Current perspectives on the role of oxytocin receptor (OXTR) gene variants in panic disorder: Associations with the disease liability and separation anxiety

Keywords
separation anxiety, Panic disorder, oxytocin receptor gene, OXTR rs53576, OXTR rs237902, OXTR rs2254298

Abstract

Introduction
Oxytocin receptor (OXTR) gene variations are associated with empathy, trust, emotional stability, stress reactivity, social bonding and attachment behaviors. We aimed to explore the impact of three OXTR gene variations (rs53576, rs237902, rs2254298) in susceptibility to panic disorder (PD). We also investigated the possible effects of these variants with separation anxiety scales in the patients with a comprehensive approach covering environmental adversity effects.

Material and methods
The hypothesis was studied in PD patients and healthy controls with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. By applying the Separation Anxiety Symptom Inventory (SASI) and the Adult Separation Anxiety Questionnaire (ASA), the relationships between the OXTR gene variants and these scales were also evaluated comprehensively.

Results
A statistically significant association was found in terms of OXTR rs237902; A allele frequency increased PD probability by 1.585-fold. Moreover, all of the analyzed OXTR variants were found to be associated with childhood and adult separation anxiety possibilities in the patients in the combined analyses of various demographic and clinical data; a striking effect of AA genotype with SASI and ASA was observed in these models.

Conclusions
The study proves the involvement of oxytocinergic gene variants in PD and it also represents one of the most profound models in terms of gene-environment (GxE) interactions.

Current perspectives on the role of oxytocin receptor (OXTR) gene variants in panic disorder: Associations with the disease liability and separation anxiety

Abstract

Introduction: Oxytocin receptor (OXTR) gene variations are associated with empathy, trust, emotional stability, stress reactivity, social bonding and attachment behaviors. We aimed to explore the impact of three OXTR gene variations (rs53576, rs237902, rs2254298) in susceptibility to panic disorder (PD). We also investigated the possible effects of these variants with separation anxiety scales in the patients with a comprehensive approach covering environmental adversity effects. **Material and methods:** The hypothesis was studied in PD patients and healthy controls with Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. By applying the Separation Anxiety Symptom Inventory (SASI) and the Adult Separation Anxiety Questionnaire (ASA), the relationships between the OXTR gene variants and these scales were also evaluated comprehensively. **Results:** A statistically significant association was found in terms of OXTR rs237902; A allele frequency increased PD probability by 1.585-fold. Moreover, all of the analyzed OXTR variants were found to be associated with childhood and adult separation anxiety possibilities in the patients in the combined analyses of various demographic and clinical data; a striking effect of AA genotype with SASI and ASA was observed in these models. **Conclusions:** The study proves the involvement of oxytocinergic gene variants in PD and it also represents one of the most profound models in terms of gene-environment (GxE) interactions.

Key words: Panic disorder, oxytocin receptor gene, OXTR rs53576, OXTR rs237902, OXTR rs2254298, separation anxiety

Introduction

Panic disorder (PD) is an anxiety disorder characterized by spontaneous and recurrent panic attacks which display multiple physical symptoms [1]. Lifetime prevalence of PD can vary from 0.5 to 4%, with peak age of onset being 25 years. Besides, PD is more commonly encountered in some groups such as females, unemployed, divorced/separated/widowed, lower education, and low household income [2]. The treatment approach involves psychopharmacological interventions and cognitive behavioral therapy (CBT) or a combination of both [1]. Diagnostic delay may result with morbidity in psychiatric disorders [3]. A meta-analysis study reported that subjects with PD are nearly 4 times as likely to attempt suicide as compared to subjects without PD [4]. Though the studies up to now have revealed some of the genetic factors, the pathophysiology of the disorder seems to have a complicated genetic background covering the interaction of both genetic and environmental factors [5].

The evolutionary conserved neuropeptide oxytocin (OXT) is involved in many physiological and behavioral mechanisms. Besides with its well-established role in pair-bonding and reproductive behaviors, it has been shown to play significant roles in emotional stability, empathy, trust, mood, social cognition, affiliation behavior [6-8]. Moreover, in the last few years, it also drew attention as a cardiovascular hormone [9]. OXT regulates neurotransmitter and emotional mechanisms via oxytocin receptors (OXTRs) which are expressed in distinct brain regions associated with anxiety and fear such as hypothalamus, amygdala and hippocampus, and on target neuronal cells [7, 10].

The comprehensive evaluation of the role of both oxytocin and its receptor may pave the way for innovative psychiatric approaches in future. The application of

intranasal oxytocin seems to be promising in both animal [11-12] and human clinical studies in terms of anxiety and aggressive behavior management [13-14].

The ocytocin receptor gene (OXTR) is located on chromosome 3p25, consists of four exons and three introns. It encodes a 389-aa polypeptide with seven transmembrane domains belonging to the class I G protein-coupled receptor family [15]. Since the physiologic effects of oxytocin are mediated through its specific receptor (OXTR), both are involved in the regulation of behavioral patterns such as trust, empathy, altruism, affiliation behavior, stress responsiveness [7, 16]. OXTR variants were associated with outcomes at various levels in disorders, including aggression [7], depression and separation anxiety [16-17], autistic disorders [6], alcohol withdrawal symptoms [10], schizophrenia [18], post-traumatic stress disorder [19-20], social cognition in bipolar disorder type I [21].

