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Carboxylesterase1, alpha 2a adrenergic receptor and noradrenalin transporter gene polymorphisms and their clinical effects in attention deficit hyperactivity disorder in Turkish children

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ABSTRACT

The objective of this study was to examine the association between ADHD and G1287A polymorphism in the NET1 gene, C1291G polymorphism in the ADRA2A gene on the adrenergic pathway and Gly143Glu polymorphism in the CES1 gene on the metabolic pathway, and their clinical effects.

The study population included 114 children with ADHD and 83 healthy controls. 103 patients are followed for 6 months, their scale points are recorded and side effects are questioned in each interview.

Every patient in both control and ADHD group are found to have GG genotype when Gly143Glu polymorphism in the CES1 gene is examined, thus we came to a conclusion that Turkish population is homozygote in the mentioned polymorphism. No significant association between NET1 gene G1287A polymorphism genotypes and ADHD was found. It was found that ADRA2A C1291G polymorphism C allele and CC genotype is a risk factor for ADHD ($p = 0.003$, OR: 2.17, CI: 12.8–37.0) and the risk is higher in males ($p = 0.013$, OR: 2.43, CI: 12.0–49.5). There was no significant relation between ADRA2A C1291G polymorphism and clinical parameters but it was found that individuals with NET1 G1287A polymorphism AA genotype have less concurrent Oppositional Defiant Disorder diagnosis (18.8% vs. 81.2%, $p = 0.039$), their initial CTRS-attention deficit points are higher (17.47 ± 3.73 vs.16.15 ± 4.58, $p = 0.045$).

In conclusion, our study showed that the ADRA2A C1291G polymorphism C allele and CC genotype is associated with ADHD. NET1 G1287A polymorphism AA genotype is mainly associated with attention deficit cluster.

1. Introduction

Attention Deficit Hyperactivity Disorder (ADHD) is a neuropsyhiatric disorder, and a number of genetic factors play role in its etiopathogenesis (Association, 2013). The genes and their polymorphisms in dopaminergic and adrenergic pathways have been most extensively studied as candidate genes due to high efficacy rates of psychostimulants; a non-stimulant agent, atomoxetine; and alpha agonists; and because a number of studies have indicated dopamine and noradrenalin as the main neurotransmitters related to attention function (Barnes et al., 2011; Biederman and Spencer, 1999; Swanson et al., 2007). Currently, the association of gene polymorphisms in those pathways with ADHD has been clearly put forward (del Campo et al., 2011). Therefore, in the light of heterogeneous clinical picture, the investigators tend to study the effects of genetic polymorphisms on clinical parameters including clinical presentation, treatment response, and adverse effect frequency taking the heterogeneous clinic, endophenotype concept, and the minor effects of a number of genes instead of a major gene into account (Banaschewski et al., 2010; Kebir and Joober, 2011). From this point of view, metabolic pathways that affect the pharmacokinetics of the drugs used in the treatment and the candidate genes in those pathways have been the topics of the current studies (Froehlich et al., 2010; Kieling et al., 2010).

NET is a sodium chloride-paired transporter protein that is

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responsible for noradrenalin reuptake from the synaptic cleft. It has 12 transmembrane regions, is composed of 617 aminoacids, and coded by NET1 gene (Porzgen et al., 1995). NET1 is a suitable gene for research in the genetics of ADHD since NET, placed mainly in the frontal lobe, is responsible for both noradrenalin and dopamine reuptake and noradrenalin-dopamine balance in synaptic cleft is only maintained by NET in this region, noradrenergic signal transduction results in ADHD-like symptoms in rodents, low noradrenalin levels in orbital and dorsal frontal regions have been associated with poor concentration and increased motor activity, and the only FDA-approved non-stimulant treatment option of ADHD, atomoxetine, is a strong and selective NET inhibitor (Faraone and Mick, 2010; Gizer et al., 2009; Russell, 2011). NET1 gene is located at chromosome 16q12.2 (Gelernter et al., 1993). NET1 gene, also known as *Solutecarrierfamily 6, member 2* (SLC6A2), is composed of 14 exons and 13 introns (Pacholczyk et al., 1991).

Alpha-2 receptors are located in the presynaptic region, and carry inhibitory properties. They have 3 subtypes: A, B and C (Aoki et al., 1994). Among them, ADRA2A is the most widely distributed subtype in the brain including prefrontal cortex, amygdala, hippocampus, and locus ceruleus (Aoki et al., 1994). ADRA2A has been noted as a candidate gene of ADHD since noradrenergic system play role in executive functions such as wakefulness and regulation of attention, ADRA2A is mainly responsible for a number of executive functions particularly in medial prefrontal cortex, animal studies indicated that stimulation of noradrenergic projections resulted in improvement in cortical functions, and presynaptic alpha-2 receptor agonists, clonidine and guanfacine, have been used in treatment of ADHD for a long time (Arnsten et al., 1996; Bidwell et al., 2010; Biederman and Spencer, 1999). ADRA2A is situated at chromosome 10q23-q25 (Yangfeng et al., 1987).

The only psychostimulant treatment option in Turkey is methylphenidate (Çetin et al., 2015). It is broken down by carboxylase enzyme (CES1) (Sun et al., 2004). From the point of pharmacokinetics, it may be suggested that CES1 gene polymorphisms may be the key responsible factors in patients resistant to treatment, or in the ones that experience adverse effects (Zhu et al., 2008). On the other hand, pharmacogenetic researches of ADHD mostly focused on MPH target receptors and transporters, and investigations on MPH metabolism are only small in number (Nemoda et al., 2009). It may be suggested that limited data on MPH metabolism compared to data on mechanism of action MPH is related to this (Froehlich et al., 2010).

The objective of this study is to investigate the association between ADHD and G1287A polymorphism in the NET1 gene, C1291G polymorphism of ADRA2A gene on adrenergic pathway, Gly143Glu polymorphism of the CES1 gene on the metabolic pathway, their clinical effects on treatment response, and adverse effects on ADHD subtypes in children with ADHD. Our hypothesis is that candidate gene polymorphisms play role in ADHD etiology, and affect clinical parameters.

2. Material and methods

2.1. Participants and procedures

The first part of this study is a cross-sectional and descriptive study performed on children newly diagnosed with ADHD, and scheduled for medical treatment in Gazi University, Adolescent and Child Psychiatry Clinic between September–December 2012. We aimed to investigate probable associations of Gly143Glu, G1287A and C1291G polymorphisms situated on CES1, NET1 and ADRA2A genes, respectively, with ADHD, and compare the frequencies of the genotypes with healthy controls. The second part of the study is a naturalistic follow up study performed on the same patients until June 2013 in order to determine probable clinical reflections of the polymorphisms.

The study population consisted of 114 children with ADHD that admitted to the outpatient clinic of Gazi University Child and Adolescent Psychiatry Department, and 83 healthy children.

The patients included in the study were the patients that admitted to the outpatient clinic of Gazi University Child and Adolescent Psychiatry Department. The children and their families were informed about the study after they had been examined by a child psychiatrist, and prediagnosed with ADHD if they met the inclusion criteria to the study. The patients were included in the study after their families provided their informed consents for the study. In addition, the patients older than 12 years of age provided their verbal informed consents. The patients included in the study were re-examined by the executive researcher, a faculty of Child and Adolescent Psychiatry Department, and the ones that were diagnosed with ADHD according to DSM-IV-TR were administered *Turkish version of Schedule for Affective Disorders and Schizophrenia for School Age Children-Present and Lifetime Version* (K-SADS-PL), a semi-structured diagnostic tool, by the responsible child psychiatrist of the study. Therefore, clinical diagnoses of the patients were based on semi-structured interview of the patients and their families, and scales were filled in by the family/teachers.

