

Association of SNAP-25 Gene *Ddel* and *MnII* Polymorphisms with Adult Attention Deficit Hyperactivity Disorder

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Objective The synaptosomal-associated protein of 25 kDa (SNAP-25) gene is a presynaptic plasma membrane protein and an integral component of the vesicle docking and fusion machinery mediating secretion of neurotransmitters. Previously, several studies reported association between SNAP-25 and attention deficit hyperactivity disorder (ADHD). We investigated whether these SNAP-25 polymorphisms (*MnII* T/G and *DdelI* T/C) were also associated with ADHD in the Turkish population.

Methods Our study comprised unrelated 139 subjects who met DSM-IV criteria for ADHD and 73 controls and all were of Turkish origin. Genetic analyses were performed and patients were evaluated with Wender-Utah Rating Scale and Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale.

Results SNAP-25 *DdelI* polymorphism was not associated with ADHD but there was a statistically significant difference between ADHD patients and controls for SNAP-25 *MnII* polymorphism. For SNAP-25 *MnII* polymorphism patients with G/G genotype of the SNAP-25 gene *MnII* polymorphism had higher Wender-Utah scores and higher scores in the 1st and 3rd parts of adult ADD/ADHD Scale.

Conclusion We detected a significant association of the *MnII* polymorphism in our ADHD sample which was similar to previous findings. Our study also revealed that SNAP-25 *MnII* polymorphism was also associated with symptom severity of ADHD. This study is also, the first report on the association of SNAP-25 with ADHD in the Turkish population. **Psychiatry Investig 2014;11(4):476-480**

Key Words ADHD, SNAP-25 gene, Genetic.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is a developmental disorder which is characterized by inattention, impulsiveness and hyperactivity.¹ The prevalence of ADHD was reported as 5.3% in childhood and estimated as 1–4% in adulthood.² The high prevalence of ADHD made it necessary to understand the etiology of this disorder and also to further refine the diagnosis and treatment of it.³

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The etiology of ADHD is not completely understood. ADHD is a complex disorder in which multiple genes exert moderate effects. The twin studies find little evidence of shared environmental influences on familiarity, although the role of the environment may still be pivotal, acting through mechanisms of gene-environment interactions. Based on twin and adoption studies, genetic factors can play an important role in the etiology of ADHD.⁴ Many candidate genes were reported in the literature associated with ADHD. Significant associations between ADHD and DAT-1, DRD4, DRD5, 5HTT, HTR1B and SNAP-25 (*The synaptosomal-associated protein, 25 kDa*) were identified.⁵

Recently, there is increasing evidence to suggest that polymorphisms within the synaptosomal-associated protein, 25 kDa (SNAP-25) may play a role in susceptibility to ADHD. Human SNAP-25 gene is located on chromosome 20p11.2. SNAP-25, a pre-synaptic plasma membrane protein, an inte-

gral part of SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) protein complex. This complex is composed of SNAP-25, syntaxin 1A and synaptobrevin. SNAP-25 plays an important role in synaptic vesicle membrane docking and fusion, which is involved in the regulation of neurotransmitter release by Ca^{+2} -mediated exocytosis.⁶ Thus, at least it is possible that any variation in SNAP-25 might interfere in the susceptibility of ADHD by influencing the release of dopamine as well as other neurotransmitters.⁷

Wilson⁸ reported that hyperkinesia was ameliorated by low doses of the psychostimulant d-amphetamine and could be rescued genetically by a transgene encoding SNAP-25, located within the mutation coloboma (Cm) deletion. He also emphasized that despite the ubiquitous role of SNAP-25 in synaptic transmission and uniformly decreased expression in the mutants; coloboma mice had shown marked deficits in Ca^{+2} dependent dopamine release selectively in dorsal but not ventral striatum. He suggested that haploinsufficiency of SNAP-25 revealed a specific vulnerability of the nigrostriatal pathway which regulated motor activity and might provide a model for impaired striatal input into executive functions encoded by the prefrontal cortex associated with ADHD.

Initially, Barr et al.⁹ identified two single-nucleotide polymorphisms (SNPs) (*Mnll*, *Ddel*) located four bases apart in the 3' UTR of SNAP-25. Since then, the association of these two SNPs and ADHD has been investigated. Some of the studies reported a significant association with ADHD and *Ddel*, *Mnll* or both.¹⁰

To the best of our knowledge, there is no genetic study about the association of genetic polymorphisms of SNAP-25 gene with adult ADHD in the English literature. In this study, we aimed to investigate whether *Ddel* and *Mnll* polymorphisms of SNAP-25 gene were associated with ADHD in an adult Turkish sample.

