Original Article

Evaluating of Solute Carrier Family 6 Member 4 Gene (SLC6A4) Promoter Polymorphisms with Escitalopram Plasma Levels for Precision Medicine in Major Depressive Disorder

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Abstract

Aim and Objective: Escitalopram (SCT) shows an antidepressant effect due to its mechanism of increasing the serotonin level by inhibiting the serotonin transporter protein (5HTT). 5HTT is encoded by solute carrier family 6 member 4 gene (SLC6A4) in the brain. Recognition of SCT plasma level of patients and pharmacodynamics of individuals during SCT treatment will increase the expected response to the treatment and reduce the adverse effects. This study aims to determine the effect of SLC6A4 promoter long/short polymorphism and the SCT plasma level of patients on the response to treatment during the SCT drug therapy. **Materials and Methods:** Blood and plasma samples of 30 major depressive patients using 20 mg SCT for 8 weeks between the ages of 18 and 65 were analyzed to determine SCT plasma level and SLC6A4 promoter polymorphism. The treatment response level was determined by using the Hamilton Depression Rating Scale at patient files. **Results:** SCT plasma level of the nine patients with LL polymorphism was found to be in the range of 13.40–63.36 ng/mL. For 13 patients with LS polymorphism, SCT plasma level was found to be in the range of 2.93–57.48 ng/mL. For eight patients with SS polymorphism, the SCT plasma level was found to be in the range of 0.95–49.32 ng/mL. **Conclusion:** When the association between SCT plasma level and response to the drug treatment was examined, we had significant results to show that SCT level affected the response to treatment, especially in the LS group, as well as the SLC6A4 promoter variation. This study may lead to a more profound understanding of rational drug therapy as well as to a careful application of pharmacogenetics in psychiatry..

Keywords: *Escitalopram plasma level, response, SLC6A4 promoter polymorphism*

Introduction

Escitalopram (S-CT) is one of the most commonly utilized selective serotonin reuptake inhibitors (SSRIs) for depression and general anxiety treatment.[1,2] S-CT shows an antidepressant effect, as it increases the serotonin level in the presynaptic area by inhibiting the serotonin transporter protein (5-HTT) in the brain.[3,4] S-CT has proven to be effective in treating depression and anxiety disorder after it is administered with an oral dose of daily 10–20 mg.[1] As far as the effects of S-CT in the body is concerned, it is seen in past studies that the effect of S-CT begins when reaching a level higher than 80% of 5-HTT occupancy.[5,6] During S-CT treatment, the knowledge of the genotypic characteristics of individuals and the plasma level of S-CT may be effective in determining therapeutic targets.[7] Polymorphism can change the gene expression or gene activity where they are

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Ethics committee approval: The ethics committee approval has been obtained on June 5, 2017 (ethics committee decision number: B.08.6.YOK.2.US.0.05.0.06/2017/154) for the blood sampling part of the work.

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present. Solute carrier family 6 member 4 gene *(SLC6A4)* encodes 5-HTT. The polymorphism exists in the promoter region of *SLC6A4* where a 44 bp GC (Guanine, Cytosine) consisting of 20–22 bp double repeats occurs depending on the repetitions of a rich sequence of insertions/deletions. Bp long (L: L) form consists of 16 repeats resulting from the insertion of the 44 bp repeat sequence. In the case of the deletion, however, the allele that is called as bp short (S) form consisting of 14 repeats occurs. L and S variants have been identified in a variety of studies showing different transcriptional effects.[8] S variant is associated with the lower transcriptional activity of the promoter when compared to the L variant.[4,7]

In addition, determining the appropriate individual drug and dose depends on taking the individual differences into consideration. Furthermore, the severity of side effects and interactions as well as possible adverse drug reactions can be decreased, and in addition, the efficacy of treatment can be increased as well.[9] While genotyping methods are used to determine polymorphisms in enzymes, carrier proteins, and receptors, drug levels in body fluids (e.g., plasma, blood, and urine) have been evaluated by therapeutic drug monitoring (TDM).[9] TDM, which has been widely used in the world, is one of the methods that can be used with the purpose of personalized treatment.