The control group consisted of 83 subjects that admitted to Gazi University Child and Adolescent Psychiatry Department with problems of adolescence, school adaptation, family relations, or similar problems requiring counseling, but they were not diagnosed with any psychiatric disorders after clinical examination and psychometric tests. The control group had similar sociodemographic characteristics with the ADHD group, and their ages were between 6 and 12 years.

The participants of the study group were between the ages of 6 and 12 years, they were diagnosed with ADHD according to K-SADS-PL and DSM-IV-TR, they did not have any additional psychiatric disorders other than Oppositional Defiant Disorder (ODD). They did not have any neurological/chronic diseases, and had a total IQ score > 90. Conners' Teacher Rating Scale (CTRS) scores and other psychometric analyses of the patients included in the study group were first recorded, they were administered medical therapy, and they were re-examined by the responsible resident researcher at 1st, 3rd, and 6th months of the treatment. The CTRS scores were recorded in every visit, and the adverse effects were questioned using a questionnaire composed of open-ended questions. Revisions including increasing or decreasing the dose of the drug, changing the medicine, or stopping the medicine were made, as needed.

2.2. Data collection tools

2.2.1. Patient report form

This form was prepared for this study, and it was filled in during the interview with the children and adolescents, and their parents. Age, educational level (class), weight, ADHD subtype, the drug used for ADHD and its dose, the revisions in treatment at control visits, and the adverse effects were noted in this form.

2.2.2. Turkish version of schedule for affective disorders and schizophrenia for school age children-present and lifetime version (K-SADS-PL)

It was used to determine the diagnosis and additional diagnoses of the patients in the study group. The patients were diagnosed after interview of the responsible resident researcher with the patients and one of their parents, and then the clinical evaluation of the executive faculty researcher. Scale is a semi-structured interview form developed by Kaufman et al. to determine past and current psychopathologic disorders in children and adolescents (6–18 years of age) (Kaufman et al., 1997). Its validity and reliability in Turkish were studied by Gökler et al. (Gökler et al., 2004). The child psychiatrist that performed the interviews has been educated for this semi-structured interview, and has a certificate.

2.2.3. Conners attention deficit hyperactivity disorder teacher rating scale (CTRS)

This scale was developed by Conners (Conners, 1969), and revised by Goyotte et al. (Goyette et al., 1978). It was adapted into Turkish, and its validation and reliability studies were done by Dereboy et al.

(Dereboy et al., 2006). The scale contains questions about hyperactivity, attention deficit, and behavioral problems of the child in school, and the teachers are asked to grade them. In this study, this scale was used in order to analyze the probable relationships of the polymorphisms with clinical characteristics.

2.2.4. Wechsler Intelligence Scale for Children-Revised (WISC-R)

This scale was developed by Wechlesler in 1949, and revised in 1974 (WISC-R) to increase the age range of the scale between 6 and 16 years of age. Savaşır et al. performed the reliability and validity studies in Turkish (Savasır and Sahin, 1995; Wechsler, 1974).

2.3. Genotyping

Molecular analysis was performed at Life Sciences Research and Application Center, Gazi University. Genomic DNA was extracted from venous blood by Heliosis® DNA extraction kit (Metis Biotechnology, Turkey) according to manufacturer's instructions.

The polymorphism of CES1 (Gly143Glu: rs71647871), ADRA2A (C1291G: rs1800544) and NET1 (G1287A: rs5569) were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Sequences of the PCR amplification primers were as follows: CES1, 5′-CCCAGGTGATGGTGTGGAT-3′ (forward) and 5′-GCCTT ACTGTGGAACCTAGCTAAGC-3′ (reverse); ADRA2A, 5′-TCACACCGGA GGTTACTTCCCTCG-3′ (forward) and 5′-GGTACCTTGAGCTAGAG ACT-3′ (reverse); NET1, 5′- TCCAGGGAGACCCTAATTCC-3′ (forward) and 5′-TTGACTTTATTG AAATGCGGC-3′ (reverse). DNA amplification was performed in a 50 μL reaction mixture including 50 ng DNA, 100 μM of each dNTP, 200 mM (NH4)2SO4, Tween-20 (0.1%), 75 mM Tris-HCl (pH 8.8), 1 unit Taq DNA polymerase (Fermentas, Vilnius, Lithuania), 2.5 mM (for ADRA2A rs1800544 polymorphism) or 1.5 mM MgCl2 and 50 pmol of primers for each polymorphism. Thermal cycling conditions were 940C for 5 min, followed by 30 cycles of 940C for 30 s, 550C or 580C (for ADRA2A rs1800544 polymorphism) for 45 s and then 720C for 5 min. The sizes of the amplified products were 251 bp for CES1, 216 bp for ADRA2A, and 241 bp for NET1. PCR products were digested overnight with *Alu*I, *Msp*I ve *Sau*96I restriction enzymes (MBI, Fermentas, Vilnius, Lithuania) at 37 °C for the CES1 (rs71647871), ADRA2A (rs1800544) and NET1 (rs5569) polymorphisms, respectively. The digested PCR products were resolved on 3% or 4% (for NET1 rs5569 polymorphism) agarose gel stained with ethidium bromide, and photographed using the KODAK Gel Logic 100 Imaging System (Kodak, Rochester, NY, USA).

After digestion with AluI, A allele generated five fragments of 168, 32, 27, 19 and 5 bp, whereas the G allele produced four fragments of 200, 27, 19 and 5 bp. Following digestion with MspI, C allele was cut into three fragments of 175, 35, 6 bp, while G allele was cut into four fragments of 120, 55, 35, 6 bp. After digestion with Sau96I, the G allele produced four fragments of 113, 76, 31 and 21 bp, while the A allele generated three fragments of 113, 97, 31 bp.

2.4. Statistical analysis

The statistical analysis of data was done with SPSS 15.0 package program. Chi square ($\chi 2$) and Fisher's Exact-test were used to determine relationships of the categorical variables. t-test was used for pairwise comparisons of continuous variables while One Way Analysis of Variance was used to analyze variables > 2 in number. The suitability of allele distributions to Hardy Weinberg equation was analyzed with $\chi 2$ test. Probability rates and 95% confidence interval (CI) estimations were done for genotype and alleles to compare the study and the control groups. Probable haplotypes were determined, and the analyses of frequencies were done with Haploview program. Number and percent were used for categorical variables, and arithmetic mean and standard deviation were used for continuous variables as descriptive values. The level of significance was set at 0.05.

Table 1
Age and gender distributions in ADHD and control groups.

	ADHD Group (n = 114)	Control Group (n = 83)	Total (n = 197)	p
Age (*Mean* ± *SD*)	9.35 ± 2.10	9.79 ± 2.93	9.53 ± 2.49	0.217
Gender (male/female) (n, %)	97/17 (85.1/ 14.9)	62/21 (74.7/ 25.3)	159/38 (80.7/19,.)	0.068

2.5. Approval by Ethics Committee

The study protocol was approved by Clinical Research Ethics Committee of T.C. Gazi University, Faculty of Medicine (date: Feb 08, 2012, decree no: 036).