METHODS

Subjects

The study was conducted at psychiatry clinics of a university hospital. Informed consent was obtained from all subjects according to the Helsinki Declaration as revised in 1996. A total of 139 patients between ages 18 and 60, meeting DSM-IV criteria for adult ADHD were admitted to the study. All patients were of Turkish origin. Patients were evaluated with Adult Attention Deficit Hyperactivity Disorder Diagnosis and Evaluation Scale (ADD/ADHD Scale). Patients, who answered at least 6 of the 9 questions as 2 or 3 points in the first and/or second parts of Adult ADD/ADHD Scale were diagnosed as ADHD. In addition, 139 patients were evaluated with Wender Utah Rating Scale (WURS) and the obtained

scores were examined in terms of SNAP-25 gene.

The control group consisted of 73 healthy subjects between the ages of 18 and 60 without any history of neuropsychiatric disorder. They were also of Turkish origin. The control group did not have any clinically significant organic disorders or mental retardation and control subjects were literate. The control group was also evaluated with Adult ADD/ADHD Scale and subjects who met adult ADHD criteria were excluded from the control group.

Instruments

Wender-Utah Rating Scale

This scale can be used to assess adults for ADHD with a subset of 25 questions associated with that diagnosis. WURS was developed by Ward and Wender in 1993.¹¹ Turkish validity and reliability of WURS was established by Oncu et al.¹² and the cut-off score point was 36.

Adult ADD/ADHD DSM IV-based diagnostic screening and rating scale

Adult Attention Deficit Hyperactivity Disorder Diagnosis and Evaluation Scale were developed by Turgay in 1995. It is a self assessment scale and patients can complete the questionnaire after being duly informed. When developing adult ADD/ADHD Scale, 18 symptoms of the diagnostic criteria in DSM-IV were reframed, so patients can understand them. Turkish validity and reliability was established by Gunay et al.¹³

DNA isolation and molecular analysis

DNA was isolated from peripheral blood leukocytes by standard phenol/chloroform method and genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR was performed with a personal thermal cycler (Techgene, NJ, USA), using the primers, forward 5'-TTCTCCTCCAAATGCTGTCG-3'; reverse 5'-CCACCGAGGAGAGAAAATG-3', to amplify of the SNAP-25 gene. PCR-RFLP assay was used to determine SNAP-25 gene (GenBank Accession Number D21267), *Ddel*(rs1051312) and *Mnll* (rs3746544) polymorphisms.

Statistical analysis

SPSS (Statistical Package for Social Sciences) version 16.0 for Windows computing program was used for statistical analysis of the data. Chi-square test was used to compare categorical variables and independent samples t-test was used to compare continuous variables. A value of $p < 0.05$ was accepted statistically significant.

RESULTS

A total of 139 patients with adult ADHD and 73 healthy controls were included into the study. Mean age of the study group was 27.24±9.62 and mean age of the control group was 26.64±6.71. There was no statistically significant difference between the study and the control groups in terms of age (p>0.05). The study group consisted of 38 women (27.3%) and 101 men (72.7%) and the control group consisted of 23 women (31.5%) and 50 men (68.5%). There was also no statistically significant difference between the study and the control groups in terms of gender (p>0.05). Of the 139 patients with adult ADHD, 44 (31.7%) were diagnosed as predominantly inattentive type, 33 (23.7%) were diagnosed as predominantly hyperactive-impulsive, and the rest of them (44.6%) were diagnosed as combined type ADHD.

SNAP-25 gene *Ddel* polymorphism was analyzed in both of the study and the control groups. T allele was the most common allele in both groups (study: 84.3%, control: 89.0%). C allele distribution in both groups was as follows; study: 15.7%, control: 11.0%. The most common genotypes were T/T (study: 71.2%, control: 80.8%). In the study group, 35 (25.2%) of them had T/C genotype and 2 (2.5%) of them had C/C genotype. In the control group, 12 (16.5%) of them had T/C genotype and 5 (3.6%) of them had C/C genotype. There was no statistically significant difference between the study and the control groups regarding *Ddel* polymorphism (p>0.05).

