Original Paper

Role of the norepinephrine transporter polymorphisms in atomoxetine treatment: From response to side effects in children with ADHD

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Abstract

Objective: Atomoxetine (ATX), one of the most commonly used drugs after stimulants in attention deficit hyperactivity disorder (ADHD) treatment, is an inhibitor of the norepinephrine transporter (*NET/SLC6A2*), which is also associated with the etiology of ADHD. In this study, we aimed to investigate the effect of *NET* gene polymorphisms on response to ATX treatment and to find the answers to the questions about whether there is a relationship between the severity of the disorder and the observed side effects in children with ADHD.

Method: About 100 children with ADHD and 80 healthy controls (HCs) were included in this study. The dose of ATX was started at 0.5 mg/kg/day and titrated at 1.2 mg/kg/day. Response to treatment of 78 patients was evaluated 2 months after the beginning of the treatment. After whole blood samples were obtained, DNAs were isolated, and samples were stored at –80°C. Two single-nucleotide polymorphisms (SNPs) (rs12708954 and rs3785143) were analyzed by real-time quantitative PCR (qRT-PCR).

Results: The patients with both rs12708954 and rs3785143 heterozygous genotype had better treatment response and more side effects than patients with wild type. There was not found any association between any of the investigated *NET* polymorphisms and ADHD severity.

Conclusion: It was, however, found that the *NET* rs12708954 and rs3785143 genotypes affect the treatment response to ATX in our study; thus, further studies with a large population are needed to understand the effects of *NET* polymorphisms on treatment, side effects, and also the severity of ADHD.

Keywords

Children, ADHD, atomoxetine, *NET*, treatment

Introduction

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that starts in childhood and continues in adulthood, characterized by inattentiveness, hyperactivity, and impulsivity symptoms that are not appropriate for the age of the individual (DSM-5 APA, 2013). The ADHD prevalence was reported 7.2% in a meta-analysis of 175 worldwide research studies on ADHD in children (Thomas et al., 2015). It has a complex etiology in which genetic and environmental factors act together to create a neurobiological spectrum. Taking into account to high heritability of ADHD; numerous genes have been tested for an association with the etiology including *NET* (Bobb et al., 2005).

NET, which is responsible for the primary destruction mechanism of noradrenaline (NE), is found in the plasma membrane of noradrenergic neurons and especially in the prefrontal cortex, reuptake of dopamine (DA) and NE into the presynaptic neuron is modulated by *NET* (Hohmann et al., 2015; Zhou, 2004). While NE is the main substrate of *NET*, *NET* is also mediating the reuptake of DA in the prefrontal cortex, so the treatment of ADHD targets both the DA and NE balanced equilibration (Zhou, 2004). The *NET*/*SLC6A2* gene is localized at 16q12.2 and encodes a protein of 617 amino acids (Ramoz et al., 2009). In studies evaluating the *NET* gene, rs3785157, rs998424, rs3785143, rs11568324, and rs28386840 are associated with the development of ADHD (Bobb et al., 2005; Brookes et al., 2006; Kim et al., 2008; Young, 2011).

Atomoxetine (ATX) is the most commonly used drug in a non-stimulant group in the ADHD treatment, acts by increasing the levels of DA and NE by inhibition of presynaptic *NET* in the prefrontal cortex (Bobb et al., 2005). Although there are many studies on the role of polymorphisms in this gene in the etiology of ADHD (Bobb et al., 2005; Kim et al.,2008), there are only three studies that have been investigating the association with response and resistance to ATX treatment. In a study examining whether *NET* and CYP2D6 polymorphisms were effective in response to ATX treatment, patients were treated with ATX dosed