It is thought that besides knowing the S-CT plasma level of patients, the pharmacodynamics of individuals has an essential role in increasing the success of the treatment. Recognition of the S-CT plasma level of patients and pharmacodynamics of individuals during the S-CT treatment will increase the expected response to the treatment and reduce the adverse effects.

The goal of this study is to determine the effect of *SLC6A4* promoter polymorphism and the S-CT plasma level of the patients on their response to treatment during the S-CT drug therapy. Therefore, in this study, the level of S-CT has been analyzed in plasma samples of patients treated by S-CT therapy in depression. Furthermore, *SLC6A4* promoter polymorphism and evaluation of response to treatment have been identified in the same patients. When *SLC6A4* promoter polymorphism and S-CT plasma levels of these patients have been identified and evaluated together, the physician can choose and set an effective drug regimen according to their 5-HTT activity. With this attempt, the side effects of the drug could be reduced more effectively and the desired result could be increased as well.

Materials and Methods

The ethics committee approval has been obtained on June 5, 2017 (ethics committee decision number: B.08.6.Y OK.2.US.0.05.0.06/2017/154) for the blood sampling part of the work.

Sample selection

Between June 1, 2017, and June 1, 2018, blood and plasma samples of 30 patients (males and females), between the ages of 18 and 65, who were using 20 mg S-CT for 8 weeks, were analyzed in Üsküdar University, Clinical Pharmacogenetics Laboratory, to determine the S-CT plasma level and *SLC6A4* promoter polymorphism. Approval of the Ethics Committee from Üsküdar University was obtained on June 5, 2017 (ethics committee decision number: B.08.6.YOK.2.US.0.05.0.06/2017/154) for the blood sampling part of the work.

Inclusion criteria of the present study were as follows: (1) Patients who showed evidence of a diagnosis of major depressive disorder according to Diagnostic and Statistical Manual of Mental Disorders-IV; (2) patients who had a score of at least 18 on the 17-item Hamilton Rating Scale for Depression (HRSD) (Hamilton, 1960)[31] in the patient files; and (3) patients taking monodrug therapy with S-CT. To be in the inclusion list, the patients must also not be taking any drugs or foods that affect (inhibit or induce) the S-CT metabolic pathway during the drug treatment of selected patients. Patients who showed evidence of bipolar or anxiety disorders, psychosis, substance use disorders, pregnancy, or breastfeeding were excluded from the study.

Samples were selected from patients, whose samples were sent to the laboratory within the last 12 months. Samples were taken 24 h after the last dose to determine the plasma S-CT trough level of the patient by using liquid chromatography–tandem mass spectrometry (LC-MS/MS). The blood and plasma samples sent to the laboratory were kept at –20°C until analysis. Treatment response level was found by examining patient files (patients were classified as a responder if there was at least 50% reduction in initial HRSD score at the endpoint by clinicians).

Chemicals and reagents

For TDM analysis, all reference standards (escitalopram oxalate and desipramine hydrochloride (as internal standard [IS]) were purchased from Sigma (Sigma-Aldrich, St. Louis, Missouri, USA). Furthermore, high-performance liquid chromatography (HPLC) grade methanol, HPLC grade acetonitrile, formic acid, and ammonium formate were purchased from Merck (Merck, Kenilworth, New Jersey, USA). For *SLC6A4* genotyping, all other reagents (DNA isolation kits, forward and reverse primers [to and from] and Taq polymerase enzyme) were purchased from Invitrogen (Germany).

DNA sample collection

DNA was isolated by using Invitrogen DNA isolation kits (Germany). The procedure of the manufacturer's instructions was followed for isolation. The purity of the

isolation was confirmed according to the OD260/OD280 ratio. Values between 1.60 and 2.00 were accepted as pure and used for amplification.