T allele was determined in 96.4% of the ADHD group and in 97.3% of the control group, and C allele was determined in 28.8% of the ADHD group and in 19.2% of the control group and the difference between the groups was not found statistically significant (p>0.05) (Table 1).

SNAP-25 gene *MnII* polymorphism was analyzed in both of the study and the control groups. While 58.3% of them were

having T allele and 41.7% of them were having G allele in the study group, 77.4% of them had T allele and 22.6% of them had G allele in the control group. The difference between the groups was found statistically significant (p<0.001). Among the ADHD patients, 50 (36.0%) of them had T/T, 62 (44.6%) of them had T/G and 27 (19.4%) of them had G/G genotype. In the control group, 47 (64.4%) of them had T/T, 19 (26.0%) of them had T/G and 7 (9.6%) of them had G/G genotype. The difference between the groups was found statistically significant (p<0.001).

T allele was determined in 80.6% of ADHD group and in 90.4% of the control group and there was no significant difference between the groups. When the groups were compared with respect to the G allele, ADHD group and the control group were found to have significantly different (p<0.001) rates of G allele which were 64.0% and 35.6% respectively (Table 2).

When ADHD patients were evaluated in terms of scale scores, the subjects having G/G genotype of the SNAP-25 gene *MnII* polymorphism had higher Wender-Utah scores and higher scores in the 1st and 3rd parts of adult ADD/ADHD Scale (p<0.05) (Table 3).

DISCUSSION

The relation between ADHD and SNAP-25 gene was investigated in many studies among children. Feng et al.¹⁴ examined 12 SNPs in two independent samples of ADHD families and found significant over-transmission of rs66039806-C, rs362549-A, rs362987-A and rs362998-C alleles in families. Mill et al.¹⁵ have reported a strong relation between rs363006 and ADHD. Renner et al.¹⁶ and Zhang et al.⁴ have reported an association between rs362549 and ADHD similar to Feng et al.¹⁴ The studies investigating the relation between ADHD

Table 1. Frequencies of SNAP-25 gene *Ddel* polymorphism

Allele frequencies	ADHD, N (%)	Control, N (%)	p* value
T	231 (84.3)	130 (89.0)	0.184
C	43 (15.7)	16 (11.0)	
Total	274 (100.0)	146 (100.0)	
Genotype frequencies	N (%)	N (%)	
T/T	99 (71.2)	59 (80.8)	0.309
T/C	35 (25.2)	12 (16.5)	
C/C	5 (3.6)	2 (2.7)	
Total	139 (100.0)	73 (100.0)	
Presence or absence of T and C alleles	N (%)	N (%)	
T/T+T/C	134 (96.4)	71 (97.3)	0.740
C/C+T/C	40 (28.8)	14 (19.2)	

*chi-square test was performed

Table 2. Frequencies of SNAP-25 gene *MnlI* polymorphism

Allele frequencies	ADHD, N (%)	Control, N (%)	p* value
T	162 (58.3)	113 (77.4)	0.000**
G	116 (41.7)	33 (22.6)	
Toplam	278 (100.0)	146 (100.0)	
Genotype frequencies	N (%)	N (%)	
T/T	50(36.0)	47 (64.4)	0.000**
T/G	62 (44.6)	19 (26.0)	
G/G	27 (19.4)	7 (9.6)	
Total	79 (100.0)	73 (100.0)	
Presence or absence of T and G alleles	N (%)	N (%)	
T/T+T/G	112 (80.6)	66 (90.4)	0.064
G/G+T/G	89 (64.0)	26 (35.6)	0.000**

*chi-square test was performed, **p>0.05. ADHD: attention deficit hyperactivity disorder

Table 3. Relationship between the SNAP-25 gene *MnlI* polymorphism and scores of wender-utah scale and adult ADD/ADHD scale

	SNAP-25 Gene <i>MnlI</i> polymorphism				Comparison
	T/T X±SD	T/G X±SD	G/G X±SD	p*	
Wender -Utah score	45.1±12.4	42.5±13.6	52.4 ±8.8	0.036	G/G>T/G (p=0.028)†
1st parts score of adult ADD/ADHD scale	15.4±4.6	13.3±5.0	18.3±5.1	0.007	G/G>T/G (p=0.005)†
3rd parts score of adult ADD/ADHD scale	16.3±5.4	13.4±7.1	19.1±6.0	0.014	G/G>T/G (p=0.012)†