3. Results

3.1. Sociodemographic data

This study included 114 children with ADHD, and 83 healthy controls. The mean ages of the ADHD and control groups were 9.35 ± 2.10 and 9.79 ± 2.93 years, respectively. The age range of the participants was 6–12 years in both ADHD and the control groups. There were 97 (85.1%) males in ADHD group, and 62 (74.7%) males in the control group. ADHD and control groups were similar for mean ages and distribution of the genders. Mean age, gender distribution, and p values of ADHD and control groups are presented in Table 1.

Fourteen (12.3%) patients in ADHD group were diagnosed with ADHD-AD. There was ODD comorbidity in 32 (28.1%) patients. Eleven of 114 patients did not come to follow up visits after the diagnosis. Therefore, pharmacogenetic analyses were performed on 103 patients. In addition, 69 patients were given WISC-R. Thirty-four patients that were included in the study but not given WISC-R had already had this scale in other centers, and the results were normal. Those data were not included in the study due to difference of the executers.

Among the patients that had been followed up, 70 (68%) used MPH, and 33 (32%) used ATX. The treatment efficacy was determined separately for each subscale score of CTRS (HP: Hyperactivity, AD: Attention deficit, BP: Behavioral problem). Efficacy was determined as a 40% decrease compared to baseline since similar studies did so, and the decrease was determined by taking baseline and 6th month CTRS sores of the patients into account (Newcorn et al., 2008). The patients responded to treatment were 57 (55.4%) patients according to decrease in CTRS-HP scores, 74 (71.9%) patients according to decrease in CTRS-AD scores, and 61 (59.2%) patients according to decrease in CTRS-BP scores. There were adverse effects in 36 (35%) patients. The clinical and sociodemographic data of ADHD group are presented in Table 2.

3.2. CES1 gene polymorphism and ADHD

When we analyzed the distribution of CES1 gene (rs71647871) polymorphism in ADHD and control groups, we determined all genotypes as GG. We did not encounter GA or AA genotypes (Table 3).

3.3. NET1 gene polymorphism and ADHD

We first investigated NET1 gene G1287A (rs5569) polymorphism in ADHD and control groups, their conformities with Hardy-Weinberg equation were analyzed, and it was determined that both groups deviated from Hardy-Weinberg equation (*Study group* $\chi 2$:44.275, *degree of freedom*: 1, $p < 0.05$; *Control group* $\chi 2$: 18.96, *degree of freedom*: 1, $p < 0.05$).

Analysis of G1287A polymorphism in NET1 gene revealed GG genotype in 55 (48.3%) subjects, GA genotype in 21 (18.4%) subjects, and AA genotype in 38 (33.3%) subjects in ADHD group. In the control

Table 2
ADHD group: sociodemographic and clinical characteristics.

Characteristics	Number	Percent (%)
Gender		
Male	97	85.1
Female	17	14.9
Comorbidity		
ODD negative	82	71.9
ODD positive	32	28.1
ADHD subtype		
ADHD-mixed	100	87.7
ADHD-AD	14	12.3
WISC-R (*Mean ± SD*)		
WISC-R performed	69	60.5
Verbal IQ	99.78 ± 12.29	
Performance IQ	103.73 ± 12.22	
TOTAL IQ	101.78 ± 14.09	

CÖDÖ Puanları	Baseline	Month 6
CTRS-HP (*Ort ± Ss*)	12.72 ± 3.60	7.33 ± 2.98
CTRS-AD (*Ort ± Ss*)	16.59 ± 4.34	8.34 ± 3.75
CTRS-BP (*Ort ± Ss*)	8.71 ± 3.70	4.33 ± 2.70
İlaç (*n* = 103)		
Atomoxetine	33	32
Methylphenidate	70	68
İlaç Dozu (*n* = 103)		
Atomoxetine (*mg/kg*)	1.13	
Methylphenidate (*mg/kg*)	0.72	
Treatment response (*n* = 103)		
No response in CTRS-HP	46	44.6
Response positive	57	55.4
No response in CTRS-AD	29	28.1
Response positive	74	71.9
No response in CTRS-BP	42	40.7
Response positive	61	59.2
Adverse effect (*n* = 103)		
Absent	67	65.0
Present	36	35.0

Table 3
Genotype and allele frequencies of CES1 Gene (rs71647871) Polymorphism in ADHD and control groups.

	Control Group (*n* = 83)	ADHD Group (*n* = 114)
Genotype frequencies		
GG	83 (100%)	114 (100%)
GA	–	–
AA	–	–
Allele frequencies		
G	166 (100%)	228 (100%)
A	–	–

group, there was GG genotype in 43 (51.8%), GA genotype in 20 (24.1%), and AA genotype in 20 (24.1%) subjects. In ADHD group, G allele frequency was 57.5% and A allele frequency was 42.5% while those frequencies were 63.8% and 36.2%, respectively, in the control group. Comparison of ADHD and the control groups for genotype distributions of G1287A polymorphisms described in NET1 gene did not yield any statistically significant difference ($p > 0.05$) (Table 4). Distribution analysis of genotypes of G1287A polymorphism in relation with gender in ADHD and control groups showed that gender did not make a difference for ADHD for the polymorphism described ($p > 0.05$). The results are presented in Table 5.

3.4. ADRA2A gene polymorphism and ADHD

We analyzed the concordance of genotype distributions of ADRA2A gene C1291G (rs1800544) polymorphisms to Hardy-Weinberg equation in ADHD and control groups, and determined that they diverged from the equation in both groups (*ADHD group* $\chi 2$: 24.398, *degree of freedom*: 1, $p < 0.05$; *Control group* $\chi 2$: 16,760, *degree of freedom*: 1, $p < 0.05$).

Analysis of ADRA2A gene C1291G (rs1800544) polymorphism revealed CC genotype in 92 (80.7%), CG genotype in 14 (12.3%), and GG genotype in 8 (7%) in ADHD group. In the control group, there was CC genotype in 54 (65.1%), CG genotype in 17 (20.5%), and GG genotype in 12 (14.5%) individuals. C allele frequency was 86.8%, and G allele frequency was 13.2% in ADHD group while those frequencies were 75.3% and 24.7%, respectively, in the control group.

There was a significant difference between ADHD and control groups for distribution of ADRA2A gene C1291G (rs1800544) polymorphisms ($p = 0.044 < 0.05$). Two groups showed a significant difference for distribution of alleles (OR: 2.17; $p = 0.003 < 0.05$). Similarly, comparison of homozygous CC genotype with other genotypes yielded a significant difference (OR: 2.24; $p = 0.013 < 0.05$). Therefore, the individuals with C allele had 2.17-fold more risk to have

Table 4
NET1 gene G1287A (rs5569) polymorphism's genotype distributions and allele frequencies in ADHD and control groups.

	Control group (*n* = 83)	ADHD group (*n* = 114)	Total (*n* = 197)	*p*[b]	OR (95% CI)
Genotype frequencies (*n*, %)					
GG	43 (51.8)	55 (42.8)	98 (49.7)	0.323	
GA	20 (24.1)	21 (18.4)	41 (20.8)		
AA	20 (24.1)	38 (33.3)	58 (29.4)		
Allele frequencies (*n*, %)					
G[a]	106 (63.8)	131 (57.5)	237 (60.1)	0.200	1.31
A	60 (36.2)	97 (42.5)	157 (39.9)		(8.7–19.7)
Homozygous allele frequencies (*n*, %)					
GG[a]	43 (51.8)	55 (48.2)	98 (49.7)	0.622[c]	1.15
GA + AA	40 (48.2)	59 (51.8)	99 (50.3)		(6.5–20.3)
AA	20 (24.1)	38 (33.3)	58 (29.4)	0.160[d]	1.57
GA + GG[a]	63 (75.9)	76 (66.7)	139 (70.6)		(8.3–29.7)

OR: Odds ratio; CI: Confidence interval.
[a] Reference genotype/allele.
[b] Chi square test.
[c] Calculated with respect to GG vs. GA + AA.
[d] Calculated with respect to GA + GG vs. AA.