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0.5–1.8 mg/kg/day for 6 weeks. Responders to treatment were shown to be carriers of alleles rs3785152 and rs12708954 (Ramoz et al., 2009) and also, between the 4 and 9 exons of the norepinephrine transporter gene, where the 20 single nucleotide polymorphisms (SNPs) are located and have been identified as part of the response to therapy. In another study, it was found that rs3785143-C allele carriers responded better to the ATX treatment, and it was reported that the T allele was responsible for resistance to treatment in the Asian population (Yang et al., 2013). Also in a study in India, 64 patients were divided into two groups; one group was treated with methylphenidate (MPH), and the other group was treated with ATX. According to the results of the study, rs28363170, a variable number tandem repeat of *SLC6A3*, and rs3785143 plays a major role in the treatment response. It was found that carriers of rs28363170 10R and T allele of rs3785143 were better responded to MPH treatment, whereas carriers of *r*s28363170 9R and C alleles of rs3785143 responded better to ATX treatment. In the same study, irritability and loss of appetite were reported more than others in rs3785143 T allele carriers during ATX treatment (Ray et al., 2017).

Taking into consideration of limited studies, this study aims to investigate the effect of different polymorphisms of a *NET* gene on the ATX treatment response and side effects in children with ADHD and assess whether there is a relationship with the severity of the ADHD. The other important point to be emphasized is that our study is the first study conducted in the Turkish population to our knowledge. Analyzing *NET* gene polymorphisms related to good or poor response could help clinicians identify which treatment would be more useful and would not have more side effects for improving the treatment compliance, considering the increased usage of ATX in the children with ADHD.

Methods

Participants and design of the study

About 100 children aged 6–15 years with a diagnosis of ADHD according to DSM-5 were included in this study at Child and Adolescent Psychiatry Outpatient Clinic, Faculty of Medicine, Erciyes University. All children participating in the study were evaluated with Kiddie-Schedule for Affective Disorders and Schizophrenia, Present and Lifetime Version **(**KD-SADS-PL), which is a semistructured interview form, and it was developed according to DSM-III-R and DSM-IV criteria to screen psychopathology among children and adolescents aged 6–18 years (Gökler et al., 2004; Kaufman et al., 1997). Children and adolescents with comorbid psychiatric diagnoses, mental retardation, neurological, metabolic, and endocrinological diseases were excluded. Considering the patient's age and gender, the healthy control (HC) group consisted of 80 children and adolescents whose parents were volunteers and who had neither mental retardation nor psychiatric or chronic disease. None of the patients had conduct disorder (CD), oppositional defiant disorder (ODD), major depression, anxiety, and tic disorder. Participants who enrolled in the study did not have any medication use within the last 3 months and according to their examination, there was no clinical infection or endocrine findings in screening laboratory results. Exclusion criteria also included a history of other developmental disorders, genetic disorders, such as Fragile X Syndrome, and other congenital diseases. Furthermore, the participants were selected from the same location and ethnic origin in this study. Information about the demographic characteristics of patients was collected using a sociodemographic form and scale, which were prepared by the researchers. The age, gender, academic achievement, number of siblings, medical history, level of education, location, and ethnic origin were obtained.

The dose of ATX was started at 0.5 mg/kg/day in the ADHD group and titrated to 1.2 mg/kg/day by increasing the dose at 1-week intervals. Response to treatment was evaluated 2 months after the beginning of the treatment. In addition to the clinical interview, the effectiveness of the treatment was evaluated with the Clinical Global Impression (CGI) Scale and the Conners' Parent Rating Scale (CPRS). CGI has been developed to evaluate the progress of all psychiatric disorders at any age for clinical research purposes. The CGI is a three-dimensional scale and is filled in during a semi-structured interview conducted by the physician to assess the response to treatment of people with psychiatric disorders (Leucht and Engel, 2006). CPRS consists of 48 items in total and consists of subtests that question psychopathology. These subtests question the symptoms of attention deficit, hyperactivity, ODD, and anxiety disorder (Conners et al., 1998). The Turkish adaptation and validity study of CPRS was performed by Dereboy et al. (2007).

Positive treatment response was defined as having a decrease of 25% in CPRS and a CGI score of equal or less than 2 after 2 months after the ATX treatment. Also, Barkley's Stimulant Side Effect Rating Scale (BSSERS) was used to assess the side effects. On this scale developed by Barkley, side effects associated with stimulant therapy are questioned in terms of their frequency and severity. About 17 questions are asked on the scale, and questions are answered by parents on a 10-point Likert scale. The scores above 7 indicate that the side effect is "severe" (Barkley et al., 1990).