*one-way ANOVA test was performed, †Tukey HSD correction was performed. ADD: attention deficit disorder, ADHD: attention deficit hyperactivity disorder, ANOVA: analysis of variance

and *MnlI* and *DdeI* polymorphisms of SNAP-25 gene reported different results. Kim et al.¹⁷ examined 61 SNPs in 229 families and found a statistically significant association with only a single SNP (rs3787283), which was in strong linkage disequilibrium with *MnlI* and *DdeI* polymorphisms (p=0.002). Although, they also emphasized that combined analysis of the pooled data of the whole SNPs was modestly significant for *MnlI* (p=0.048) and rs6077690 (p=0.031). Choi et al.¹⁸ have detected a significant association between ADHD and *MnlI* polymorphism. In the present study, we determined a significant association between ADHD and *MnlI* polymorphism, which is similar to the findings of Kim et al.¹⁷ and Choi et al.¹⁸

While Brophy et al.¹⁹ have reported no significant relation between ADHD and *MnlI* polymorphism; they have reported significant relation between ADHD and *DdeI* polymorphism. Kustanovich et al.³ reported an increased transmission of the *Ddel* polymorphism of SNAP-25 gene. On the other hand, Barr et al.⁹ have reported significant relation between ADHD and *MnlI* and *DdeI* polymorphisms of SNAP-25 gene. Faraone and Mick²⁰ have performed a meta-analysis, consisted of four family-based studies of SNAP25 examining two SNPs (*Ddel* and *MnlI*), and they have shown significant evidence for an association with ADHD and *MnlI* (OR=1.19, 95% CI: 1.03–1.38); similar to our results.

Recently, a relation between ADHD and *MnlI* and *DdeI* polymorphisms of SNAP-25 gene is mentioned in the pharmacogenetic studies. Öner et al.²¹ have reported that hemodynamic changes in the brain induced by methylphenidate might be related with SNAP-25 gene *DdeI* T/T and *MnlI* T/T genotypes in a fNIRS (*Functional Near-Infrared Spectroscopy*) study performed with 15 ADHD adults and 16 ADHD children. They suggested that their results were in line with Barr et al.⁹ findings; combination of *DdeI* and *MnlI* polymorphisms of SNAP-25 gene might be important in ADHD. In a study investigating the response of methylphenidate in adult ADHD and *DdeI* and *MnlI* polymorphisms of SNAP-25 gene, Ünal et al.²² reported that considerable increase was determined in N-acetylaspartate (NAA) levels after methylphenidate at anterior cingulate cortex in the samples having SNAP-25 *Ddel* polymorphism T/T genotype and *MnlI* polymorphism G/G genotype. In the present study, an association between ADHD and *MnlI* polymorphism G allele was determined. Also, the subjects having *MnlI* polymorphism G/G genotype had higher Wender-Utah scores and higher scores in the adult ADD/ADHD Scale. Therefore, these findings may suggest much more impairment of neuronal integrity and functionality. When it is considered that NAA is a marker of neuronal integrity and functionality, our results is consistent with

the findings of Ünal et al.,²² indicating considerable increase in NAA levels after methylphenidate in the samples having *Mn1l* polymorphism G/G genotype. Aforementioned studies performed among adult ADHD patients involving SNAP-25 gene are pharmacogenetic studies, but not association studies. Our study was the first report on the association of SNAP-25 gene with adult ADHD.

Limitations of the study are the number of the patient and the control groups not to be much enough, impossibility of excluding familial genetic load completely, interaction of the genes with each other besides their relation with the disorder.

In conclusion, it is supposed that the supplied evidence supporting the association of SNAP-25 gen polymorphisms and ADHD may be important in the genetic etiology of ADHD.