Table 5
Association of NET1 Gene G1287A Polymorphism with gender in ADHD and control groups.

Gender	Genotype	Control group *n* = 83	ADHD group *n* = 114	Total	*p*[b]	OR (95% CI)
Male	GG	33 (41.8)	46 (58.2)	79	0.240	
	AG	16 (45.7)	19 (54.3)	35		
	AA	13 (28.9)	32 (71.1)	45		
Female	GG	10 (52.6)	9 (47.4)	19	0.827	
	AG	4 (66.7)	2 (33.3)	6		
	AA	7 (53.8)	6 (46.2)	13		
Male	GG[a]	33 (41.8)	46 (58.2)	79	0.475[c]	1.26
	AG + AA	29 (36.3)	51 (63.8)	80		(6.6%–23.8%)
Female	GG[a]	10 (52.6)	9 (47.4)	19	0.744[c]	0.80
	AG + AA	11 (57.9)	8 (42.1)	19		(2.2%–29.0%)
Male	AA	13 (28.9)	32 (71.1)	45	0.101[d]	1.85
	AG + GG[a]	49 (43.0)	65 (57.0)	114		(8.8%–39.0%)
Female	AA	7 (53.8)	6 (46.2)	13	0.899[d]	0.91
	AG + GG[a]	14 (56.0)	11 (44.0)	25		(2.3%–35.2%)

OR: Odds ratio; CI: Confidence interval.
[a] Reference genotype/allele.
[b] Chi square test.
[c] Calculated with respect to GG vs. GA + AA.
[d] Calculated with respect to GA + GG vs. AA.

Table 6
ADRA2A gene C1291G (rs1800544) polymorphism's genotype distributions and allele frequencies in ADHD and control groups.

	Control group (n = 83)	ADHD group (n = 114)	Total (n = 197)	p[b]	OR (95% CI)
Genotype frequencies (n, %)					
CC	54 (%65.1)	92 (80.7)	146 (74.1)	0.044	
CG	17 (20.5)	14 (12.3)	31 (15.7)		
GG	12 (14.5)	8 (7.0)	20 (10.2)		
Allele frequencies (n, %)					
C	125 (75.3)	198 (86.8)	323 (81.9)	0.003	2.17
G[a]	41 (24.7)	30 (13.2)	71 (18.1)		(12.8%–37.0%)
Homozygous allele frequencies (n, %)					
CC	54 (65.1)	92 (80.7)	146 (74.1)	0.013[c]	2.24
CG + GG[a]	29 (34.9)	22 (19.3)	51 (25.9)		(11.7%–42.9%)
GG[a]	12 (14.5)	8 (7.0)	20 (10.2)	0.088[d]	2.23
CG + CC	71 (85.5)	106 (93.0)	177 (89.8)		(8.7%–57.5%)

OR: Odds ratio; CI: Confidence interval.
[a] Reference genotype/allele.
[b] Chi square test.
[c] Calculated with respect to GG vs. GA + AA.
[d] Calculated with respect to GA + GG vs. AA.

the disease when compared to the individuals with G allele; and the ones with CC genotype had 2.24-fold increased risk for the disease compared to the individuals with other genotypes (Table 6).

Analysis of distribution of C1291G polymorphism genotypes in relation with gender in ADHD and control groups revealed that gender was a determinant for ADHD. Importance of C1291G polymorphism for ADHD risk was higher in males (p = 0.045) (Table 7). Therefore, the males with homozygous CC genotype have 2.43-fold more risk for the disease when compared to other genotypes (0R: 2.43; p = 0.013) (Table 7).

3.5. Clinical reflections of NET1 and ADRA2A Gene Polymorphisms

The statistical analyses in this section were performed only on 103 patients in ADHD group. The effects of genotypes of polymorphisms were investigated on eight clinical parameters (*age*, *gender*, *ADHD subtype*, *baseline CTRS scores*, *presence of comorbidities*, *results of WISC-R test*, *response to treatment*, *and presence of adverse effects*). Before investigating the probable effects of polymorphisms on treatment response and presence of adverse effects, the probable effects of other non-clinical parameters on those two parameters were analyzed. Presence or absence of the adverse effects did not show any associations with age, gender, ADHD subtype, baseline CTRS scores, presence of comorbidities, results of WISC-R test, drug used in treatment, or mean dose of the drug ($p > 0.05$). Table 8 shows the statistical analysis of other clinical parameters on presence of adverse effects.

Treatment response was graded as weak or good in relation with 40% decrease in Conners Attention Deficit Hyperactivity Disorder Teacher Rating Scale's hyperactivity, attention deficit, and behavioral problems scores (CTRS-HP, CTRS-AD and CTRS-BP). Weak or good response did not show any correlations with age, gender, ADHD subtype, baseline CTRS scores, presence of comorbidities, WISC-R test results, drug used in treatment, or the mean dose of the drug ($p > 0.05$). It was determined that the ones that had good response in CTRS-HP scores had significantly lower baseline CTRS-AD scores compared to the ones that had a weak response (p = 0.001). Table 9 shows statistical analysis of the probable effects of other clinical parameters on treatment response.

Table 7
Association of ADRA2A Gene C1291G Polymorphism with gender in ADHD and control groups.

Gender	Genotype	Control Group n = 83	ADHD Group n = 114	Total	p[b]	OR (95% CI)
Male	CC	38 (33.0)	77 (67.0)	115	0.045	
	CG	15 (55.6)	12 (44.4)	27		
	GG	9 (52.9)	8 (47.1)	17		
Female	CC	16 (51.6)	15 (48.4)	31	0.267	
	CG	2 (50.0)	2 (50.0)	4		
	GG	3 (100.0)	0 (0.0)	3		
Male	CC	38 (33.0)	77 (67.0)	111	0.013[c]	2.43
	CG + GG[a]	24 (54.5)	20 (45.5)	44		(12.0%–49.5%)
Female	CC	16 (51.6)	15 (48.4)	31	0.341[c]	0.42
	CG + GG[a]	5 (71.4)	2 (28.6)	7		(0.0%–25.4%)
Male	GG[a]	9 (52.9)	8 (47.1)	17	0.212[d]	1.88
	CG + CC	53 (37.3)	89 (62.7)	142		(6.8%–51.9%)
Female	GG[a]	3 (100.0)	0 (0.0)	3	0.104[d]	1.94
	CG + CC	18 (51.4)	17 (48.6)	35		(14.0%–26.8%)

OR: Odds ratio; CI: Confidence interval.
[a] Reference genotype/allele.
[b] Chi square test.
[c] Calculated with respect to GG vs. GA + AA.
[d] Calculated with respect to GA + GG vs. AA.