The primary purpose of this study was to investigate the relationship between OXTR gene variants and panic disorder liability. We also evaluated Separation Anxiety Symptom Inventory (SASI) and the Adult Separation Anxiety Questionnaire (ASA) in panic disorder patients and compared them with variations of the OXTR gene as the second aim. To the best of our knowledge, this is one of the most comprehensive models conducted in panic disorder in terms of evaluating the effects of OXTR variants in terms of both the disease liability and childhood and adult separation anxiety levels.

Material and methods

Participants

In the study, 134 PD patients (Age range: 18-71 years mean: 36.34±11.53; gender: 59.0% female and 41% male) who met the criteria according to the Diagnostic and

Statistical Manual of Mental Disorders, 5th edition were recruited from Department of Psychiatry, Faculty of Medicine, Ondokuz Mayıs University, Samsun, Turkey. University staff (n=140) without personal/family history of psychiatric disorders served as controls (Mean age: 39.14±9.50; gender: 60.71% female and 39.29% male). The study was approved by the Medical Ethics Committee of Ondokuz Mayis University (OMU KAEK 2020/709) and complied with the Declaration of Helsinki.

Separation anxiety evaluations

Two validated interviewer-administered instruments were used: Separation Anxiety Symptom Inventory (SASI) [22] and Adult Separation Anxiety Questionnaire (ASA) [23]. Turkish versions of the instruments were proven to be reliable and valid [24]. The cut-off values used for SASI and ASA evaluations were 12≥ and 25≥, respectively.

Genotyping analyses

Peripheral blood samples were collected in EDTA containing tubes and DNA extractions were performed with PureLink™ Genomic DNA Mini Kit (Invitrogen-Thermo Fisher Scientific, MA, USA). Genotyping analyses were made with PCR-RFLP method previously reported in the literature for OXTR rs53576 [16], OXTR rs237902 [6] and OXTR rs2254298 [16] with modifications. PCR reaction was carried out in a 25 µl mixture containing 1x Taq buffer, 2 mM $MgCl_2$, 0.2 mM dNTP, 0.5 µM of forward and reverse primer pair, 1.5 U Taq DNA polymerase (Thermo Fisher Scientific, MA, USA) and ~200 ng of DNA sample. Thermal cycler conditions for OXTR rs53576 were as follows: 35 cycles of 40 s at 94°C, annealing for 45 s at 58°C and extension for 45 s at 72°C. A predenaturation step for 6 min at 94°C and a final extension step for 12 min

at 72°C were included. The same thermal cycler conditions were used for OXTR rs2254298 with a difference at annealing temperature (60.5ºC). Thermal cycler conditions for OXTR rs237902 consisted of a pre-denaturation step at 95ºC for 10 min, 35 cycles of denaturation at 95ºC for 45 s, annealing at 62.3ºC for 45 s and extension at 72ºC for 45 s. A final extension step at 72ºC for 12 min completed the reaction. The expected amplicon sizes of 340 bp, 527 bp and 307 bp for OXTR rs53576, OXTR rs237902 and OXTR rs2254298, respectively were confirmed. For the analysis of the OXTR rs53576 genotypes, amplified fragments were digested with BamHI restriction enzyme (Thermo Fisher Scientific, MA, USA) at 37ºC for 2 h and were separated by electrophoresis. The unrestricted PCR product (GG genotype) had a size of 340 bp, complete restriction (AA genotype) produced bands of 120 bp and 220 bp and heterozygotes had all the bands. For the analysis of the OXTR rs237902 genotypes, amplicons were digested with Tsp509l restriction enzyme (Thermo Fisher Scientific, MA, USA) at 65ºC for 2 h. The complete restriction (AA genotype) resulted with the production of 321 bp and 206 bp fragments while the GG genotype remained unrestricted (527 bp). For the analysis of OXTR rs2254298, amplicons were digested with Bsrl restriction enzyme (Thermo Fisher Scientific, MA, USA) at 65°C for 2 h. The expected fragment sizes for the analyzed genotypes were: GG: 163+101+34+9 bp and AA: 163+135+9 bp. A minor set of all the samples (20%) were randomly re-genotyped for the analyzed variants to assure genotyping success and the results were found concordant.

Statistical analyses

In order to test the sample size with power analysis, Python programming language and its statsmodels module were used. Python language was chosen because of its high scientific property, open source code and very good 3rd party module support [25]. Statsmodels module was preferred to be able to perform power analysis and determine the minimum limit of sample size. This module is known as the gold standard for making statistical calculations safely [26]. All necessary visualizations were made with matplotlib module [27]. For sample size determination, effect size was chosen as 0.25 and it indicates a small-medium effect size. According to Cohen's guidelines, this size is often observed in fields such as education, psychology and social sciences. α=0.05 was chosen as the classical significance level, which accepts a Type I error risk of 5% in hypothesis testing. The target power was determined as 80% in this study. This ensures that when a real effect exists, the probability of correctly detecting this effect is at least 80% [28]. In the light of this data, necessary calculations were made with the Python script developed, and the sample size was found to be at least 128, which was interpreted as samples of 128 and above supporting the purpose. The sample size power interaction graph was created with the values given below. At this point, the power target of 80% is achieved with a sample size of n = 128.

