# Association of Synapsin III Gene with Adult Attention Deficit Hyperactivity Disorder

Ayşe Nur İnci Kenar,[1] Tuba Edgünlü,[2] Hasan Herken,[3] and Mehmet Emin Erdal[4]

It was aimed to investigate the association of the synapsin III gene $-196\,G > A$ and $-631\,C > G$ polymorphisms that takes place in an encoding presynaptic protein, with adult attention deficit hyperactivity disorder (ADHD). One hundred thirty-nine patients having adult ADHD and 106 controls were included in the study. DNA samples were extracted from whole blood and genetic analyses were performed. A significant difference was determined between ADHD and synapsin III gene $-631\,C > G$ polymorphism compared to the control group. No significant difference was determined between ADHD and synapsin III gene $-196\,G > A$ polymorphism. These polymorphisms were found not to be associated with subtypes of ADHD. It is supposed that synaptic protein genes together with dopaminergic genes might have roles in the etiology of ADHD.

## Introduction

ATTENTION DEFICIT HYPERACTIVITY disorder (ADHD) is one of the psychiatric disorders that genetic factors play a major role in the etiology (Curran and Taylor, 2000). Genetic investigations, which are performed to study the role of genetic factors in the etiology, are focused especially on the dopamine pathways and receptors. However, it is suggested that the genes encoding presynaptic proteins that have roles in neurotransmitter releasing and synaptogenesis may play a role in the etiology of ADHD (Kovács-Nagy et al., 2009).

There are a lot of studies reporting the relation of ADHD and SNAP-25 gene (the synaptosomal-associated protein, 25 kDa), which is one of the genes encoding the proteins that have roles in the modulation of neurotransmitter release (Kimura et al., 2003). SNAP-25 gene Mnll and Ddel polymorphisms are the mostly studied polymorphisms in ADHD, and either or both Mnll and Ddel polymorphisms are found to be related with ADHD in various studies (Barr et al., 2000; Brophy et al., 2002; Choi et al., 2007). Our clinic has studied the relation between ADHD and synaptobrevin-2 (VAMP2) and syntaxin 1A genes, which are the genes encoding proteins that have roles in the modulation of neurotransmitter release, and reported a significant correlation between ADHD and these genes (Kenar et al., 2012). In another study, a decrease in the syntaxin 1A m-RNA levels and a downregulation in synaptic proteins was determined after the administration of methylphenidate, which is used in the treatment of ADHD (Bartl et al., 2010).

The biology of synapsin III is not as well understood as synapsins I and II, this gene is emerging as an important factor in the regulation of the early stages of neurodevelopment and dopaminergic neurotransmission, and in certain neuropsychiatric illnesses. Synapsins are neuron-specific synaptic vesicle-related phosphoproteins that effect the modulation of neurotransmitter release, synaptogenesis, and axonal formation (Ohmori et al., 2000). Three types of synapsins are determined; synapsin I, II, and III (Kao et al., 1998). Feng et al. (2002) reported that the generation of a synapsin III knockout mouse has provided evidence of a unique function of this gene compared with synapsin I and II, indicating a role in early axon development and the regulation of neurotransmitter release in mature synapses with evidence for decreased basal transmission at inhibitory synapses, but not at excitatory synapses. ADHD has been hypothesized to be a neurodevelopment disorder. Furthermore, it has been reported that not only synapsins, but also many synaptic proteins such as synaptobrevins, synaptophysins, synaptotagmins, SNAP-25, and syntaxins are involved in synaptic plasticity (Söllner et al., 1993; Südhof, 1995). These proteins are also involved in neurotransmitter release. Thus, these synaptic proteins are worthy of investigation as an approach to understand the pathogenesis of ADHD.

We present our current understanding of the newest member of the synapsin gene family, synapsin III, with regard to its relationship with the other synapsins, its role in neurodevelopment and neurotransmission, and its potential relevance to human disease.

[1]Denizli State Hospital, Denizli, Turkey.
[2]School of Health, Muğla University, Muğla, Turkey.
[3]School of Medicine, Pamukkale University, Denizli, Turkey.
[4]School of Medicine, Mersin University, Mersin, Turkey.

Synapsin III (SYN3) has been mapped to the long arm of chromosome 22 at 22q12–q13 (Ohtsuki *et al.*, 2000). As this region is supposed to be responsible from schizophrenia, the synapsin III gene is studied mostly in schizophrenia among psychiatric disorders (Ohmori *et al.*, 2000). No relationship was found between synapsin III gene polymorphisms and schizophrenia (Ohmori *et al.*, 2000; Imai *et al.*, 2001). There is only one study that studied the relationship between rs242089, rs3788459, rs1056484, −196 G > A and −631 C > G polymorphisms of the synapsin III gene and ADHD in English literature and it reported no relationship between this gene and ADHD (Makkar *et al.*, 2007). However, polymorphism studies can show ethnic differences.