3.5.1. Clinical reflections of NET1 gene G1287A polymorphism

The associations of genotypes of G1287A polymorphism with age, gender, ADHD subtype, baseline CTRS scores, presence of comorbidity, WISC-R test results, treatment response, and presence of adverse effects were first analyzed by grouping the patients as having GG, GA or AA genotypes, and then grouping them into homozygous genotypes (GG and AA) and other genotypes. The results indicated that ADHD patients with GG genotype had a significantly higher simultaneous ODD rate (65.6% vs. 34.45; p = 0.020), and ADHD patients with AA genotype had a significantly lower simultaneous ODD rate (18.8% vs. 81.2%; p = 0.039). Baseline CTRS-AD score was significantly higher in ADHD patients with AA genotype compared to the ADHD patients with other genotypes (17.47 ± 3.73 vs. 16.15 ± 4.58; p = 0.045). In relation with CTRS-BP score, the rate of ADHD patients with AA genotype that had a good treatment response was significantly lower compared to other genotypes (25% vs. 75%; p = 0.023). Genotypes of G1287A polymorphism did not show any significant correlations with other clinical parameters ($p > 0.05$) (Table 10).

3.5.2. Clinical reflections of ADRA2A gene C1291G polymorphism

The associations of C1291G polymorphism's genotypes with age, gender, ADHD subtype, baseline CTRS scores, presence of comorbidity, WISC-R test results, treatment response, and presence of adverse effects were first analyzed after grouping them for CC, GC and GG genotypes, then grouping them as homozygous genotypes (CC and GG), and other genotypes. Genotypes of C1291G polymorphism did not show any correlations with clinical parameters ($p > 0.05$) (Table 11).

4. Discussion

In this study, we found that all individuals in ADHD and control groups had homozygous GG genotype for CES1 gene Gly143Glu polymorphism, and it was determined that Turkish population was homozygous for this polymorphism. It was found that genotypes of NET1 gene G1287A polymorphism did not show any significant associations with ADHD. We found a significant association between ADRA2A gene C1291G polymorphism and ADHD in Turkish population, and this is an important result of our study. Having C allele and CC homozygous genotypes were determined as risk factors for ADHD. We found significant correlation of gender distribution and ADRA2A gene C1291G polymorphism in ADHD and control groups, and C allele and CC homozygous genotype were found as a significant risk factor for ADHD in males. An important finding in relation with clinical reflections is presence of significant associations between some genotypes of NET1 gene G1287A polymorphism and clinical parameters. ADHD patients

Table 8
Clinical and demographic characteristics with respect to presence of adverse effects in ADHD group.

Clinical characteristic	Adverse effect		*p*
	Present (*n* = 67)	Absent (*n* = 36)	
Age (*Ort* ± *Ss*)	9.52 ± 2.04	8.97 ± 1.97	0.191[b]
Gender (*n*, %)			
Male	57 (85.1)	30 (83.3)	0.816[a]
Female	10 (14.9)	6 (16.7)	
ADHD subtype (*n*, %)			
ADHD-Mixed	58 (86.6)	32 (88.9)	0.735[a]
ADHD-AD	9 (13.4)	4 (11.1)	
Baseline CTRS scores (*mean* ± *SD*)			
CTRS-HP	12.62 ± 3.38	12.91 ± 3.91	0.696[b]
CTRS-AD	16.44 ± 4.47	16.80 ± 4.04	0.690[b]
CTRS-BP	8.50 ± 3.64	8.69 ± 3.63	0.804[b]
Comorbidity (*n*, %)			
ODD negative	51 (76.1)	24 (66.7)	0.304[a]
ODD positive	16 (23.9)	12 (33.3)	
WISC-R (*Mean* ± *SD*)			
Verbal IQ	100.00 ± 12.29	99.37 ± 12.56	0.842[b]
Performance IQ	103.97 ± 17.45	103.29 ± 17.14	0.876[b]
Total IQ	102.20 ± 14.22	101.00 ± 14.11	0.739[b]
Drug (*n*, %)			
Atomoxetine	18 (26.9)	15 (41.7)	0.125[a]
Methylphenidate	49 (73.1)	21 (58.3)	
Mean drug dose (*mean* ± *SD*)			
Atomoxetine (*mg/kg*)	1.16 ± 0.10	1.08 ± 0.17	0.202[b]
Methylphenidate (*mg/kg*)	0.73 ± 0.21	0.80 ± 0.26	0.643[b]

[a] Chi square test.
[b] *t* test.

Table 9
Clinical and demographic characteristics of ADHD group with respect to treatment response.

Clinical characteristic	Treatment response (HP)		*p*	Treatment response (DE)		*p*	Treatment response (DB)		*p*
	Weak (*n* = 46)	Good (*n* = 57)		Weak (*n* = 30)	Good (*n* = 73)		Weak (*n* = 43)	Good (*n* = 60)	
Age (*mean* ± *SD*)	9.63 ± 2.16	9.08 ± 1.90	0.179	8.93 ± 199	9.49 ± 2.03	0.205	9.27 ± 1.95	9.36 ± 2.09	0.830
Gender (*n*, %)									
Male	36 (78.3)	51 (89.5)	0.118	25 (83.3)	62 (84.9)	0.839	36 (83.7)	51 (85.0)	0.860
Female	10 (21.7)	6 (10.5)		5 (16.7)	11 (15.1)		7 (16.3)	9 (15.0)	
ADHD subtype (*n*, %)									
ADHD-mixed	37 (80.4)	53 (93.0)	0.057	28 (93.3)	62 (84.9)	0.243	38 (88.4)	52 (86.7)	0.797
ADHD-AD	9 (19.6)	4 (7.0)		2 (6.7)	11 (15.1)		5 (11.6)	8 (13.3)	
Baseline CTRS scores (*mean* ± *SD*)									
CTRS-HP	12.41 ± 3.93	12.98 ± 3.25	0.423	13.53 ± 2.54	12.39 ± 3.87	0.143	13.48 ± 3.21	12.12 ± 3.72	0.067
CTRS-AD	18.13 ± 3.76	15.31 ± 4.34	0.001	15.93 ± 4.16	16.83 ± 4.37	0.337	17.11 ± 4.46	16.18 ± 4.19	0.281
CTRS-BP	8.73 ± 3.80	8.43 ± 3.51	0.678	8.10 ± 3.76	8.76 ± 3.58	0.399	7.90 ± 9.05	3.42 ± 372	0.115
Comorbidity (*n*, %)									
ODD negative	31 (67.4)	44 (77.2)	0.266	22 (73.3)	53 (72.6)	0.940	32 (74.4)	43 (71.7)	0.757
ODD positive	15 (32.6)	13 (22.8)		8 (26.7)	20 (27.4)		11 (25.6)	17 (28.3)	
WISC-R (*mean* ± *SD*)									
Verbal IQ	100.96 ± 11.19	98.75 ± 13.24	0.460	102.85 ± 11.30	98.43 ± 12.58	0.171	99.77 ± 11.02	99.78 ± 13.18	0.998
Performance IQ	104.40 ± 16.53	103.16 ± 18.00	0.767	105.52 ± 17.55	102.95 ± 17.20	0.573	101.74 ± 16.78	105.02 ± 17.57	0.444
Total IQ	102.71 ± 13.03	100.97 ± 15.08	0.612	104.28 ± 12.78	100.68 ± 14.62	0.333	100.51 ± 12.41	102.59 ± 15.17	0.554
Drug (*n*, %)									
Atomoxetine	16 (34.8)	17 (29.8)	0.592	11 (36.7)	22 (30.1)	0.519	16 (37.2)	17 (28.3)	0.341
Methylphenidate	30 (65.2)	40 (70.2)		19 (63.3)	51 (69.9)		27 (62.8)	43 (71.7)	
Mean drug dose (*mean* ± *SD*)									
Atomoxetine (*mg/kg*)	1.09 ± 0.10	1.16 ± 017	0.152	1.08 ± 0.11	1.15 ± 015	0.434	1.10 ± 011	1.15 ± 017	0.382
Methylphenidate (*mg/kg*)	0.72 ± 0.21	077 ± 0.24	0.313	0.77 ± 0.24	0.74 ± 0.22	0.767	0.69 ± 0.21	0.78 ± 0.23	0.121