R statistical software (Version 4.2.2) was used. Using the HWE.chisq subroutine of the genetics package in R, each of the variants was found compatible with Hardy-Weinberg equilibrium (HWE). Pearson Chi-square test with the Yates' continuity correction and Fisher's exact test were used to evaluate the associations of the OXTR genotypes/alleles with PD. Statistical significance level was defined as $p<0.05$. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to interpret the findings.

Using simple Bayesian logistic regression models on SASI and ASA, we investigated the association between the genotypes of OXTR variants and separation anxiety.

Results

Panic disorder data

Nocturnal panic attacks were observed in 41.8% of the patients. Triggering life event was present in 66.5% (death of a close person: 23.60%, life-threatening event: 38.20%, psychosocial stress factor: 38.20%) of the patients. Object carrying was present in 45.5%. Nearly half of the patients had family history of psychiatric disease (panic disorder: 33.6%, others: 12.7%). Mean age of disease onset was 27.49±10.38. Mean age of treatment onset was 30.25±10.38.

Case-control study: Relationship between OXTR genotypes and panic disorder

The AA genotype and (A allele) were taken as the reference categories. Based on the results of Pearson Chi-Square test with the Yates' continuity correction, no significant relationship was detected between the genotypes and panic disorder. For OXTR rs237902, the allele frequency appeared to have a significant impact on panic disorder (χ^2 = 6.328, p = 0.012). When compared to the G allele frequency, the A allele frequency increases the probability of panic disorder by 1.585 times (Figure 1 (A), Table 1).

Based on the Fisher's exact test results, no significant relationship was observed between the levels of the categorical variables and OXTR gene variants (p > 0.05). The analysis of variance (ANOVA) results indicate a difference between the OXTR rs237902 genotypes in terms of the average age of disease onset (F = 3.218, p = 0.043)

(Figure 1 (B)). The corrected p-values of the Tukey multiple mean comparison tests after ANOVA indicated that the patients with the GG genotype have a higher mean age of disease onset when compared to the patients with the AA genotype (μ_{GG} - μ_{AA} = 7.414, p_{adj} = 0.046).

Bayesian logistic regression: The relationship of the OXTR gene variants with SASI and ASA

We examined the associations between OXTR rs237902, OXTR rs53576, and OXTR rs2254298 and the childhood & adulthood separation anxieties with respect to each demographic and clinical variable in the data, separately. In each Bayesian analysis, we first obtained posterior samples, and then, odds ratios and 90% credible intervals.

By examining the investigated genotypes in terms of the probability of separation anxiety using the SASI and ASA inventory results, we observed significant differences between the patients with the AA genotype and those with the AG or GG genotype. Strikingly, the patients with the AA genotype were found to be more likely to have the childhood and adult separation anxiety than other genotypes in the presence of several entities (Table 2 and Table 3).

OXTR gene variants and SASI

When considering the medium or high socioeconomic status and the OXTR rs237902 gene variant together, the patients with the AA genotype are OR = 1/0.335 = 2.99 times more likely to have the childhood separation anxiety than the patients with the AG genotype. For this gene variant, we observed that the patients with the nocturnal

panic and the AA genotype are OR = 1/0.159 = 6.29 times more likely to have the childhood separation anxiety than the patients in the same group with the AG genotype (Table 2, Colon 2).

For OXTR rs53576 and the married patients, the probability of having the childhood separation anxiety is OR = 4.97 times higher for the GG genotype when compared to the AA genotype. For OXTR rs53576, we observed that the single patients with the AA genotype are OR = 1/0.033 = 30.30 and OR = 1/0.027 = 37.04 times more likely to experience the childhood separation anxiety when compared to the single patients with the AG and GG genotypes, respectively. For the same gene variant, we observed that the probability of having the childhood separation anxiety in the unemployed patients with the AA genotype is OR = 1/0.046 = 21.74 and OR = 1/0.040 = 25 times higher than those with the AG and GG genotypes, respectively. In the patients without the anxiety triggering life event, this probability is OR = 1/0.042 = 23.81 and OR = 1/0.034 = 29.41 times higher for those with the AA genotype when compared to those with the AG and GG genotypes, respectively (Table 2, Colon 3).

For OXTR rs2254298, in the case of a family history of psychiatric disease caused by panic disorder, the probability of childhood separation anxiety for the patients with the AA genotype is OR = 1/0.010 = 100 times more likely than those with the AG genotype (Table 2, Colon 4).