While evaluating the treatment response, the data of 22 patients from 100 patients were excluded because 10 patients did not take the drug regularly, 4 patients did not continue treatment, and the CPRS scores of 8 patients filled by the parents were not evaluated as reliable. All the assessments were made by the two experienced clinicians, and one of the clinicians was a treating clinician.

This study was approved by the local ethical committee of the university. Detailed information was provided to all subjects and controls regarding the objective and protocol of the study. Written informed consent was obtained from both the children who participated in the study and their parents. The procedures followed the ethical standards of the responsible committee on human experimentation (institutional or regional) or with the Helsinki Declaration of 1975 (as revised in 1983).

Genomic DNA isolation and rs12708954 and rs3785143 analysis

The samples were analyzed at Genome and Stem Cell Center (GENKOK), Erciyes University. Two milliliters of blood samples was collected from each participant by a standard method in an ethylenediaminetetraacetic acid (EDTA) tube. DNA was extracted from whole blood samples of each participant by using the High Pure PCR Template Preparation Kit (Roche, Mannheim, Germany). The extracted DNA was stored at −80°C until used. Genotyping of rs12708954 (located intron 8) and rs3785143 (located intron 1) polymorphisms of the *NET* gene were performed by using the Real-Time PCR (Rotor-Gene Q; Qiagen, Hilden, Germany) with

Table 1. The LD calculation in study cohort.

L1	L2	D′	LOD	r^2	CI_{low}	CI_{hi}	Distance	T-int
rs3785143	rs12708954	0.759	0.38	0.017	0.08	0.97	36493	0.38

LD: linkage disequilibrium; LOD: Logarithm of odds.

specific primers (Primerdesign, Chandler's Ford, UK). About 5 µl of DNA was added to produce 20 µl of PCR solution mixture that contained 10 µl qPCR master mix, 1 µl of each primer, and 4 µl ddH_2O. And 5 µl of diluted positive control and wild-type (WT) control was added as control of the study. The PCR was performed with an initial denaturation for 2 min at 95°C followed by 10 cycles for 10 s at 95°C denaturation step, 60 s at 60°C annealing step, 30 s at 72°C, and 35 cycles for 10 s at 95°C, 60 s at 60°C. We used the melting curve analysis to determine the genotypes.

Statistical analysis

Data were analyzed using the IBM Statistical Program for Social Sciences (IBM SPSS for Windows, Version 22.0; IBM Corp., Armonk, NY, USA). The summary statistics of the data were given as a unit number (*n*), percentage (%), mean, standard deviation, median, minimum, and maximum value. The Shapiro–Wilk test and Q–Q plot normality were used for the data distribution. The Levene's test was used to check the variance homogeneity. An independent sample *t*-test was used for comparison of continuous variables with a normal distribution. The Mann–Whitney *U*-test was used for comparison of standard non-distributed variables. A paired sample *t*-test was used for dependent repetitive variables. The Pearson chi-square test, Fisher's exact test, Monte Carlo test, Bonferroni correction, and Logistic Regression Analysis were used to compare the categorical data between the groups. The statistical significance level for all analyses was $p < 0.05$.

Results

The study sample consisted of 75 male, 25 female patients, aged 6–15 years, who were diagnosed as having a combined presentation of ADHD (77%), or predominantly inattentive presentation of ADHD (23%) and had not previously used any medication for ADHD. No statistically significant difference was found between the ADHD and HC groups in terms of age and gender distributions ($p > 0.05$). About 39 of 78 patients responded to ATX treatment according to clinical assessment scales.