"Chi square" test was used for categorical variables, numerical variables were compared with "T Testi" in case of pairwise comparisons, and with "one-way variance analysis" in case of triple comparisons.

with GG genotype had ODD diagnosis more frequently, the ones with AA genotype had ODD diagnosis less frequently, and the ones with AA genotype had lower baseline CTRS-AD scores. Those results suggested that A allele was mainly associated with attention deficit cluster, and the individuals with AA genotype expressed their behaviors less.

4.1. ADRA2A gene C1291G polymorphism

Although a number of SNPs have been demonstrated in ADRA2A gene, most extensively studied two SNPs are C1291G polymorphism that results in occurrence of an endonuclease called as *Msp*I region at the promotor region of the gene, and C > G polymorphism located at *Dra*I part of 3′UTR region (Bono et al., 1996; Lario et al., 1997). The

Table 10
The association of NET1 Gene G1278A Polymorphism's genotypes and clinical and demographic characteristics in ADHD group.

Clinical characteristic		GG	GA	AA	p^*	GG	GA + AA	p^*	AA	GA + GG	p^*
Age (*mean* ± *SD*)		9.34 ± 1.97	9.23 ± 2.18	9.42 ± 2.28	0.951	9.34 ± 1.97	9.35 ± 2.23	0.551	9.42 ± 2.28	9.31 ± 2.02	0.490
Gender (*n*, %)											
Male		46 (47.49)	19 (19.6)	32 (33.0)	0.743	46 (47.4)	51 (52.6)	0.674	32 (33.0)	65 (67.0)	0.853
Female		9 (52.9)	2 (11.8)	6 (35.3)		9 (52.9)	8 (47.1)		6 (35.3)	11 (64.7)	
ADHD subtype (*n*, %)											
ADHD-mixed		50 (50.0)	18 (18.0)	32 (32.0)	0.597	50 (50.0)	50 (50.0)	0.316	32 (32.0)	68 (68.0)	0.420
ADHD-AD		5 (35.7)	3 (21.4)	6 (42.9)		5 (35.7)	9 (64.3)		6 (42.9)	8 (57.1)	
Baseline CTRS scores (*mean* ± *SD*)											
CTRS-HP		12.69 ± 3.66	12.19 ± 3.65	13.07 ± 3.52	0.663	12.69 ± 3.66	12.76 ± 3.56	0.835	13.07 ± 3.52	12.55 ± 3.64	0.921
CTRS-AD		16.52 ± 4.81	15.19 ± 3.88	17.47 ± 3.73	0.153	16.52 ± 4.81	16.66 ± 3.91	0.061	17.47 ± 3.73	16.15 ± 4.58	0.045
CTRS-BP		9.20 ± 3.75	8.95 ± 3.23	7.86 ± 3.77	0.224	9.20 ± 3.75	8.25 ± 3.63	0.441	7.86 ± 3.77	9.13 ± 3.62	0.853
Comorbidity (*n*, %)											
ODD negative		34 (41.5)	16 (19.5)	32 (39.0)	0.055	34 (41.5)	48 (58.5)	0.020	32 (39.0)	50 (61.0)	0.039
ODD positive		21 (65.6)	5 (15.6)	6 (18.8)		21 (65.6)	11 (34.4)		6 (18.8)	26 (81.2)	
WISC-R (*mean* ± *SD*)											
Verbal IQ		102.55 ± 10.51	96.64 ± 14.4	96.84 ± 13.15	0.148	102.55 ± 10.51	96.75 ± 13.50	0.254	96.84 ± 13.15	100.90 ± 11.90	0.763
Performance IQ		106.75 ± 17.04	100.14 ± 18.04	100.68 ± 16.53	0.320	106.75 ± 17.04	100.45 ± 17.07	0.812	100.68 ± 16.53	104.90 ± 17.50	0.499
Total IQ		105.02 ± 12.81	98.28 ± 16.80	98.21 ± 13.54	0.136	105.02 ± 12.81	98.24 ± 14.76	0.431	98.21 ± 13.54	103.14 ± 14.19	0.946
Treatment response											
HP	Weak	21 (45.7)	6 (13.0)	19 (41.3)	0.303	21 (45.7)	25 (54.3)	0.598	19 (41.3)	27 (58.7)	0.159
	Good	29 (50.9)	12 (21.1)	16 (28.1)		29 (50.9)	28 (49.1)		16 (28.1)	41 (71.9)	
AD	Weak	14 (46.7)	4 (13.3)	12 (40.0)	0.638	14 (46.7)	16 (53.3)	0.807	12 (40.0)	18 (60.0)	0.408
	Good	36 (49.3)	14 (19.2)	23 (31.5)		36 (49.3)	37 (50.7)		23 (31.5)	50 (68.5)	
BP	Weak	18 (41.9)	5 (11.6)	20 (46.5)	0.063	18 (41.9)	25 (58.1)	0.251	20 (46.5)	23 (53.5)	0.023
	Good	32 (53.3)	13 (21.7)	15 (25.0)		32 (53.3)	28 (46.7)		15 (25.0)	45 (75.0)	
Adverse effect											
Present		32 (47.8)	14 (20.9)	21 (31.3)	0.428	32 (47.8)	35 (52.2)	0.828	21 (31.3)	46 (68.7)	0.441
Absent		18 (50.0)	4 (11.1)	14 (38.9)		18 (50.0)	18 (50.0)		14 (38.9)	22 (61.1)	

“Chi square” test was used for categorical variables, numerical variables were compared with “T Testi” in case of pairwise comparisons, and with “one-way variance analysis” in case of triple comparisons.

Table 11
Association of ADRA2A Gene C1291G Polymorphism with clinical and demographic characteristics in ADHD group.