OXTR gene variants and ASA

By examining OXTR rs237902 in terms of the probability of adulthood separation anxiety using the ASA inventory results, we observed significant differences between the patients with the AA genotype and those with the AG or GG genotype. In some

cases, the patients with the AA genotype were found to be more likely to have the adult separation anxiety than those with the AG genotype. The corresponding odds ratios were as follows: OR = 1/0.027 = 37.04 in the female patients, OR = 1/0.026 = 38.46 in the unemployed patients, OR = 1/0.257 = 3.89 in the patients with the medium or high socioeconomic status, OR = 1/0.027 = 37.04 in the patients with the nocturnal panic, OR = 1/0.190 = 5.26 in the patients with the anxiety triggering life event, and OR = 1/0.026 = 38.46 in the patients with the object carrying behavior. Similarly, the odds ratios indicating that the patients with the AA genotype are more likely to have the adulthood separation anxiety than those with the GG genotype are as follows: OR = 1/0.046 = 21.74 in the female patients, OR = 1/0.022 =45.45 in the unemployed patients, OR = 1/0.040 = 25 in the patients with the nocturnal panic, and OR = 1/0.060 = 16.67 in the patients with the object carrying behavior (Table 3, Colon 2).

For OXTR rs53576, the probability of having adulthood separation anxiety was found to be higher in some cases for the patients with the AA genotype when compared to those with the AG genotype. The significant odds ratios are as follows: OR =1/0.055 = 18.18 in the single patients, OR = 1/0.032 = 31.25 in the patients with the nocturnal panic, and OR = 1/0.052 = 19.23 in the patients without the anxiety triggering life event. Similarly, this probability was found to be higher in some groups for the patients with the AA genotype when compared to those with the GG genotype. The significant odds ratios are as follows: OR = 1/0.042 = 23.81 in the single patients, OR = 1/0.043 = 23.26 in the unemployed patients, OR = 1/0.058 = 17.24 in the patients with the nocturnal panic, and OR = 1/0.057 = 17.54 in the patients without the anxiety triggering life event (Table 3, Colon 3).

For OXTR rs2254298 and the patients without the anxiety triggering life event, the probability of having adulthood separation anxiety is OR = 1/0.038 = 26.32 times higher when comparing the AA genotype against the AG genotype. For the same gene variant and the patients with the family history of psychiatric disease caused by panic disorder, the probability of having adulthood separation anxiety is OR = 1/0.030 = 33.33 times higher for the AA genotype compared to the GG genotype (Table 3, Colon 4).

Discussion

OXTR variations have been associated with behavioral patterns and predisposition to psychiatric diseases. To the best of our knowledge, there has been only one study which evaluated the effects of OXTR variants in panic disorder. In this study, the possible relationships between two OXTR variants (rs2254298 and rs53576) and panic disorder, social anxiety disorder and major depressive disorder were investigated in Japanese participants. G allele of OXTR could render the people more susceptible to panic and major depressive disorder [29]. Though we did not find a statistically significant association for rs2254298 and rs53576, OXTR rs237902 analysis revealed a significant association partially contrary to Onodera et al. [29]. A allele frequency resulted with a 1.585-fold increased probability for panic disorder.

Most of the studies have focused on SASI and only so few covered ASA. We evaluated the possible associations of the genotypes with SASI and ASA in a combined approach model covering several demographic and clinical data (Table 2 and Table 3). Genetic liability to complex traits could be better analyzed in the context of environmental and social factors, which are markedly linked to genetic liability. All of the analyzed variants displayed significant associations with several traits. The

combinations of AA genotype of the analyzed variants + several other risk factors (Table 2 and Table 3) were associated with SASI & ASA possibilities. Although the studied models are not the same, our results seem partly discordant with the studies of Costa et al. [17], Schiele et al. [30] and Costa et al. [16] since the GG genotype effect was more prominent in these studies. On the other hand, the potential detrimental effect of the rs2254298 A allele in the presence of an environmental factor as in the case of a childhood emotional neglect [31] or a childhood maltreatment [20] seems to be supported by our results. Nocturnal panic attacks which increase the severity of the disease were reported in half of the panic disorder patients [32]. Compatible with more detrimental potential of AA genotype, OXTR rs237902 AA genotype in panic disorder patients with nocturnal panic attacks was found to be associated with SASI (Table 2). Both OXTR rs237902 AA and OXTR rs53576 AA genotypes in panic disorder patients with nocturnal panic attacks were found to be associated with ASA (Table 3). It seems like that AA genotypes of the OXTR gene in the presence of nocturnal panic attacks worsen the course of the disease. The only exception referring to the effect of the GG genotype in our study was the combined analysis of OXTR rs53576 with marital status (Table 2). While the probability of childhood separation anxiety for the married people with the GG genotype was 4.97-fold compared with the AA genotype group, single patients with the AA genotype had 30.30-fold and 37.07-fold increased risks compared with the AG and GG genotypes, respectively. The GG genotype of married people seems to reflect a lower childhood separation anxiety possibility. This finding indirectly seems to support the results of Monin et al. [8] who drew attention to the association between GG genotype (self or partner effect) and marital satisfaction.