The LD calculation in the study cohort was carried out (Table 1). When the distribution of rs12708954 and rs3785143 genotypes between ADHD and HC was examined; no statistically significant difference was found between the groups (Table 2). The distribution of rs12708954 polymorphism was different, and WT was more frequent in males than females in patients with ADHD ($p = 0.03$) (Figure 1). When the severity of ADHD was compared between the different carriers of genotypes rs12708954 and rs3785143, no significant difference was found between the groups in terms of severity ($p = 0.114$). When the response to ATX treatment was compared between different rs12708954 genotype carriers, it was determined that the treatment response was higher in the heterozygous carriers than WT carriers (Table 2 and Figure 2) (odd's ratio: 2.51; %95 CI: 1.13–6.34) and considering that the WT carriers were higher in males, we compared the CPRS decreasing point (males; C1; R^2; 0.53) in a regression model between different rs12708954 genotypes carriers and it is just explaining 5% of the response. Like rs12708954, it was observed that heterozygous carriers of rs3785143 genotypes had higher treatment response than WT carriers (odd's ratio: 2.47; %95 CI: 1.10–5.43) (Table 3 and Figure 3). The effects of rs12708954 and rs3785143 genotypes on response to the ATX treatment were examined together; individuals who were heterozygous in both polymorphisms and also heterozygous carriers of rs12708954/WT of rs3785143 were found to respond higher to the treatment (Table 4).

Table 2. Distribution of rs12708954 and rs3785143 polymorphisms in ADHD and HC.

Polymorphism	ADHD group (*n*/%)	HC (*n*/%)	*p*	df
rs12708954				
CC	64 (64%)	55 (68.75%)		
CA	32 (32%)	24 (30%)	0.492	2
AA	4 (4%)	1 (1.25%)		
rs3785143				
CC	79 (79%)	60 (75%)		
CT	20 (20%)	20 (25%)	0.499	2
TT	1 (1%)	0 (0%)		

The Pearson χ^2 test. Allele frequency C/A: 0.8/0.2 (ADHD group) and 0.84/0.16 (control group). Allele frequency C/T: 0.89/0.11 (ADHD group) and 0.88/0.12 (control group).
ADHD: attention deficit hyperactivity disorder; HC: healthy control.

In both rs12708954 and rs3785143 genotypes, side effects were more common in heterozygous carriers than in WT carriers (Table 5, Figures 4 and 5). When the side effects during treatment were examined, different carriers of both rs12708954 and rs3785143 genotypes together side effects were more frequent in patients who were heterozygous carriers in both rs12708954 and rs3785143 genotypes than in individuals who were WT carriers in both polymorphisms (Table 6).

Discussion

The influence of variation in genes, affecting the mechanism, transport, or metabolism of drug-related treatment response, has been mainly focused on dopaminergic genes and MPH response in ADHD. However, polymorphisms in the *NET* gene can modulate the effectiveness of ATX treatment like MPH response. Considering the increased usage of ATX therapy in the treatment of ADHD, two variants of the gene encoding for *NET* were associated with ATX treatment in children with ADHD in the present study.

In the IMAGE project, the relationship between ADHD and rs3785143 was determined (Brookes et al., 2006), and the presence of this association was also confirmed by two different studies (Kim et al., 2008; Xu et al., 2008). Also, there was only one study found in the literature on the association of rs12708954 with ADHD (Ramoz et al., 2009). In our study, the distribution of rs12708954 and rs3785143 genotypes were found to be similar in both ADHD and HC, and all the rs12708954 and rs3785143 genotypes have no effects on the severity of the disease. When the distribution of rs12708954 genotype polymorphism in gender was investigated, it was found that the expression of rs12708954