SLC6A4 genotyping

To genotype *SLC6A4* promoter polymorphism, conventional polymerase chain reaction (PCR) was carried out by using the forward primer 5'-TCCCAGCAACTCCCTGTA-3 and reverse primer 5'-GGAATACTGGTAGGGTGCAA-3'. The PCR conditions were previously described.[10] Long allele (L) and short allele amplicons gave rise to 317 bp and 272 bp, respectively [Figure 1].

Therapeutic drug monitoring analysis of patient samples

The quantitative determination method has been applied for S-CT in plasma considering the publications of bioanalytical method validation.[11-14-30] The validation results of the method were published in our previous study.[15]

Liquid chromatography–tandem mass spectrometry conditions

Agilent 6470 HP-1200 LC series (USA) was used for the analysis. ACE-3 C 8 (3 µm, 3.0 mm 150 mm) column was used for analytical separation. The column temperature was 45°C. Mobile phase conditions in the previous study were applied.[15] The total analysis run time was 8 min at a flow rate of 0.5 mL/min. Quantitative analysis was carried out by multiple reaction modes with an electrospray positive ionization (ES+). Quantitation was based on monitoring precursor ion and product ion for S-CT m/z 325.1 >109.1 and for ISs, desipramine m/z 267.0 >72.0.

Preparation of standard and quality control samples

The stock standard solution was prepared by dissolving 12 mg of escitalopram oxalate in methanol ($c_{S\text{-}CT}$: 0.92 mg/mL). Then, a diluted stock standard solution was prepared by diluting the stock standard solution with methanol ($c_{S\text{-}CT}$: 3.7 µg/mL). To prepare eight calibration standards and five quality control samples for S-CT in plasma, the diluted stock standard solution was spiked in different volumes to the plasma. The limit of quantification that can be used for quantitative assay in plasma for S-CT was found to be 5.9 ng/mL. The calibration range for S-CT was 5.9–441.8 ng/mL.

Analysis of plasma samples*:* 100 µL of IS solution (c: 550 µg/mL) and 400 µL cold acetonitrile were spiked to the 500 µL plasma sample and further vortexed for 30 s and then it was centrifuged at 15,000 rpm for 5 min. Subsequently, 5 µL from the clear portion was injected into the system.

Statistical analysis

Statistical analysis was carried out by descriptive methods. For comparisons between groups, the nonparametric statistical method (Kruskal–Wallis and Mann–Whitney U-tests) was applied.

Results

The patients' demographic data, mean with standard deviations, as well as the minimum and maximum value for S-CT plasma level (for TDM), are displayed in Table 1. The percentage of females was found to be 66.7%. The mean age of the patients was 39.00 ± 10.55 (years). The mean S-CT plasma level of the patients was 27.59 ± 16.05 ng/mL.

There was no statistically significant difference between the males and females in the mean plasma level of S-CT ($P > 0.05$) [Table 1 and Figure 2]. When the relationship between S-CT plasma level and age groups was examined using the Kruskal–Wallis test, no statistically significant difference was found ($P > 0.05$) [Table 1 and Figure 3]. The frequency distribution of the LL, LS, and SS groups for the *SLC6A4* promoter polymorphism

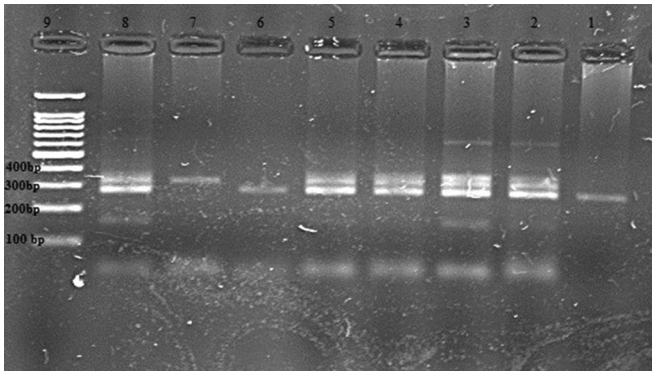

Figure 1: Representetive figure of agarose gel electrophoresis and *SLC6A4* Promoter Polymorphism Results. Lanes; 1: SS genotype (272 bp); 2: LS genotype (317 and 272 bp); 3: LS genotype (317 and 272 bp); 4: LS genotype (317 and 272 bp); 5: LS genotype (317 and 272 bp); 6: SS genotype (272 bp); 7: LL genotype (317 bp); 8: LS genotype (317 and 272 bp); 9: DNA standart marker