In this study, it is aimed to research the relationship between adult ADHD and the synapsin III gene −196 G > A and −631 C > G polymorphisms in a Turkish population.

## Methods

### Subjects

A total of 139 patients between ages of 18 and 60, meeting DSM-IV criteria for adult ADHD were included in the study. All patients were recruited from the research center and were of Turkish origin. Patients were evaluated with the Wender-Utah Rating Scale (WURS) and Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale. Patients who scored 36 points or more on the WURS and answered at least six of nine questions as two or three of first and second parts of the Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale were diagnosed as ADHD. The control group consisted of 106 healthy subjects between 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 the WURS and Adult ADHD Diagnosis and Evaluation Scale and, subjects who met adult ADHD criteria were excluded from the control group.

### Instruments

Social demographic data form. A data sheet was developed by the researchers for studying the sociodemographic characteristics of study groups.

Wender-Utah Rating Scale. sWURS was developed by Ward and Wender (McCann *et al.*, 2000). Turkish validity and reliably of the WURS was established by Öncü *et al.* (2005) and the cutoff score point was 36.

Adult ADD/ADHD DSM IV-Based Diagnostic Screening and Rating Scale. Adult ADHD 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 the 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 Günay *et al.* (2006).

DNA isolation and molecular analysis. For the amplification of the synapsin III gene −196 G > A (rs133945) poly-

morphism, DNA was isolated from peripheral blood leukocytes by the standard phenol/chloroform method and genotyped by the polymerase chain reaction–restriction fragment length polymorphism method. PCR was performed with a personal thermal cycler (Techgene), using SYN2 F-5'-TCCTTTCCAGAAGGATGTCC-3'/SYN2 R-5'-AAGCCAACAAATACATAAGTGGAGA-3' primers.

For the amplification of the synapsin III gene −631 C > G (rs133946) polymorphism, DNA was isolated from peripheral blood leukocytes by the Standard phenol/chloroform method and genotyped by the PCR-RFLP method. PCR was performed with a personal thermal cycler (Techgene), using SYN1 F-5'-AGGCATGTACTTGCGTTACC-3'/SYN1 R-5'-ACCAAATGACTACAAAGATGTACCA-3' primers.

### Statistical analyses

Statistical Package for Social Sciences (SPSS) version 10.0 for Windows computing program was used for statistical analysis of the data. The chi-square test was used to compare categorical variables and the independent samples *t*-test was used to compare continuous variables. Logistic regression analysis was used to assess the risk. A *p*-value of < 0.05 was accepted as statistically significant.

## Results

A total of 139 patients with adult ADHD and 106 healthy controls were admitted to the study. The mean age of the study group was $27.12 \pm 9.77$ and mean age of the control group was $27.86 \pm 7.88$. There was no significant difference between the study and control groups regarding age ($p > 0.05$). The study group consisted of 61 women (43.9%) and 78 men (56.1%) and the control group consisted of 41 women (38.7%) and 65 men (61.3%). There was also no significant difference between the study and control groups regarding gender ($p > 0.05$).

Of the 139 patients with adult ADHD, 40 (28.8%) were diagnosed as predominantly inattentive type, 39 (28.1%) were diagnosed as predominantly hyperactive-impulsive type, and 60 (43.2%) were diagnosed as combined type of ADHD.

### Synapsin III gene −196 G > A polymorphism results of the groups

Because of technical problems, genomic DNAs for the synapsin III gene −196 G > A polymorphism could not be obtained from 5 of the 139 patients and 6 of the 106 control subjects, and they were not included into the results.

The groups were compared according to the synapsin III gene −196 G > A polymorphism. The G allele (patient: 64.9%, control: 62.0%) ($p = 0.515$) and the G/A genotype (patient: 47.8%, control: 54.0%) ($p = 0.610$) were determined most often among the groups and no significant difference was found between the groups.

No significant difference was found between the groups when they were compared according to whether they have the G ($p = 0.963$) or A allele ($p = 0.347$) (Table 1).

Because no significant difference was determined between the groups in the point of the synapsin III gene −196 G > A polymorphism, genotype distribution of ADHD subtypes was not compared.