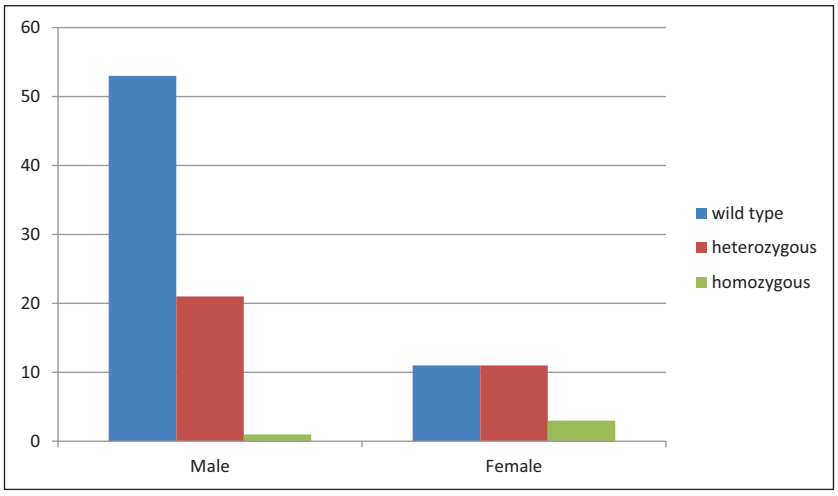

Figure 1. The distribution of rs12708954 polymorphism in males and females in the ADHD group.
ADHD: attention deficit hyperactivity disorder.

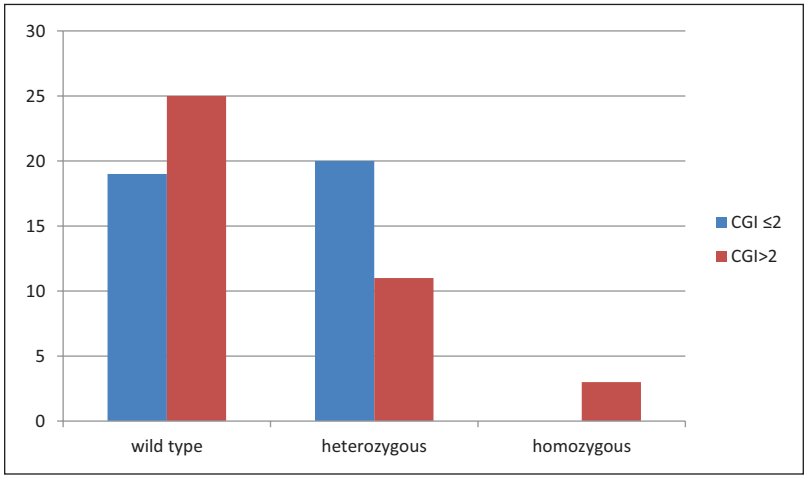

Figure 2. Distribution of response to ATX treatment in patients with rs12708954 genotype.
ATX: atomoxetine.

Table 3. Response to ATX treatment in polymorphisms of rs12708954 and rs3785143.

Polymorphism	Response to treatment (*n*/%)		*p*	df
	CGI ⩽ 2	CGI ⩾ 2		
rs12708954				
CC	19 (48.7%)[a]	25 (64.1%)[b]	**0.019***	2
CA	20 (51.3%)[b]	11 (28.2%)[a]		
AA	0 (0%)[a,b]	3 (7.7%)[a,b]		
rs3785143				
CC	27 (69.2%)[a]	34 (87.2%)[b]	**0.015**	2
CT	12 (30.8%)[b]	4 (10.3%)[a]		
TT	0 (0%)[a,b]	1 (2.6%)[a,b]		

The Monte Carlo test and Bonferroni correction; b > a.
ATX: atomoxetine; CGI: Clinical Global Impression; CPRS: Conners' Parent Rating Scale.
*Also Logistic Regression Analysis was done by assessing the CPRS decreasing point and polymorphism of rs12708954/males; C1; R^2: 0.53, just explaining 5%.
Significant association ($p < 0.05$) is indicated in bold.

was higher in the male patients with ADHD, and there was no difference in the term of rs3785143 expression between genders.

To the best of our knowledge, no study evaluating gender relationship with rs12708954 has been found in the literature. In a study, the relation of subtypes and gender with the *NET* gene in children with ADHD was assessed, and it was found that the rs3785143 was related with ADHD subtypes and with the female gender (Sengupta et al., 2012). Different from the results in our study, it was found that the T allele of rs3785143 displayed a stronger effect in females than in males in another study (Biederman et al., 2008). The difference between these studies and the results of our study may be due to the diversity of the gene pool and the methodological differences. Besides, the limited number of female patients in our study may have contributed to this result.