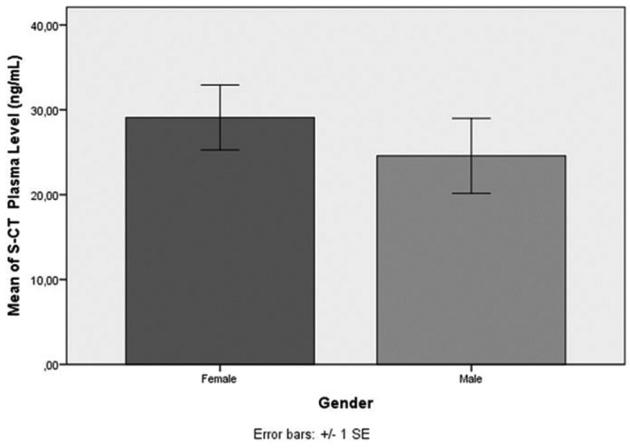

Figure 2: Relationship of Gender and S-CT Plasma Level. Mann–Whitney U-test shows that there is no statistically significant effect of gender difference on S-CT plasma level ($P > 0.05$). Number of samples (N_{female}: 20, N_{male}: 10), mean S-CT plasma level (female: 29.10 ng/mL, male: 24.58 ng/mL), standart deviation S-CT plasma level (female: 16.69, male: 13.28)

of 30 patients is summarized in Table 2. In our study, out of 30 patients, nine patients (30.0%) with LL polymorphism, 13 patients (43.3%) with LS polymorphism, and eight patients (26.7%) with SS polymorphism were detected [Table 2]. Patients with LL, LS, and SS polymorphisms were compared in three different groups using the Kruskal–Wallis and Mann–Whitney U-tests to determine the effect of *SLC6A4* promoter polymorphism to response during the drug treatment. The difference between the groups was found to be statistically significant. When the Mann–Whitney U-test was applied to determine the difference between the groups, the difference between the LL and SS groups was found to be statistically significant ($P \leq 0.05$) in the 95% confidence interval in Table 2 [Figure 4]. Mann–Whitney U-test was used to determine the effect of S-CT plasma level on the response to treatment. S-CT plasma level was found to be statistically significant on the response to treatment ($P \leq 0.05$) [Table 2 and Figure 5].

S-CT plasma level of the nine patients with LL polymorphism was found to be in the range of 13.40–63.36 ng/mL [Table 2]. When the patient files were examined, it was reported that no side effects were observed in nine patients during S-CT administration, and the desired drug response was obtained. For 13 patients with LS polymorphism, S-CT plasma level was found to be in the range of 2.93–57.48 ng/mL. It has been notified in patient files that no treatment response was obtained from four patients with LS polymorphism whose S-CT plasma level was below the therapeutic range. In this group, the drug side effects (insomnia and loss of appetite) have been reported to have appeared in one person. For eight patients with SS polymorphism, S-CT plasma level was found to be in the range of 0.95–55.25 ng/mL. When the patient files have been reviewed, it has been reported that the five nonresponder patients were obtained from these patients. Among these patients, two with S-CT plasma levels below the therapeutic range and three with S-CT plasma levels within the therapeutic range were identified. Furthermore, it has been reported that a response has been obtained for three patients whose S-CT level was within the therapeutic range.