REFERENCES

- American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IVTR). Washington, DC: American Psychiatric Association; 2000.
- Franke B, Neale BM, Faraone SV. Genome-wide association studies in ADHD. *Hum Genet* 2009;126:13-50.
- Kustanovich V, Merriman B, McGough J, McCracken JT, Smalley SL, Nelson SF. Biased paternal transmission of SNAP-25 risk alleles in attention-deficit hyperactivity disorder. *Molecular Psychiatry* 2003;8:309-315
- Zhang H, Zhu S, Zhu Y, Chen J, Zhang G, Chang H. An association study between SNAP-25 gene and attention-deficit hyperactivity disorder. *Eur J Paediatr Neurol* 2011;15:48-52.
- Gizer IR, Ficks C, Waldman ID. Candidate gene studies of ADHD: a meta-analytic review. *Hum Genet* 2009;126:51-90.
- Cai F, Chen B, Zhou W, Zis O, Liu S, Holt RA, et al. SP1 regulates a human SNAP-25 gene expression. *J Neurochem* 2008;105:512-523.
- Thapar A, O'Donovan M, Owen MJ. The genetics of attention deficit hyperactivity disorder. *Hum Mol Genet* 2005;14:R275-R282.
- Wilson MC. Coloboma mouse mutant as an animal model of hyperkinesis and attention deficit hyperactivity disorder. *Neurosci Biobehav Rev* 2000;24:51-57
- Barr CL, Feng Y, Wigg K, Bloom S, Roberts W, Malone M, et al. Identification of DNA variants in the SNAP-25 gene and linkage study of these polymorphisms and attention-deficit hyperactivity disorder. *Mol Psychiatry* 2000;5:405-409.
- Faraone SV, Perlis RH, Doyle AE, Smoller JW, Goralnick JJ, Holmgren MA, et al. Molecular genetics of attention-deficit/hyperactivity disorder. *Biol Psychiatry* 2005;57:1313-1323.
- McCann BS, Schele L, Ward N, Roy-Byrne P. Discriminant Validity of the Wender Utah Rating Scale For Attention-Deficit/Hyperactivity Disorder in Adults. *J Neuropsychiatry Clin Neurosci* 2000;12:240-245.
- Öncü B, Ölmez S, Şentürk V. Validity and reliability of the Turkish version of the Wender Utah Rating Scale for attention-deficit/hyperactivity disorders in adults. *Turk J Psychiatry* 2005;16:252-259.
- Günay Ş, Savran C, Aksoy UM, Maner F, Turgay A, Yargıç İ. The norm study, transliterational equivalence, validity, reliability of adult hyperactivity scale in Turkish adult population. *Psychiatry Turk* 2006;8:98-107.
- Feng Y, Crosbie J, Wigg K, Pathare T, Ickowicz A, Schachar R, et al. The SNAP25 gene as a susceptibility gene contributing to attention-deficit hyperactivity disorder. *Mol Psychiatry* 2005;10:998-1005, 973.
- Mill J, Richards S, Knight J, Curran S, Taylor E, Asherson P. Haplotype analysis of SNAP-25 suggests a role in the aetiology of ADHD. *Mol Psychiatry* 2004;9:801-810.
- Renner TJ, Walitza S, Dempfle A, Eckert L, Romanos M, Gerlach M, et al. Allelic variants of SNAP25 in a family-based sample of ADHD. *J Neural Transm* 2008;115:317-321.
- Kim JW, Biederman J, Arbeitman L, Fagerness J, Doyle AE, Petty C, et al. Investigation of variation in SNAP-25 and ADHD and relationship to co-morbid major depressive disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007;144B:781-790.
- Choi TK, Lee HS, Kim JW, Park TW, Song DH, Yook KW, et al. Support for the MnlI polymorphism of SNAP25; a Korean ADHD case-control study. *Mol Psychiatry* 2007;12:224-226.
- Brophy K, Hawi Z, Kirley A, Fitzgerald M, Gill M. Synaptosomal-associated protein 25 (SNAP-25) and attention deficit hyperactivity disorder (ADHD): evidence of linkage and association in the Irish population. *Mol Psychiatry* 2002;7:913-917.
- Faraone SV, Mick E. Molecular genetics of attention deficit hyperactivity disorder. *Psychiatr Clin North Am* 2010;33:159-180.
- Öner Ö, Akın A, Herken H, Erdal ME, Çiftçi K, Ay ME, et al. Association Among SNAP-25 Gene DdeI and MnlI Polymorphism and Hemodynamic Changes During Methylphenidate Use: A Functional Near-Infrared Spectroscopy Study. *J Atten Disord* 2011;15:628-637.
- Ünal GA, Kenar Aİ, Herken H, Tepeli E, Kiroğlu Y. The Relationship Between SNAP-25 Gene Polymorphism and Response to The Treatment of Methylphenidate HCl in ADHD. *Bull Clin Psychopharmacol* 2012;22(Suppl1):S157.