Table 1. Frequencies of Synapsin III Gene −196 G>A Polymorphism

| Allele frequencies | ADHD n (%) | Control n (%) | p-Value[a] |
|---|---|---|---|
| G | 174 (64.9%) | 124 (62.0%) | |
| A | 94 (35.1%) | 76 (38.0%) | 0.515 |
| Total | 268 (100.0%) | 200 (100.0%) | |
| *Genotype frequencies* | n (%) | n (%) | |
| G/G | 55 (41.0%) | 35 (35.0%) | |
| G/A | 64 (47.8%) | 54 (54.0%) | 0.610 |
| A/A | 15 (11.2%) | 11 (11.0%) | |
| Total | 134 (100.0%) | 100 (100.0%) | |
| *Presence or absence of G and A alleles* | n (%) | n (%) | |
| G/G+G/A | 119 (88.8%) | 89 (89.0%) | 0.963 |
| A/A+G/G | 79 (59.0%) | 65 (65.0%) | 0.347 |

[a]Chi-square test was performed.
ADHD, attention deficit hyperactivity disorder.

### Synapsin III gene −631 C>G polymorphism results of the groups

Because of technical problems, genomic DNAs for the synapsin III gene −196 G>A polymorphism could not be obtained from 5 of the 139 patients and 7 of the 106 control subjects, and they were not included into the results.

When allele frequencies were compared according to the synapsin III gene −631 C>G polymorphism, the C allele (patient: 69.4%, control: 70.2%) ($p=0.853$) was determined most often in both of the groups and the difference between the groups was not found as significant. When genotype frequencies were compared, the genotype distribution was determined as follows: ADHD group; 40.3% had C/C, 58.2% had C/G, 1.5% had G/G genotype, control group; 47.5% had C/C, 45.4% had C/G, 7.1% had G/G genotype. The difference between the groups was found statistically significant ($p=0.030$).

Fifty-nine point seven percent of the ADHD group and 52.5% of the control group had the G allele and there was no significant difference between the groups ($p>0.05$). When the groups were compared with respect to the C allele, the ADHD group and the control group were found to have statistically significantly different ($p=0.039$) rates of the C allele, which were 98.5% and 92.9%, respectively (Table 2).

When C/G and G/G genotypes of the synapsin III gene −631 C>G polymorphism were compared, the C/G genotype was found statistically significantly higher (97.5%) in the ADHD group and the G/G genotype was found statistically significantly higher (13.5%) in the control group ($p=0.028$). When C/C and C/G genotypes and C/C and G/G genotypes were compared with each other, no significant difference was determined ($p>0.05$) (Table 2).

According to the logistic regression analysis, even after adjusted by age and gender, when the C/C+C/G genotype was regarded as reference, the possibility of having the G/G genotype in the subjects that are not ADHD was not found statistically significant according to the subjects diagnosed as ADHD ($p>0.05$).

Table 2. Frequencies of Synapsin III Gene −631 C>G Polymorphism

| Allele frequencies | ADHD n (%) | Control n (%) | p-Value[a] |
|---|---|---|---|
| C | 186 (69.4%) | 139 (70.2%) | |
| G | 82 (30.6%) | 59 (29.8%) | 0.853 |
| Total | 268 (100.0%) | 198 (100.0%) | |
| *Genotype frequencies* | n (%) | n (%) | |
| C/C | 54 (40.3%) | 47 (47.5%) | |
| C/G | 78 (58.2%) | 45 (45.4%) | 0.030[b] |
| G/G | 2 (1.5%) | 7 (7.1%) | |
| Total | 134 (100.0%) | 99 (100.0%) | |
| *Presence or absence of C and G alleles* | n (%) | n (%) | |
| C/C+C/G | 132 (98.5%) | 92 (92.9%) | 0.039[b] |
| G/G+C/G | 80 (59.7%) | 52 (52.5%) | 0.275 |
| *Dual comparison of genotypes* | n (%) | n (%) | |
| C/C | 54 (40.9%) | 47 (51.1%) | |
| C/G | 78 (59.1%) | 45 (48.9%) | 0.132 |
| Total | 101 (100.0%) | 92 (100.0%) | |
| | n (%) | n (%) | |
| C/C | 54 (96.4%) | 47 (87.0%) | |
| G/G | 2 (3.6%) | 7 (13.0%) | 0.091 |
| Total | 56 (100.0%) | 54 (100.0%) | |
| | n (%) | n (%) | |
| C/G | 78 (97.5%) | 45 (86.5%) | |
| G/G | 2 (2.5%) | 7 (13.5%) | 0.028[b] |
| Total | 80 (100.0%) | 52 (100.0%) | |

[a]Chi-square test was performed.
[b]$p<0.05$.

When ADHD subtypes and genotypes were compared, the C/C genotype was found the most often in the combined type (44.8%) and the C/G genotype was found the most often in the predominantly inattentive type (64.1%). No G/G genotype was found in the predominantly inattentive type, but only one case was determined in each of the predominantly hyperactive-impulsive type and combined type (2.7% and 1.7%, respectively). The difference between the subtypes was not found statistically significant ($p=0.741$) (Table 3).