In conclusion, our study has many powerful dimensions excluding a relatively considerable limitation factor. Limitation of our study design was the analyses of three OXTR variants; enriched studies with much more variants would be so desirable. Though this limitation, our study analyzed the effects of OXTR variants and presented one of the most comprehensive models in panic disorder in terms of gene-environment (GxE) interactions. The enrichments of our findings would better enlighten the genetic underpinnings of PD and provide early intervention implications for clinicians to identify at-risk clinical subgroups. Since the defects of the oxytocin system are reported in various psychiatric disease models, intranasal oxytocin use for therapeutic purposes might be on agenda as an innovative approach in future.

References

1. Kyriakoulis P, Kyrios M. Biological and cognitive theories explaining panic disorder: A narrative review. Front Psychiatry 2023; 14:957515.
2. Manjunatha N, Ram D. Panic disorder in general medical practice- A narrative review. J Family Med Prim Care 2022;11(3):861-869.
3. Saunders EFH, Mukherjee D, Waschbusch DA, et al. Predictors of diagnostic delay: Assessment of psychiatric disorders in the clinic. Depress Anxiety 2021;38(5):545-553.
4. Tietbohl-Santos B, Chiamenti P, Librenza-Garcia D, et al. Risk factors for suicidality in patients with panic disorder: A systematic review and meta-analysis. Neurosci Biobehav Rev 2019; 105:34-38.
5. Schumacher J, Kristensen AS, Wendland JR, et al. The genetics of panic disorder. Journal of Medical Genetics 2011; 48:361-368.

6. Wermter AK, Kamp-Becker I, Hesse P, Schulte-Körne G, Strauch K, Remschmidt H. Evidence for the involvement of genetic variation in the oxytocin receptor gene (OXTR) in the etiology of autistic disorders on high-functioning level. Am J Med Genet B Neuropsychiatr Genet 2010;153B (2):629-639.
7. Shao D, Zhang HH, Long ZT, et al. Effect of the interaction between oxytocin receptor gene polymorphism (rs53576) and stressful life events on aggression in Chinese Han adolescents. Psychoneuroendocrinology 2018; 96:35-41.
8. Monin JK, Goktas SO, Kershaw T, DeWan A. Associations between spouses' oxytocin receptor gene polymorphism, attachment security, and marital satisfaction. PLoS One 2019;14(2): e0213083.
9. Wsol A, Gondek A, Podobinska M, et al. Increased oxytocinergic system activity in the cardiac muscle in spontaneously hypertensive SHR rats. Arch Med Sci 2019;18(4):1088-1094.
10. Shen G, Yang S, Wu L, et al. The oxytocin receptor rs2254298 polymorphism and alcohol withdrawal symptoms: a gene-environment interaction in mood disorders. Front Psychiatry 2023; 14:1085429.
11. Calcagnoli F, de Boer SF, Althaus M, et al. Antiaggressive activity of central oxytocin in male rats. Psychopharmacology (Berl) 2013;229(4):639-51.
12. Joushi S, Esmaeilpour K, Masoumi-Ardakani Y, et al. Intranasal oxytocin administration facilitates the induction of long-term potentiation and promotes cognitive performance of maternally separated rats. Psychoneuroendocrinology 2021; 123:105044.

13. Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry 2003;54(12):1389-98.
14. Berends YR, Tulen JHM, Wierdsma AI, et al. Intranasal administration of oxytocin decreases task-related aggressive responses in healthy young males. Psychoneuroendocrinology 2019; 106:147-154.
15. Chen FS, Kumsta R, von Dawans B, et al. Common oxytocin receptor gene (OXTR) polymorphism and social support interact to reduce stress in humans. Proc Natl Acad Sci U S A 2011;108(50):19937-42.
16. Costa B, Pini S, Gabelloni P, et al. Oxytocin receptor polymorphisms and adult attachment style in patients with depression. Psychoneuroendocrinology 2009;34(10):1506-14.
17. Costa B, Pini S, Baldwin DS, et al. Oxytocin receptor and G-protein polymorphisms in patients with depression and separation anxiety. J Affect Disord 2017; 218:365-373.
18. Nakata Y, Kanahara N, Kimura A, et al. Oxytocin system dysfunction in patients with treatment-resistant schizophrenia: Alterations of blood oxytocin levels and effect of a genetic variant of OXTR. J Psychiatr Res 2021; 138:219-227.
19. Cao C, Wang L, Wu J, et al. Association between the OXTR rs53576 genotype and latent profiles of post-traumatic stress disorder and depression symptoms in a representative sample of earthquake survivors. Anxiety Stress Coping 2020;33(2):140-147.