In our study, the response rate at ATX treatment was 50%. In a review, it was stated that the response rate to ATX was approximately 60% in children and adolescents according to the results of

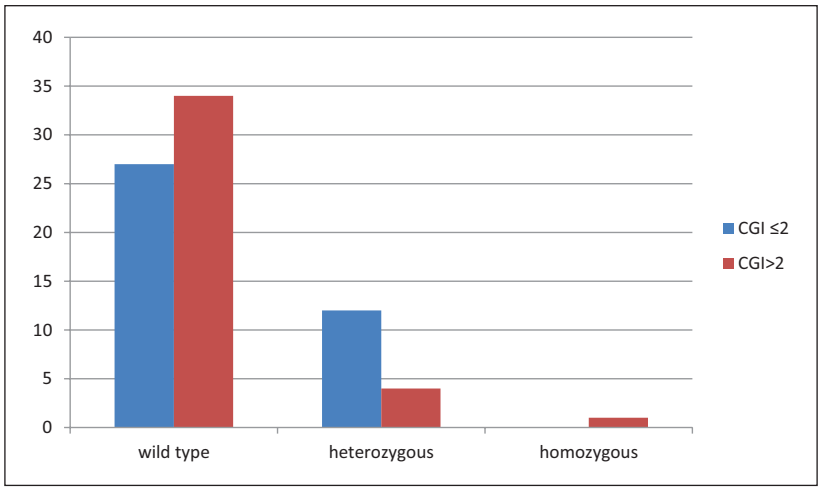

Figure 3. Distribution of response to ATX treatment in patients with rs3785143 genotype.
ATX: atomoxetine.

Table 4. Response to ATX treatment rs12708954 and rs3785143 polymorphisms evaluated together.

Polymorphism	Response to treatment (*n*/%)		*p*	df
	CGI ⩽ 2	CGI ⩾ 2		
rs12708954/rs3785143				
CC/CC	14 (35.9%)[a]	20 (51.3%)[b]		
CC/CT	3 (7.7%)[a]	4 (10.3%)[a]		
CA/CC	15 (38.5%)[a,b]	11 (28.2%)[a,b]	**0.001**	5
CA/CT	7 (17.9%)[b]	0 (0%)[a]		
CC/TT	0 (0%)[a]	1 (2.6%)[a]		
AA/CC	0 (0%)[a]	3 (7.7%)[a]		

The Monte Carlo test and the Bonferroni Correction; b > a.
ATX: atomoxetine; CGI: Clinical Global Impression.
Significant association ($p < 0.05$) is indicated in bold.

Table 5. Side effects of ATX treatment in polymorphisms of rs12708954 and rs3785143.

Polymorphism	Side effects (*n*/%)		*p*	df
	Yes	No		
rs12708954				
CC	15 (41.7%)[a]	29 (69%)[a]	**0.008**	2
CA	20 (55.6%)[b]	11 (26.2%)[b]		
AA	1 (2.8%)[a,b]	2 (4.8%)[a,b]		
rs3785143				
CC	24 (66.7%)[a]	37 (88.1%)[a]	**0.004**	2
CT	12 (33.3%)[b]	4 (9.5%)[b]		
TT	0 (0%)[a,b]	1 (2.4%)[a,b]		

The Monte Carlo test and the Bonferroni correction; b > a.
ATX: atomoxetine.
Significant association ($p < 0.05$) is indicated in bold.

six randomized controlled trials (Clemow and Bushe, 2015), and in a meta-analysis, the effect size of ATX was found to be 0.62 (Faraone et al., 2006). When the effect of rs12708954 SNP on the treatment response was assessed; it was found that the response of the heterozygote genotype was better than the normal genotype. There was only one study about the effect of rs12708954 SNP on response to ATX treatment in the literature (Ramoz et al., 2009). ADHD samples have comorbidity, including CD, ODD, separation anxiety disorder, and major depression. Two independent samples were taken in this study, and SNPs were investigated after 6 weeks of ATX treatment. Different SNPs were found to be associated with ATX response, and rs12708954-A allele carriers were the best responders to the treatment. When the two groups were evaluated together, it was found that the strongest relationship with the response was related to rs3785152, and this SNP was specific to the treatment response in the white race. Besides, since the 20 SNPs associated with the ATX response are among the four to nine exons of the norepinephrine transporter gene, this region has been identified as the part associated with response to the treatment (Ramoz et al., 2009). The results of our study support the relationship of rs12708954 polymorphism with treatment response, and patients who carried AA genotype and CA genotype had better ATX treatment response than CC genotype carriers in our study. The absence of any difference in terms of ethnicity, the exclusion of comorbidity, and the presence of a control group is the difference in our study.