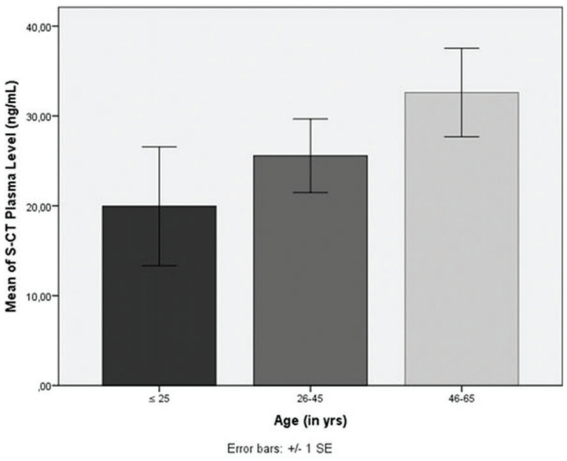

Figure 3: Relationship of Age and S-CT Plasma Level. Kruskal–Wallis test shows that there is no statistically significant effect of age groups on S-CT plasma level ($P > 0.05$). Number of samples ($N \leq_{25\ age}$: 3, $N_{26\text{-}45\ age}$: 16, $N_{46\text{-}65\ age}$: 11), mean S-CT plasma level (≤25 age: 19.96 ± 11.44 ng/mL, 26–45 age: 25.58 ± 16.37 ng/mL, 46–65 age: 32.60 ± 16.34 ng/mL)

Discussion

S-CT is used in the treatment of major depression at a wide range of ages.[16,17] It is reported that the physician can more

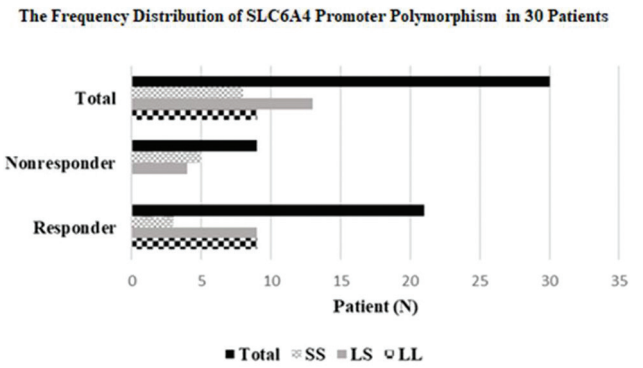

Figure 4: Evaluation of the Association between *SLC6A4* Promoter Polymorphism and Response to Treatment. Out of 30 patients, nine patients (30.0%) with LL polymorphism ($N_{responder}$; 9), 13 patients (43.3%) with LS polymorphism ($N_{responder}$; 9 and $N_{nonresponder}$; 4), and eight patients (26.7%) with SS polymorphism ($N_{responder}$; 3 and $N_{nonresponder}$; 5) were detected

Table 1: Results of descriptive statistic for gender, age, and escitalopram plasma level (ng/ml)

Patient		S-CT plasma level (ng/mL)			Mann-Whitney U-test, *P**
Gender	***n*; %**	**Minimum**	**Maximum**	**Mean±SD**	
Female	20; 66.7	0.95	63.36	29.10±16.69	*P*>0.05
Male	10; 33.3	9.66	57.48	24.58±13.28	
Total	30;100.0	0.95	63.36	27.59±16.05	
Age (years), mean±SD: 39.00±10.55					Kruskal-Wallis test
≤25	3;10.0	7.35	29.71	19.96±11.44	*P*>0.05
26-45	16;53.3	0.95	57.48	25.58±16.37	
46-65	11; 36.7	15.23	63.36	32.60±16.34	
Total	30; 100.0	0.95	63.36	27.59±16.05	

*The mean difference is significant at≤0.05 level in the 95% CI. CI: Confidence interval, SD: Standard deviation, S-CT: Escitalopram

Table 2: The evaluation of the relationship between escitalopram plasma level and *SLC6A4* promoter polymorphism and drug response