Table 3. Synapsin III Gene −631 C>G Polymorphism Genotype Distribution of the Attention Deficit Hyperactivity Disorder Subtypes

| Genotype | Inattentive type n (%) | Hyperactive-impulsive type n (%) | Combined type n (%) | p-Value[a] |
|---|---|---|---|---|
| C/C | 14 (35.9%) | 14 (37.8%) | 26 (44.8%) | |
| C/G | 25 (64.1%) | 22 (59.5%) | 31 (53.5%) | 0.741 |
| G/G | 0 (0.0%) | 1 (2.7%) | 1 (1.7%) | |
| Total | 39 (100.0%) | 37 (100.0%) | 58 (100.0%) | |

[a]Chi-square test was performed.

## Discussion

The studies that are performed about the genetic etiology of the ADHD are focused on the proteins, which have roles in the modulation of neurotransmitter releasing and synaptogenesis process recently (Wilson, 2000). The synapsin III gene, one of the genes encoding those proteins, has been studied mostly in schizophrenia among the psychiatric disorders, just as ADHD in which, the modulation of dopamine releasing is important in the etiology.

Imai *et al.* investigated the relation of six polymorphisms of the synapsin III gene; 1408 (exon 3) Thr136Thr, 1402 (exon 12) Pro468Ser, 1573 (exon12) Glu525Gln, 1601 (exon 12) Pro534Leu, 1769 G>C, and also −196 G>A polymorphism, with schizophrenia. They have determined the genotype frequencies of all polymorphisms except the −196 G>A polymorphism, almost the same in the patient and the control groups. Genotype frequencies of the −196 G>A polymorphism were determined as GG 43%, GA 52%, AA 5% among the schizophrenia patients and as GG 50%, GA 39%, AA 11% among the control group. Although the difference between the patient and the control groups was not significant, they studied the transcription binding site of −196 G>A polymorphism by advanced genetic analysis and have determined that the site of the −196 G>A polymorphism did not match up with motifs involving transcriptional regulation. Thus, they suggested that this polymorphism may not have a functional effect on the transcriptional activity of the synapsin III gene (Imai *et al.*, 2001). Ohmori *et al.* (2000) did not observe a significant relation between the genotypes and alleles of −196 G>A and −631 C>G polymorphisms of the synapsin III gene and schizophrenia in a study that they performed among the schizophrenia patients. Makkar *et al.* studied the relation of ADHD and rs242089, rs3788459, rs1056484, −196 G>A, and −631 C>G polymorphisms of the synapsin III gene among 177 small, nuclear families consisting of an ADHD proband, their parents, and 43 affected siblings. They reported that there was no significant relation between these 5 polymorphisms of the synapsin III gene and ADHD (Makkar *et al.*, 2007).

In the present study, no relation was observed between ADHD and −196 G>A polymorphism of the synapsin III gene as compatible with the literature. The observation such as no relation between the studied disorder and −196 G>A polymorphism of the synapsin III gene in most of the studies may support the idea that Imai *et al.* (2001) suggested as this polymorphism may not have a functional effect on the transcriptional activity of the synapsin III gene. In other words, since this polymorphism site does not have transcription, it may be thought that it does not have any effects on the synthesized synapsin III protein. Nonetheless, we found a significant relation between ADHD and −631 C>G polymorphism of the synapsin III gene opposing the reports of Makkar *et al.* The difference between the aforementioned study and ours may be due to the fact that, while their study is a family study and does not have a control group, our study is a case–control study and the subjects are from different ethnic groups. A significant relation was determined among the allele frequencies of the −631 C>G polymorphism of the synapsin III gene between the patient and the control groups in another study that was performed to investigate the association of the synapsin III gene and Alz-

heimer's disease, where learning, attention and memory problems are prominent as ADHD. It was reported that the subjects having G alleles have 1.5 times much more risk of developing Alzheimer's disease compared to the subjects having C alleles (Gökdoğan, 2009). Both of this study and ours show that the −631 C>G polymorphism of the synapsin III gene is effective on the function of the synthesized synapsin III protein.

Consequently, to our knowledge, this is the first study in English literature, which ascertains the relation of the synapsin III gene and ADHD in adults and shows that the −631 C>G polymorphism of the synapsin III gene may be associated with ADHD.

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

## Conclusion

As a conclusion, genes encoding proteins that have roles in the modulation of neurotransmitter release and synaptogenesis seem to be the candidate genes in the genetic etiology of ADHD. The present study is supposed to form a base for the forthcoming gene research.

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Address correspondence to:
*Hasan Herken, MD*
*School of Medicine*
*Pamukkale University*
*Denizli 20100*
*Turkey*

*E-mail:* hherken@pau.edu.tr