In a study, 12 SNPs related to *NET, ADRA2A*, and *ADRA1A* were investigated in the 101 ADHD patients with comorbid ODD, CD, tic disorder, generalized anxiety disorder, and social and specific phobia. ATX was titrated at a dose of 0.5 mg/kg/day, titrated at 1.2 mg/kg/day, and increased to a dose of 1.4 mg/kg/day. It was found that carriers of the C allele of rs3785143 were responders to treatment, and the T allele was responsible for resistance to treatment (Yang et al., 2013). In another study examining the association of dopamine and norepinephrine genes with ADHD treatment, rs28363170 and rs3785143 play a major role in the treatment response. It was found that carriers rs28363170 10R and T allele of rs3785143 were better responded to MPH treatment, whereas carriers rs28363170 9R and C alleles of rs3785143 responded well to ATX treatment (Ray et al., 2017). Unlike the two studies mentioned above, genotyping in our study

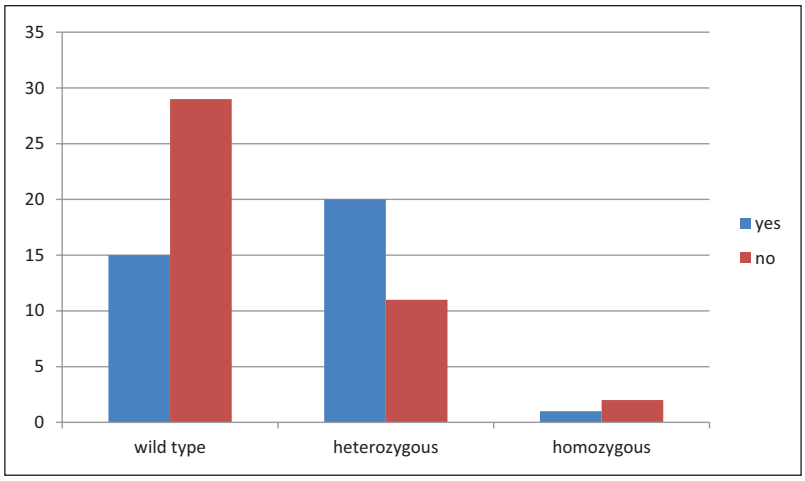

Figure 4. Distribution of side effects with ATX treatment developed in patients with rs12708954 genotype.
ATX: atomoxetine.

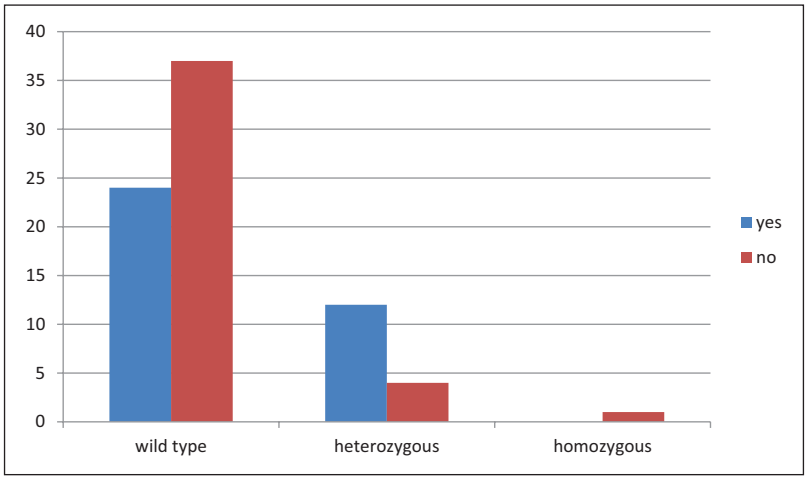

Figure 5. Distribution of side effects with ATX treatment developed in patients with rs3785143 genotype.
ATX: atomoxetine.