Clinical characteristic		CC	CG	GG	p^*	CC	CG + GG	p^*	GG	CG + CC	p^*
Age (*mean* ± *SD*)		9.28 ± 2.06	9.85 ± 1.91	9.25 ± 2.91	0.633	9.28 ± 2.06	9.63 ± 2.27	0.481	9.25 ± 2.91	9.35 ± 2.04	0.889
Gender (*n*, %)											
Male		77 (79.4)	12 (12.4)	8 (8.2)	0.461	77 (79.4)	20 (20.6)	0.394	8 (8.2)	89 (91.8)	0.219
Female		15 (88.2)	2 (11.8)	0 (0.0)		15 (88.2)	2 (11.8)		0 (0.0)	17 (100.0)	
ADHD subtype (*n*, %)											
ADHD-mixed		80 (80.0)	13 (13.0)	7 (7.0)	0.822	80 (80.0)	20 (20.0)	0.612	7 (7.0)	93 (93.0)	0.984
ADHD-AD		12 (85.7)	1 (7.1)	1 (7.1)		12 (85.7)	2 (14.3)		1 (7.1)	13 (92.9)	
Baseline CTRS scores (*mean* ± *SD*)											
CTRS-HP		12.79 ± 3.62	12.21 ± 3.37	12.87 ± 4.12	0.851	12.79 ± 3.62	12.45 ± 3.58	0.693	12.87 ± 4.12	12.71 ± 3.57	0.905
CTRS-AD		16.63 ± 4.48	15.78 ± 4.15	17.25 ± 3.15	0.712	16.63 ± 4.48	16.31 ± 3.80	0.740	17.25 ± 3.15	16.54 ± 4.43	0.661
CTRS-BP		8.69 ± 3.78	9.50 ± 3.39	7.50 ± 3.42	0.479	8.69 ± 3.78	8.77 ± 3.46	0.931	7.50 ± 3.42	8.80 ± 3.72	0.340
Comorbidity (*n*, %)											
ODD negative		65 (79.3)	10 (12.2)	7 (8.5)	0.596	65 (79.3)	17 (20.7)	0.535	7 (8.5)	75 (91.5)	0.309
ODD positive		27 (84.4)	4 (12.5)	1 (3.1)		27 (84.4)	5 (15.6)		1 (3.1)	31 (96.9)	
WISC-R (*mean* ± *SD*)											
Verbal IQ		99.50 ± 12.49	96.12 ± 8.99	107.16 ± 12.99	0.238	99.50 ± 12.49	100.85 ± 11.86	0.717	107.16 ± 12.99	99.07 ± 12.10	0.125
Performance IQ		104.39 ± 17.52	100.50 ± 14.83	102.83 ± 19.76	0.839	104.39 ± 17.52	101.50 ± 16.43	0.590	102.83 ± 19.76	103.82 ± 17.14	0.894
Total IQ		101.87 ± 14.23	98.25 ± 11.65	105.66 ± 16.86	0.625	101.87 ± 14.23	101.42 ± 14.03	0.917	105.66 ± 16.86	101.41 ± 13.90	0.484
Treatment response											
HP	Weak	37 (80.4)	5 (10.9)	4 (8.7)	0.637	37 (80.4)	9 (19.6)	0.852	4 (8.7)	41 (91.3)	0.491
	Good	45 (78.9)	9 (15.8)	3 (5.3)		45 (78.9)	12 (21.1)		3 (5.3)	54 (94.7)	
AD	Weak	24 (80.0)	3 (10.0)	3 (10.0)	0.594	24 (80.0)	6 (20.0)	0.950	3 (10.0)	27 (90.0)	0.408
	Good	58 (79.5)	11 (15.1)	4 (5.5)		58 (79.5)	15 (20.5)		4 (5.5)	69 (94.5)	
BP	Weak	35 (81.4)	3 (7.0)	5 (11.6)	0.084	35 (81.4)	8 (18.6)	0.704	5 (11.6)	38 (88.4)	0.099
	Good	47 (78.3)	11 (18.3)	2 (3.3)		47 (78.3)	13 (21.7)		2 (3.3)	58 (96.7)	
Adverse effect											
Present		52 (77.6)	10 (14.9)	5 (7.5)	0.790	52 (77.6)	15 (22.4)	0.492	5 (7.5)	62 (92.5)	0.714
Absent		30 (83.3)	4 (11.1)	2 (5.6)		30 (83.3)	6 (16.7)		2 (5.6)	34 (94,4)	

"Chi square" test was used for categorical variables, numerical variables were compared with "T Testi" in case of pairwise comparisons, and with "one-way variance analysis" in case of triple comparisons.

first study that investigated the association of ADHD and C1291G polymorphism was done by Xu et al. in 2001 on 94 families, and the authors did not find any associations (Xu et al., 2001). Later, > 10 studies on C1291G polymorphism yielded conflicting results (Park et al., 2005; Schmitz et al., 2006; Stevenson et al., 2005). A meta-analysis focusing on 11 studies did not confirm the hypothesis of "G allele in C1291G polymorphism is correlated with ADHD" (OR = 1, $p = 0.473$). The same meta-analysis reported a similar result for the polymorphism at DraI part (OR = 0.92, $p = 0.925$) (Park et al., 2005). Our study is important for being the first study that has investigated ADRA2A gene C1291G polymorphism in ADHD in our country. The results of our study indicated that the individuals with C allele had 2.17-fold higher risk for the disease compared to the individuals with G allele, and the ones with CC genotype had 2.24-fold more risk to have the disease compared to the ones with other genotypes. Therefore, C1291G polymorphism is associated with ADHD, and C allele is the risky allele. This result is contradictory to the findings of the studies that found G allele as the risky allele. However, our result is in conjunction with the results of the studies that determined C allele as the risky allele, and associated it with an unfavorable clinic picture. This result supports the hypothesis claiming that the same candidate gene causes different clinical pictures in different genders. Our study is also important for being the first one in the literature that has emphasized the role of gender on association of ADRA2A gene C1291G polymorphism with ADHD.

A study that investigated the association of treatment with ADRA2A gene C1291G polymorphism on 106 children with ADHD reported more improvement in attention deficit cluster in children with G/G and G/C alleles compared to the ones with G allele (Polanczyk et al., 2007). A study hypothesized that noradrenergic pathways were mainly associated with wakefulness and attention was performed on 59 children diagnosed with ADHD-AD. C1291G polymorphisms were analyzed, and the children were divided into 2 groups as the ones with or without G allele. The authors found > 50% improvement in SNAP-IV attention deficit score in 72.5% in G (+) group, and 47.4% in G (−) group after treatment with MPH for 4 weeks (da Silva et al., 2008). Another study performed in Korea analyzed 114 children with ADHD with Attention Deficit Hyperactivity Disorder Rating Scale after treating them with MPH for 8 weeks, and found more than 50% improvement in 76.9% of the patients with G/G allele, in 46.0% of the ones with G/C allele, and in 41.7% of the ones with C/C allele (Cheon et al., 2009).

4.2. NET1 gene G1287A polymorphism

NET1 gene polymorphisms appear as single nucleotide polymorphisms (SNP). In 2002, Barr et al. investigated 3 SNPs at NET1 gene on 122 patients with ADHD, and did not find any correlation. After that, > 100 SNPs have been demonstrated on NET1 gene (Barr et al., 2002; Shang et al., 2015). A number of studies studied SNPs at NET1 gene in the literature, and reported conflicting results about their associations with ADHD (Cho et al., 2008; Kim et al., 2006; Xu et al., 2005). Xu et al. investigated 21 SNPs on 180 patients and 334 controls, found an association between rs3785157 polymorphism and ADHD, and this finding was repeated by Bobb et al. who also found a significant association with rs998424 polymorphism (Bobb et al., 2005; Xu et al., 2005). IMAGE (International Multicentre ADHD Genetics Study) investigated 43 SNPs at NET1, did not find any significant association with any of them, however rs3785143 and rs1568324 got close to the limit of statistical significance (Brookes et al., 2006). Interestingly, it was reported that NET1polymorphisms showed their effects only in absence of the specific variant of COMT gene (Retz et al., 2008). A study that investigated the effect of genders reported a more strong association of NET1 gene polymorphisms with ADHD in girls (Biederman et al., 2008). Another most studied SNP at NET1 gene is G1287A polymorphism. It is located at exon 9, and it is a silent mutation that does not code any proteins (Gizer et al., 2009). A meta-analysis that investigated G1287A and rs2242447 polymorphisms obtained the data on G1287A polymorphism from 5 studies, did not find any association between ADHD and G1287A polymorphism (OR = 1.06, $p = 0.279$), and the data of 4 studies included in this meta-analysis did not support any association between ADHD and rs2242447 (OR = 1.03, $p = 0.641$) (106). A recent linkage analysis performed on 810 individuals (270 children and their both parents) mapped 91 SNPs at 14 noradrenergic genes, and it was reported that G1287A polymorphism showed an increased familial transition (Hawi et al., 2013). Our study is important for being the first one that investigated NET1 gene G1287A polymorphism in ADHD in our country. We compared ADHD and control groups for allele distribution of G1287A polymorphism, and did not find any significant association of ADHD with G1287A polymorphism (OR = 1.31, $p = 0.200$). This result is in conjunction with the studies that did not find any association between the aforementioned polymorphism and ADHD. Our study sheds light to further studies in relation with G1287A polymorphism in Turkish population since it is the first study that has investigated this polymorphism in our population, and this is another important point in our study.