Patient (*n*; %)	S-CT plasma level (ng/mL)		SLC6A4 promoter polymorphism			
	Mean±SD	Mann-Whitney U-test (*P**)	LL (*n*; %)	LS (*n*; %)	SS (*n*; %)	Kruskal-Wallis-Mann-Whitney U-test (*P**)
Responder (21; 70.0)	31.42±12.90	≤0.05*	9; 100.0	9; 69.23	3;37.5	≤0.05**(LL-SS)
Nonresponder (9; 30.0)	18.65±19.74		-	4; 30.77	5;62.5	
Total (30; 100.0)	27.58±18.77		9;100.0	13,100.0	8; 100.0	
SLC6A4 promoter polymorphism						
Patient (*n*; %)	**S-CT plasma level (ng/mL)**			**Therapeutic range (15-80 ng/mL)**		
	Minimum	**Maximum**	**Mean±SD**	**Below (*n*, %)**	**Within (*n*, %)**	**Above (*n*, %)**
Responder (21; 70.0)						
LL (9; 42.9)	13.40	63.36	33.15±14.33	1; 11.1	8;88.9	NR
LS (9; 42.9)	16.83	57.48	30.43±13.75	NR	9; 100.0	NR
SS (3; 14.2)	23.90	38.21	29.22±6.39	NR	3; 100.0	NR
Nonresponder (9; 30.0)						
LL (NR; NR)	NR	NR	NR	NR	NR	NR
LS (4; 44.4)	2.93	14.89	8.71±4.98	4; 100.0	NR	NR
SS (5; 55.6)	0.95	55.25	26.60±24.13	2; 40.0	3; 60.0	NR
Total (30; 100.0)						
LL (9; 30.0)	13.40	63.36	33.15±14.33	1; 11.1	8;88.9	NR
LS (13; 43.3)	2.93	57.48	23.74±15.53	4;30.8	9; 69.2	NR
SS (8; 26.7)	0.95	55.25	27.58±18.77	2; 25.0	6; 75.0	NR

*The mean difference is significant at≤0.05 level in the 95% CI, **The difference between the LL and SS groups was found to be statistically significant (*P*≤0.05) in the 95% CI. NR: Not reported, SD: Standard deviation, S-CT: Escitalopram, CI: Confidence interval

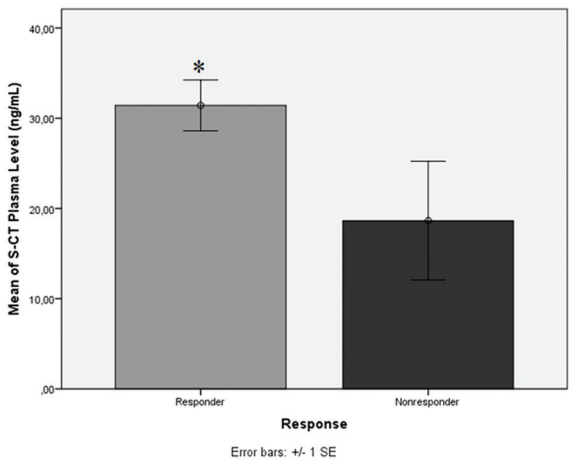

Figure 5: Evaluation of the association between S-CT Plasma Level and Response to Treatment. S-CT plasma level was found to be statistically significant on the response to treatment by Mann Whitney U test (*P* ≤ 0.05). '*' represent significant differences (*P* ≤ 0.05). Number of samples ($N_{nonresponder}$: 9, $N_{responder}$: 21), mean S-CT plasma level ($N_{nonresponder}$: 18.65 ± 19.74 ng/mL, $N_{responder}$: 31.42 ± 12.90 ng/mL)

easily adjust the drug plasma levels by following up TDM to provide the plasma therapeutic drug range when the individual factors affect the plasma drug levels differently.[18]