Table 6. Side effects of ATX treatment rs12708954 and rs3785143 polymorphisms evaluated together.

Polymorphism	Side effects (*n*/%)		*p*	df
	Yes	No		
rs12708954/rs3785143				
CC/CC	9 (25%)[a]	25 (59.5%)[b]		
CC/CT	5 (13.9%)[a,b]	2 (4.8%)[a,b]		
CA/CC	15 (41.7%)[a,b]	11 (26.2%)[a,b]	**0.001**	5
CA/CT	6 (16.7%)[b]	1 (2.4%)[a]		
CC/TT	0 (0%)[a,b]	1 (2.4%)[a,b]		
AA/CC	1 (2.8%)[a,b]	2 (4.8%)[a,b]		

The Monte Carlo test and Bonferroni correction; b > a.
ATX: atomoxetine.
Significant association ($p < 0.05$) is indicated in bold.

was evaluated as WT, homozygous, and heterozygote rather than as allele carriers. In terms of rs3785143, heterozygous individuals were found to respond better to ATX treatment. Also, in our study, the response of individuals to treatment in terms of two genotypes was examined, that is individuals who were heterozygous in both polymorphisms, and heterozygous carriers of rs12708954 and WT carriers of rs3785143 were found to have a higher response to the treatment. It was thought that factors, such as ethnicity, the size of the study sample, and the differences in methodology, could play a role in the differences in our results. Also, our study is different from other studies in terms of lack of comorbidity. As in the other two mentioned studies, the presence of ODD/CD comorbidity could be another reason for the differentiation of response to treatment in rs3785143 allele carriers. At this point, in one study, six different *NET* gene SNPs were examined in the ADHD comorbid ODD, and the relationship of the rs3785143 T allele was determined (Liu et al., 2015).

In the present study, when the relationship of rs12708954 genotypes with side effects during treatment was assessed, more side effects were observed in heterozygous carriers than normal genotypes. Similarly, in rs3785143 genotypes, side effects in heterozygous carriers have been reported more frequently than WT carriers. Also, when two SNPs were evaluated in our study, it was

found that side effects were more frequent in heterozygous carriers of both rs3785152 and rs3785143. In a previous study examining the response to ATX, it was reported that the carriers of alleles rs3785152 and rs12708954 were responders to treatment; however, the association of genotypes with side effects was not evaluated (Ramoz et al., 2009). In only one pilot study, rs3785143 T allele carriers reported a loss of appetite and irritability during ATX treatment (Ray et al., 2017). Given that there is limited information in the literature about the relationship between the side effects observed in the ATX treatment and *NET* gene polymorphisms, it is obvious that further studies are needed to improve the compliance with the treatment.

Limitations

The small sample size in both ADHD and HC groups, the limited number of female cases, the absence of ADHD subgroups, lack of a placebo group, and the evaluation of the limited number of polymorphisms are among the limitations of our study.

Conclusions

Considering the limited studies about *NET* gene polymorphism and increased usage of ATX therapy in ADHD, studies to better understand the role of genes involved in the metabolism of noradrenergic drugs in treatment response are critical. Treatment response of rs12708954 and rs3785143 heterozygous carriers were found to be better than WT carriers in our study. Similarly, side effects were found to be higher in heterozygous carriers in both rs12708954 and rs3785143 polymorphisms. Further expanded studies including other functional polymorphisms of the *NET* are needed to better understand the role of genetic variation in good or poor response to ATX treatment for ADHD.

Author contributions

Conceived and designed the study: ED and EFS. Performed the experiments or case: ED and MKG. Analyses of gene variants: EFS and MGO. Analyzed the data: ED, MKG, EFS, and MGO. Wrote the paper: ED and MKG. All authors have read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical approval

This study was approved by the local ethical committee of the university (2017/419). Detailed information was provided to all subjects and controls regarding the objective and protocol of the study. Written informed consent was obtained from both the children who participated in the study and their parents.

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