20. Lee H, King AP, Li Y, Seng JS. Oxytocin receptor gene, post-traumatic stress disorder and dissociation in a community sample of European American women. BJPsych Open 2022;8(4): e104.
21. Ríos U, Morán J, Hermosilla J, et al. The interaction of the oxytocin receptor gene and child abuse subtypes on social cognition in euthymic patients with bipolar disorder type I. Front Psychiatry 2023; 14:1151397.
22. Silove D, Manicavasagar V, O'Connell D, Blaszczynski A, Wagner R, Henry J. The development of the Separation Anxiety Symptom Inventory (SASI). Aust N Z J Psychiatry 1993;27(3):477-88.
23. Manicavasagar V, Silove D, Wagner R, Drobny J. A self-report questionnaire for measuring separation anxiety in adulthood. Compr Psychiatry 2003;44(2):146-53.
24. Diriöz M, Alkın T, Yemez B, Onur E, Eminağaoğlu N. Ayrılma Anksiyetesi Belirti Envanteri İle Yetişkin Ayrılma Anksiyetesi Anketinin Türkçe Versiyonunun Geçerlik ve Güvenirliği. Türk Psikiyatri Dergisi 2011: 22.
25. "Welcome to Python.org", Python.org. Accession: 15 January 2025. [Online]. Access adress: https://www.python.org/
26. Seabold S, Perktold J. Statsmodels: Econometric and statistical modeling with python. 9th Python in Science Conference; 2010. [Online].
27. Hunter JD. Matplotlib: A 2D Graphics Environment. Comput Sci Eng 2007;9(3): 90-95.
28. Arslan A, Zenker F. Cohen's convention, the seriousness of errors, and the body of knowledge in behavioral science. Synthese 2024; 204:163.

29. Onodera M, Ishitobi Y, Tanaka Y, et al. Genetic association of the oxytocin receptor genes with panic, major depressive disorder, and social anxiety disorder. Psychiatr Genet 2015;25(5):212.
30. Schiele MA, Costa B, Abelli M, et al. Oxytocin receptor gene variation, behavioural inhibition, and adult separation anxiety: Role in complicated grief. World J Biol Psychiatry 2018;19(6):471-479.
31. Womersley JS, Hemmings SMJ, Ziegler C, et al. Childhood emotional neglect and oxytocin receptor variants: Association with limbic brain volumes. World J Biol Psychiatry 2020;21(7):513-528.
32. Sarísoy G, Böke O, Arík AC, Sahin AR. Panic disorder with nocturnal panic attacks: symptoms and comorbidities. Eur Psychiatry 2008;23(3):195-200.

403 **Table 1. Genotype and allele comparisons for the healthy controls and panic disorder patients**

Genotype	Controls		Cases		χ^2	P value	OR (95% CI)
OXTR rs237902	n	%	n	%			
AA	16	11.43	16	11.95			
AG	58	41.43	83	61.95	0.514	0.473	1.428 [0.614 3.326]
GG	66	47.14	35	26.10	1.815	0.178	0.533 [0.220 1.288]
A allele frequency	90	32.14	115	42.91			
G allele frequency	190	67.86	153	57.09	**6.328**	**0.012**	**0.631 [0.438 0.906]**
OXTR rs53576	n	%	n	%			
AA	21	15.00	12	8.96			
AG	62	44.29	64	47.76	1.642	0.200	1.800 [0.769 4.381]
GG	57	40.71	58	43.28	1.511	0.219	1.774 [0.751 4.352]
A allele frequency	104	37.14	88	32.84			
G allele frequency	176	62.86	180	67.16	0.935	0.334	1.208 [0.837 1.746]
OXTR rs2254298	n	%	n	%			
AA	4	2.86	7	5.23			
AG	46	32.86	42	31.34	0.456	0.500	0.525 [0.105 2.241]
GG	90	64.28	85	63.43	0.434	0.510	0.541 [0.112 2.220]
A allele frequency	54	19.29	56	20.90			
G allele frequency	226	80.71	212	79.10	0.132	0.716	0.905 [0.582 1.405]

404

405

406

407

408

409

410

411

412

413

414

415

416

417

418

419

420

421

422

423

Table 2. Relationships between the OXTR gene variants and SASI

	OXTR rs237902		OXTR rs53576		OXTR rs2254298	
	OR	**90% CI**	**OR**	**90% CI**	**OR**	**90% CI**
Marital status (Married)						
AA vs AG	0.431	[0.078 1.733]	1.455	[0.326 7.020]	1.946	[0.269 21.291]
AA vs GG	0.436	[0.076 1.987]	**4.972**	[1.179 24.856]	2.104	[0.293 22.165]
Marital status (Single)						
AA vs AG	0.511	[0.138 1.765]	**0.033**	[0.001 0.454]	0.177	[0.016 1.392]
AA vs GG	0.562	[0.125 2.439]	**0.027**	[0.001 0.380]	0.532	[0.053 3.425]
Employment status (Unemployed)						
AA vs AG	0.225	[0.022 1.371]	**0.046**	[0.001 0.782]	10.114	[0.260 917.36]
AA vs GG	0.302	[0.024 2.655]	**0.040**	[0.001 0.643]	15.790	[0.466 1415.59]
Socioeconomic status (Medium/High)						
AA vs AG	**0.335**	[0.110 0.895]	0.529	[0.170 1.633]	0.400	[0.088 1.679]
AA vs GG	0.356	[0.110 1.054]	1.363	[0.418 4.206]	0.906	[0.230 3.609]
Nocturnal Panic (Yes)						
AA vs AG	**0.159**	[0.016 0.818]	0.160	[0.017 1.014]	0.083	[0.001 3.985]
AA vs GG	0.196	[0.018 1.272]	0.454	[0.050 2.748]	0.080	[0.001 3.515]
Triggering life event (No)						
AA vs AG	0.180	[0.018 1.026]	**0.042**	[0.001 0.747]	0.797	[0.048 12.959]
AA vs GG	0.274	[0.024 1.933]	**0.034**	[0.001 0.588]	1.087	[0.071 15.307]
Family history of psychiatric illness (Panic disorder)						
AA vs AG	0.356	[0.071 1.477]	0.873	[0.060 12.492]	**0.010**	[0.001 0.313]
AA vs GG	0.452	[0.072 2.491]	1.510	[0.109 21.548]	0.076	[0.001 1.839]