The expected S-CT plasma level/drug dose ratio (C/D [ng/mL/mg]) was identified to be 0.58–1.54 ng/mL/mg in the study of Hiemke *et al*.[5] When this ratio is taken into consideration, the expected S-CT plasma level range for the 20 mg S-CT can be calculated as 11.6–30.8 ng/mL. In our study, the mean plasma S-CT level of 30 patients using 20 mg S-CT was found to be 27.59 ± 16.05 ng/mL [Table 1]. The mean S-CT plasma level obtained from our study was found to be within the expected S-CT plasma level range (11.6–30.8 ng/mL), as reported by Hiemke *et al*.[5] Jin *et al*.[17] investigated the effect of age on S-CT exposure, indicating that S-CT plasma level is higher than that of younger people because they have lower clearance in elderly people.[17] In our study, however, it was found that there was no statistically significant difference between the S-CT plasma levels in the age groups formed [Table 1]. The reason for the difference between the two studies is that, in contrast to the other study, the age range of the study group is closer to each other and the general age range of the study sample is narrow (range: 20–58 age) in our study, which is why the exact distinction could not be made. Rao[1] study of S-CT pharmacokinetics showed that there was no statistically significant difference in S-CT pharmacokinetics (t_{max}, C_{max}, $t_1/_2$) between adolescents (12–17 age) and adults (18–35 age), whereas the difference between the adults and elderly was found to be significant.[1] Moreover, in the same study, it was stated that gender had no effect on S-CT level.[1] In our study, the age group formed is mainly composed of adolescents and adults. No statistically significant difference was detected in S-CT plasma levels between these age groups ($P > 0.05$). In addition, we found that gender difference did not cause a significant difference in S-CT plasma level ($P > 0.05$) [Table 1]. The results of the comparison between S-CT plasma level and gender and age in our study are similar to those of Rao's study [Figures 2 and 3].[1,17]

In many studies, the pharmacodynamic mechanism of SSRIs is explained by the effect on 5-HTT.[19] S-CT located in the SSRIs group inhibits 5-HTT and prevents serotonin reuptake and increases the level of serotonin in the synaptic region.[20] It is known that the *SLC6A4* promoter has a polymorphic characteristic. In our study, the variant distribution of LL, LS, and SS of 30 patients is given in Table 2. It has been found that nine patients (30%) have the LL variant, 13 patients (43.3%) have the LS variant, and eight patients (26.7%) have the SS variant. In a study by Samochowiec *et al*.,[21] the effect of *SLC6A4* promoter on anxiety disorders was examined.[21] Of the 202 healthy Caucasians in the control group included in this study, 42% were reported as LL, 48% as LS, and 10% as SS. The *SLC6A4* promoter variant distribution of the patients who participated in our study is similar to the variant distribution of the control group in the publication by Samochowiec *et al*.[21] However, the proportion of people with SS variant, in the patients who participated in our study, was found to be slightly higher than that in the healthy control group in the related publication. In many studies, variant variability has been reported to affect the level of 5-HTT expression.[22] The L-homozygous variant increases the transcriptional activity of the *SLC6A4* promoter, which results in a rise of 5-HTT expression relatively more than those of the S variant.[4,7] In related studies, it was noted that the 5-HTT expression decreased in those with the S variant.[23,24] It was reported by Mancama and Kerwin[22] that patients in the LL and LS groups had higher compliance with drug treatment than those in the SS group. When the response of S-CT treatment to 30 patients with *SLC6A4* promoter polymorphism distribution was examined, it was observed that the difference between the groups was statistically significant ($P \leq 0.05$) [Table 2 and Figure 4]. While a statistical significance was detected between LL and SS groups in response to the treatment ($P \leq 0.05$), no statistical significance in the response to treatment was found between the LS group and the other groups ($P > 0.05$) [Table 2]. While the response to treatment was observed in the entire LL group, patients without response to the drug treatment in the SS group were determined. In cases where the SS group failed to respond to the drug treatment, it has been determined from patient files that dose increase and different drug additions were performed to maintain the treatment. Many studies have reported that people with LL variants can respond better to SSRIs than those with SS variant, and it is difficult and long to reach a response in SS group patients.[25-27] The data obtained from our study is similar to the data in the literature.