Yang et al. investigated NET1 gene G1287A polymorphism, and reported that individuals with A/A genotype responded MPH therapy worse than the individuals with G/A or G/G genotype (Yang et al., 2004). Song et al. reported a similar result in case of G1287A polymorphism with a minor difference. They investigated MPH response in 114 children with ADHD administered for 8 weeks, and G/G genotype was found to be associated with better treatment response compared to G/A and A/A groups (Song et al., 2011b). A study with a similar design performed on 87 children with ADHD reported that patients with G/G genotype and G1287A polymorphism gave fewer false positive responses in Continuous Performance Test (CPT) when compared to G/A and A/A groups. Those results were interpreted as the protective role of G allele for deficiency of stopping behavior in ADHD (Song et al., 2011a). A study on previously untreated 101 children with ADHD investigated pre- and posttreatment reaction times with auditory/visual selective attention tests and CPT, and found a significant association between A allele frequency and elongation in reaction time (Kim et al., 2013). A study investigated clinical progress after long-acting MPH therapy administered for 8 weeks as well as 5 SNPs including G1287A polymorphism, and none of them were found to be associated with treatment response (Lee et al., 2011). A study performed in China on 111 children with ADHD investigated the associations of 12 SNPs chosen from NET1, ADRA2 and ADRA1 genes with response to ATX therapy. The patients were divided into groups as the ones responded ($n = 81$) and did not respond to therapy ($n = 30$), and rs3785143 polymorphism at NET1 gene was found to be associated with treatment response (Yang et al., 2013). Lastly, a research that used both imaging and genetic studies showed that cases with G/G genotype had more perfusion in their temporal gyri compared to the ones with other alleles, and the ones with G/G alleles had better treatment responses (Park et al., 2012). In our study, we determined that ADHD patients with AA genotype had higher baseline CTRS-AD scores, the ones with GG genotype more frequently had ODD diagnosis, and the ones with AA genotype had ODD diagnosis less frequently. When we take the results altogether, we may suggest that A allele is mostly associated with attention deficit cluster of ADHD, and therefore the cases homozygous for A allele express less extraverted behaviors. This result is in conjunction with the studies that have reposted that A allele is associated with attention deficit cluster, such as an elongated reaction time.

4.3. CES1 gene Gly143Glu polymorphism

MFD is hydrolyzed in liver by CES1 enzyme, and converted into its inactive metabolite, ritalinic acid, which is excreted with urine (Sun et al., 2004). CES1 gene is located at chromosome 16q13-q22.1 region. It contains 14 exons composed approximately of 30 base pairs

(Langmann et al., 1997). CES1 gene is approximately 99 kilobase pairs away from NET1 gene located at chromosome 16, and the proximity between two genes is remarkable for probable connection imbalance in ADHD (Johnson et al., 2013). Genders and age groups were found similar for CES1 gene expression (Zhu et al., 2009). On the other hand, CES1 enzyme activity levels of the individuals show significant differences (Zhu et al., 2008). It has been shown that alcohol, some medications including aripiprazole, fluoxetine, thioridazine, as well as environmental pollution, and genotype variations suppress CES1 enzyme activity (Hosokawa et al., 1995). Although > 30 SNPs were described in CES1 gene, 2 SNPs described by Zhu et al. result in significantly decreased enzyme activity and increased MPH levels (Zhu et al., 2008). The first one is the frameshift mutation at codon 260 of exon 6 resulting in premature stop codon, which is known as Asp260fs. The second one is G > A polymorphism at codon 143 of exon 4 resulting in transition of glutamic acid in place of glycine, and this is known as Gly143Glu (Zhu et al., 2008). Both mutations cause almost complete loss of hydrolytic activity of CES1 enzyme for MPH (Zhu et al., 2008). Both polymorphisms are rare when the ethnic origins are taken altogether, and the frequency of Gly143Glu is < 5%, and the frequency of Asp260fs is < 1% (Zhu et al., 2008).

Bruxel et al. studied −75 T > G polymorphism at CES1 gene on 213 children with ADHD, and reported that the ones with G allele had loss of appetite 3.47-fold more compared to the ones homozygous for T allele (Bruxel et al., 2013). Nemoda et al. investigated the association of treatment response with Gly143Glu polymorphism in 122 patients with ADHD, and did not find any significant difference for the rates of Gly/Glu heterozygous allele carriers when the patients were groups divided into two groups as responders and non-responders to treatment. Interestingly, Gly/Glu heterozygous allele carriers ($n = 5$) needed a significantly lower MPH dose when compared to Gly/Gly homozygous patients ($n = 85$) (0.410 ± 0.127 vs. 0.572 ± 0.153 mg/kg, $p = 0.022$) (Nemoda et al., 2009). Lastly, a study that investigated 7 SNPs at CES1 gene found a significant correlation of sadness parameter in the adverse effect scale with rs2244613 and rs20022577 (Johnson et al., 2013).

The prevalence of Gly143Glu polymorphism is < 5% when all people from all ethnic origins are considered. However, its prevalence in Caucasian, Mongolian, Spanish, and Asian people was reported as 3.7%, 4.3%, 2%, and 0%, respectively (Hosokawa et al., 1995; Zhu et al., 2008). In our study, both control and ADHD groups were homozygous for CES1 gene Gly143Glu polymorphism. In other words, all participants had GG genotype, and there were no heterozygous individuals. Therefore, the prevalence of Gly143Glu polymorphism was calculated as 0% in Turkish population. This result is in conjunction with the studies that reported the prevalence of this polymorphism as 0% in Asian people.

5. Conclusions

In conclusion, we have determined that ADRA2A gene C1291G polymorphism is associated with ADHD, and this association is more important in males. In addition, we have found that NET1 gene G1287A polymorphism's A allele is mainly associated with attention deficit. Our another important result is determination of Turkish population as homozygous for CES1 gene GLY143GLU polymorphism. Candidate gene studies have contributed understanding pathogenesis of ADHD. The results of our study will provide insight to further studies on candidate genes. In addition, the results of this and other similar studies may help screening the individuals under risk, and may enable genetic counseling for early diagnosis, guiding the patients for proper treatment, and avoidance from some preventable risk factors. Pharmacogenetic studies are also valuable for pioneering individualization of therapy, in accordance with the statement “do not consider the disease, but consider the patient”.

Conflict of interest

The authors declare that they have no conflict of interest.

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