440 **Table 3. Relationships between the OXTR gene variants and ASA**

441

	OXTR rs237902		OXTR rs53576		OXTR rs2254298	
	OR	**90% CI**	**OR**	**90% CI**	**OR**	**90% CI**
Gender (Female)						
AA vs AG	**0.027**	[0.001 0.456]	0.062	[0.001 1.085]	1.189	[0.199 5.789]
AA vs GG	**0.046**	[0.001 0.763]	0.001	[0.001 1.244]	3.952	[0.704 19.508]
Marital status (Single)						
AA vs AG	0.232	[0.025 1.222]	**0.055**	[0.001 0.952]	0.339	[0.027 2.521]
AA vs GG	0.315	[0.027 2.319]	**0.042**	[0.001 0.726]	1.391	[0.115 9.429]
Employment status (Unemployed)						
AA vs AG	**0.026**	[0.001 0.437]	0.104	[0.002 2.032]	0.080	[0.001 2.623]
AA vs GG	**0.022**	[0.001 0.531]	**0.043**	[0.001 0.809]	0.275	[0.003 9.406]
Socioeconomic status (Medium/High)						
AA vs AG	**0.257**	[0.048 0.833]	0.368	[0.073 1.379]	0.599	[0.116 2.599]
AA vs GG	0.444	[0.079 1.620]	0.478	[0.096 1.826]	0.950	[0.192 4.025]
Nocturnal Panic (Yes)						
AA vs AG	**0.027**	[0.001 0.379]	**0.032**	[0.001 0.483]	0.053	[0.001 3.122]
AA vs GG	**0.040**	[0.001 0.663]	**0.058**	[0.001 0.922]	0.169	[0.001 9.570]
Triggering life event (No)						
AA vs AG	0.385	[0.034 2.249]	**0.052**	[0.001 0.918]	**0.038**	[0.001 0.933]
AA vs GG	0.283	[0.022 2.054]	**0.057**	[0.001 0.927]	0.090	[0.001 2.167]
Triggering life event (Yes)						
AA vs AG	**0.190**	[0.022 0.957]	0.649	[0.130 2.554]	1.318	[0.198 7.099]
AA vs GG	0.362	[0.038 2.077]	0.854	[0.164 3.423]	2.125	[0.357 10.926]
Object carrying (Yes)						
AA vs AG	**0.026**	[0.001 0.316]	0.468	[0.047 2.852]	2.360	[0.364 16.567]
AA vs GG	**0.060**	[0.001 0.858]	0.699	[0.074 4.338]	4.958	[0.750 32.921]
Family history of psychiatric illness (Panic disorder)						
AA vs AG	0.251	[0.030 1.287]	0.081	[0.001 1.969]	**0.030**	[0.001 0.915]
AA vs GG	0.424	[0.041 3.117]	0.076	[0.001 1.967]	0.085	[0.001 2.333]