It has been reported that in many studies studying the association between S-CT plasma level and antidepressant effect, the antidepressant effect of S-CT initiates by occupying at least 80% of 5-HTT (Klein *et al*., 2006).[28] It has been reported that 80% of 5-HTT is loaded when S-CT reaches 15 ng/mL in plasma. The therapeutic interval was reported to be 15–80 ng/mL in previous studies.[6] In our study, the mean plasma level of 30 patients was found to be 27.59 ± 16.05 ng/mL. It was found that nine patients (30%) did not respond to the treatment. In our study, when *SLC6A4* promoter variations of these nonresponder patients were examined, it was observed that five patients were from the SS group and four patients were from the LS group. The minimum and maximum value ranges of S-CT plasma level and mean plasma level in these nonresponder patients with SS group were found to be 0.95–55.25 ng/mL and 26.60 ± 24.13 ng/mL, respectively [Table 2]. Even though the S-CT plasma levels of these patients with the SS variant were within the therapeutic range, these levels were detected to be insufficient for a response to treatment. Taylor *et al*.[29] reported that patients with the SS group had lower rates of remission than LS or LL groups in their studies. The data obtained from our study is similar to the results of Taylor *et al*.[29] However, the TDM level of nonresponder patients with SS variant in our study differs from the literature.[6]

In our study, the mean TDM levels in the nonresponder group (LS versus SS) were found to differ from each other. While the mean TDM level of the LS group that did not respond to treatment was determined to be below the therapeutic range in accordance with the literature, patients who did not respond to the treatment were observed in the SS group, even if the mean TDM level in the SS group was within the therapeutic range. Unfortunately, TDM level may could not be exactly determined in patients, who did not respond due to an insufficient number of samples. Therefore, the limitations of our study include the insufficient number of samples for each group. Hence, it is recommended to determine the mean TDM level in responder and nonresponder patients by planning studies with a higher number of samples.

Florio *et al*.[6] reported that the antidepressant effect of S-CT begins when S-CT plasma levels reach approximately higher than 20 ng/mL.[6] In our study, the minimum–maximum S-CT plasma level of the four nonresponder patients with the LS group was found to be 2.93–14.89 ng/mL [Table 2]. It has been observed that since the S-CT plasma levels of these patients with LS variants have not reached the lower limit of the therapeutic range (15 ng/mL), these levels are not sufficient for the drug response to be seen. The data obtained from our study is similar to the data in the literature. The S-CT plasma level affected the response to treatment, especially in the LS group, as well as the variation of *SLC6A4* promoter polymorphism.

It has been found that the means of S-CT plasma level of 21 responder patients with LL, LS, and SS variants were 33.15 ± 14.33 ng/mL, 30.43 ± 13.75 ng/mL, and 29.22 ± 6.39 ng/mL, respectively [Table 2]. Nine of these patients have the LL variant, nine have the LS variant, and

three have the SS variant. While the S-CT plasma levels of eight patients in the LL variant were found to be within the therapeutic range, the S-CT plasma level of only one patient was 13.40 ng/mL. Although not reaching the lower limit of the therapeutic range (15 ng/mL), it was observed that the patient received the desired response from the treatment at the detected drug level. S-CT plasma levels of patients with LS and SS variants in this group were found in the therapeutic range.

Conclusion

When the association between the S-CT plasma level and response to the drug treatment was examined, significant results were obtained which showed that the S-CT plasma level affected the response to treatment, especially in the LS group, as well as the variation of *SLC6A4* promoter polymorphism. This study may lead to a more profound understanding of drug therapy and to a careful application of pharmacogenetics in psychiatry.

Patient informed consent

There is no need for patient informed consent.

Ethics committee approval

The ethics committee approval has been obtained on June 5, 2017 (ethics committee decision number: B.08.6.Y OK.2.US.0.05.0.06/2017/154) for the blood sampling part of the work.

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Conflicts of interest

There is no conflict of interest to declare.

Author contribution subject and rate

Fadime Canbolat (40%): Design the research, sample analyses, data statistically analysis and wrote the whole manuscript.

Dilek Meltem Tasdemir (20%): Sample collection and data interpretation

Canan Sercan Dogan (15%): Sample analyses

Alper Evrensel (10%): Sample collection and data interpretation

Korkut Ulucan (%5): Sample analyses

Ahmet Aydın (%5): Contributed with comments on research design and slides interpretation.

K. Nevzat Tarhan (%5): Supervised the article write-up.

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