotypes, which suggested differential medication response in these two groups. Again, it must be kept in mind that we did not evaluate the effectiveness of MPH treatment.

What does the change in (HHb) or (HbO₂) mean? Brain activation causes increased cerebral blood flow, but not all of this oxygenated blood is used, therefore (HbO₂) increases and (HHb) decreases during sustained activation (Villringer & Chance, 1997). We found that SNAP-25 gene *DdeI* and *MnII* polymorphisms were particularly associated with change of (HHb) with treatment. Schroeter and associates (2002) reported that (HbO₂) concentration increased and (HHb) concentration decreased more during incongruent trials specifically over lateral prefrontal cortex. In the present study, we did not compare hemodynamic changes during incongruent and neutral trials but investigated the effects of methylphenidate on the difference between these two conditions. Nevertheless, following those authors, it can be suggested that higher (HbO₂) and lower (HHb) may be related with neurovascular coupling and that increased blood flow efficiently carried (HHb) from the activated brain regions (Schroeter et al., 2002). Right prefrontal (HHb) of participants with T/T polymorphisms did not increase with

MPH, suggesting that neurovascular coupling was differentially affected by genotype.

Our findings indicated prominently right-sided differences, which is consistent with previous imaging studies that reported right-sided abnormalities in ADHD participants (Seidman et al., 2005).

The findings reported in this study must be interpreted with caution due to certain limitations. First, we did not investigate the interaction of different polymorphisms since the sample size was not adequate for that analysis. Second, the reported changes in the prefrontal cortex could reflect changes in other brain regions, like basal ganglia, which could not be evaluated with fNIRS. In this study we evaluated the effect of a single administration of short-acting MPH. Thus, we did not have data on the symptomatic/behavioral response to MPH, and we could not group the patients as responders or nonresponders. We did not use a double blind placebo controlled protocol; thus in the present study it was not possible to discern placebo effect completely. We did not evaluate the effect of comorbidity and this might be a limitation. All patients had combined type ADHD; therefore, these results may not be valid for other ADHD types. The age range was very broad; however, we used each participant as its own control and also controlled the effects of age and gender statistically. Besides, mean age of each genotype subgroup was not significantly different. We could not control the effect of symptom severity since we included both adult and pediatric participants and we did not have comparable symptom ratings for these groups. It can be suggested that adult ADHD participants had relatively low severity of symptoms because they were not diagnosed and treated for many years. However, this may not be true for ADHD that has very recently been diagnosed in adults in Turkey. We did not find any association between genotype and treatment with behavioral performance. This might be due to good performance of the participants even in the off-medication condition. Considering all these limitations, confirmation of the present results is clearly necessary.

Nevertheless, our results suggested that SNAP-25 gene *DdeI* and *MnII* polymorphisms might be associated with MPH-related changes in brain hemodynamics in ADHD participants.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interests with respect to the authorship and/or publication of this article.

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