442

443

444

445

446

447

448

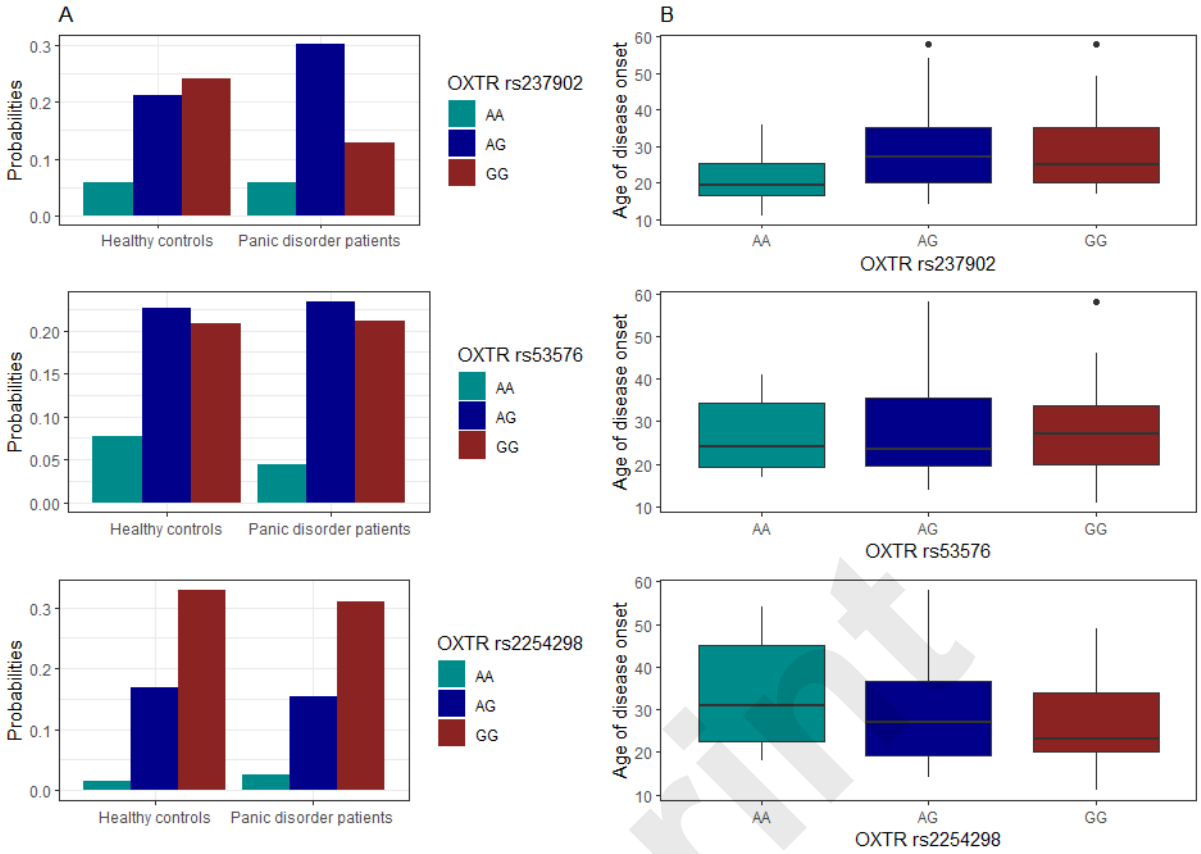
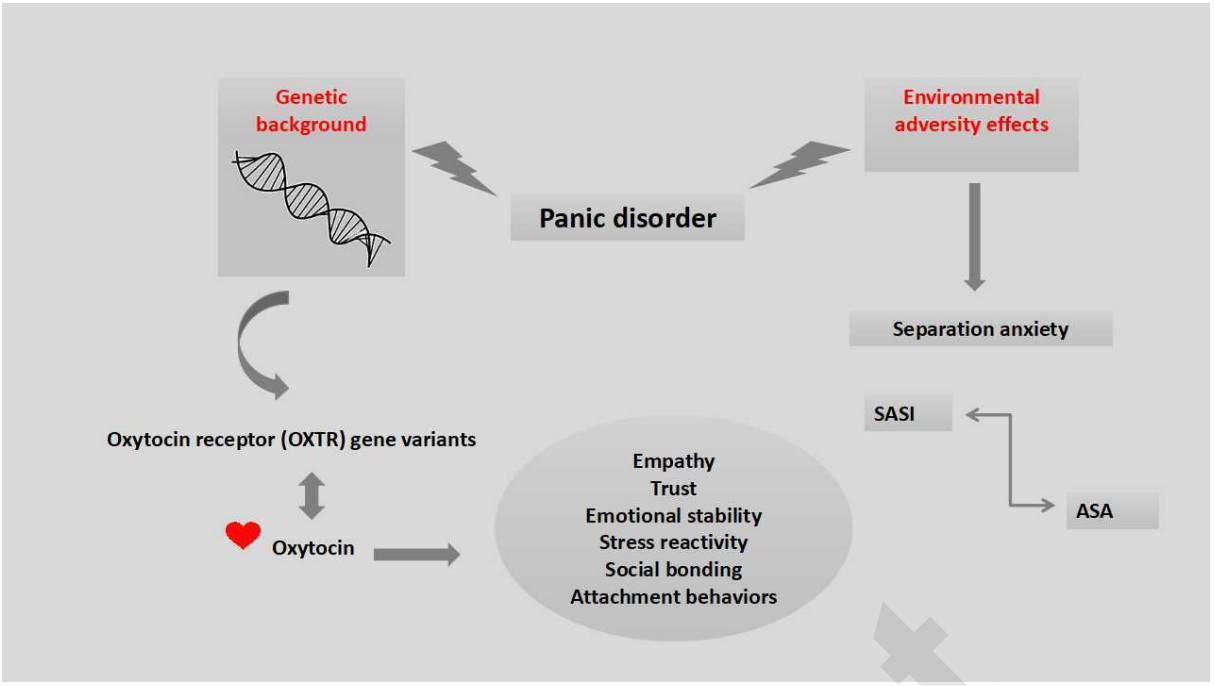


Figure 1. (A) The box plots comparing the probabilities of observing healthy controls and panic disorder patients with respect to the OXTR genotypes. **(B)** The ANOVA plots comparing the mean age of disease onset with respect to the genotypes of each OXTR gene variant.


Genetic background
Environmental adversity effects
Panic disorder
Separation anxiety
SASI
ASA
Oxytocin receptor (OXTR) gene variants
Oxytocin
Empathy
Trust
Emotional stability
Stress reactivity
Social bonding
Attachment behaviors