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Original Research

Investigation of polymorphic variants of SLC6A4, TPH-1, and TPH-2 genes in cases of completed suicide

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Abstract

Background and objective: Serotonin plays an important role in the pathophysiology of aggressive behavior. Low serotonin levels and altered functions of serotonin receptors affect suicidal behavior. TPH-1, TPH-2 and SLC6A4 genes have important roles in serotonin production and degradation pathways. In this study, polymorphic variants of the TPH-1, TPH-2 and SLC6A4 genes, rs1800532, rs7305115, rs6355 and rs1386494, were investigated in 100 completed suicides and 100 healthy individuals.

Materials and methods: After DNA isolation, a new polymerase chain reaction technology KASP TM (Competitive allele-specific polymerase chain reaction method), was used in the genotyping of the selected gene variants.

Results: No statistically significant difference was found for the genotype and individual allele frequencies of the polymorphic variants between suicide and control groups, as well as between men and women in suicide cases. However, we observed non-significant tendencies of increased minor alleles in suicidal men for all TPH SNPs.

Conclusions: More molecular genetic research studies are needed to understand the pathophysiology of suicide and to reveal its relationship with gender.

Keywords

Suicide; Polymorphism; KASP; TPH-1; TPH-2; SLC6A4

1. Introduction

Suicide, an intentional self-harming behavior, is a major health problem around the world. According to the World Health Organization, nearly 800,000 individuals die each year due to suicide [1]. According to data from the Turkish Statistical Institute (TURKSTAT), there were 3406 suicide-related deaths in 2019 in Turkey, of which 2626 (77.1%) were men and 780 (22.9%) were women [2]. Among those women who committed suicide, a majority (15.8%) were aged 15–19 years. Similarly, among the men who committed suicide, a majority (11.6%) were aged 20–24 years [2]. The number of suicides may be higher than reported because some motor vehicle accidents may be used a method for committing suicide [3]. In traffic accidents in south-east Scotland between 1993 and 2003, 17 of 597 traffic deaths appeared to be suicides [3, 4].

Many studies have been conducted on the causes of suicide

[5–11]. These studies have typically investigated the psychiatric, sociological, economic, and biological components of suicidal behavior. There are also studies on the genetic component of suicidal behavior [12–17]. Monozygotic and dizygotic twins were investigated in the initial studies on the genetic component of this behavior [18]. It has been shown that monozygotic twins are more likely to exhibit suicidal behavior than dizygotic twins [18–20]. Additionally, family studies and studies performed on adopted individuals [21–25], have shed light on molecular etiologies for suicide behavior [12–17].

The serotonergic system is linked to decision making in patients with altered behavior. Low serotonin input to the anterior cingulate and ventromedial prefrontal cortex is linked to suicide behaviors [26]. Serotonergic system genes with neurobiological effects have been investigated in studies involving suicide behavior [27–32]. Polymorphisms in genes may play a role in suicidal behavior [29, 33, 34]. These polymorphisms are usually single nucleotide polymorphisms (SNP), which are single nucleotide changes in specific regions of the genome [34–36]. Serotonin transporter (SLC6A4) gene is an important regulator of serotonin reuptake and signaling. The process after serotonin is released into the synaptic gaps is regulated by SLC6A4, which helps reuptake serotonin from nerve endings and platelets. SLC6A4 is located on chromosome 17, 17q11.1–q12 [37]. Polymorphisms in SLC6A4 that have been considered as a risk factor for suicidal behavior are 5HTTLPR, S-Tin2, rs25531, and rs6355 [38–41]. In rhesus macaques, the short allele ("s") of 5HTTLPR is associated with decreased serotonergic function among monkeys reared in stressful conditions [42, 43]. The low frequency ss genotype of the 5HTTLPR/rs25531 polymorphism increased the risk of suicidal behavior in men using citalopram [44]. In another study, SLC6A4 polymorphism was found to have a stress-reducing effect on suicidal ideation which varies between men and women [45].

The tryptophan hydroxylase (TPH) enzyme, which is a ratio and rate limiting enzyme in serotonin biosynthesis, also determines the level of serotonin in the synaptic gap [35, 46, 47]. TPH has the following two isoforms: TPH-1 and TPH-2. These two forms are located on different chromosomes. The TPH-1 gene is located on chromosome 11 and has the following two polymorphisms on the 7th intron showing A/C (adenine/cytosine) changes: A779C (rs1799913) and A218C (rs1800532). It is believed that the TPH-1 gene rs1800532 polymorphism may be a risk factor for suicidal behavior [48]. In a meta-analysis, the association of THP-1 and TPH-2 gene polymorphisms with suicidal behavioral was explored and TPH-1 gene variants showed a significantly positive association with suicide behavioral [49].

The TPH-2 gene is located on chromosome 12 (12q15) and contains 11 exons [35]. Approximately 500 SNPs have been detected in the TPH-2 gene, and they are typically located in the non-coding regions of the gene [50]. Although all of these point polymorphisms on the TPH-2 gene have not been fully investigated, a study has reported that they play a role in psychiatric diseases [51]. The rs7305115 polymorphism G allele, located on the 7th exon in the TPH-2 gene, has been shown to be a risk factor in patients with severe depression [52]. Suicidal behavior has been associated with the A allele and AA genotype of the rs7305115 polymorphism in the Mexican population [52].

Another polymorphism of the TPH-2 gene is the rs1386494 polymorphism. This intronic polymorphism has been found to be a risk factor in patients with severe depression [53, 54]. It has been reported that the frequency of the rs1386494 polymorphism G allele increased in the suicide group [55].

In this study, the polymorphic variants rs1800532, rs7305115, rs6355, and rs1386494 of TPH-2, SLC6A4, and TPH-1 genes were investigated in women and men subjects in cases of completed suicide compared to a control, non-suicidal group.

2. Materials and methods

2.1 Case selection and sampling

Ethics Committee approval was obtained from the Ankara University Faculty of Medicine for this study (Decision No: 09-387-15, May 25, 2015) and approved by the Ministry of Justice (Decision No: 21589509/694, Aug 6, 2015). The study included 100 suicide cases (85 men, 15 women) who had routinely been autopsied in the Ankara Group Presidency of the Forensic Medicine Institute, and 100 healthy donors (88 men, 12 women) who had donated blood at Ankara University Faculty of Medicine Serpil Akdağ Blood Center. Blood samples were collected after obtaining consent for the study. Inclusion criteria included the absence of a history of psychiatric illnesses other than depression, absence of schizophrenia, bipolar mood disorder, mental retardation, chronic illness, substance and alcohol addiction, and subjects who were 18–65 years. Blood samples (2 mL) from suicide and healthy cases were collected in EDTA tubes and stored at –20 °C until the DNA isolation stage.

TABLE 1. Genes and polymorphisms included in the study.

Gene	SNP rs#	Chromosome	SNP-Ensembl database information
SLC6A4	rs6355	17 (17q11.1–q12)	Missense, C/G [a]MAF: 0.01
TPH-1	rs1800532	11 (11q15.3–p14)	Intron variant, A/C [a]MAF: 0.36
TPH-2	rs7305115	12 (12q15)	Synonymous variant, A/G [a]MAF: 0,47
TPH-2	rs1386494	12 (12q15)	Intron variant, A/G [a]MAF: 0.15

[a]MAF (Minor allele frequency): The frequency of the rarest allele in a population.

2.2 SNP selection and DNA isolation

A literature review was conducted on studies involving suicidal behavior, from which 4 studies of genes and polymorphisms that could be considered candidates were selected

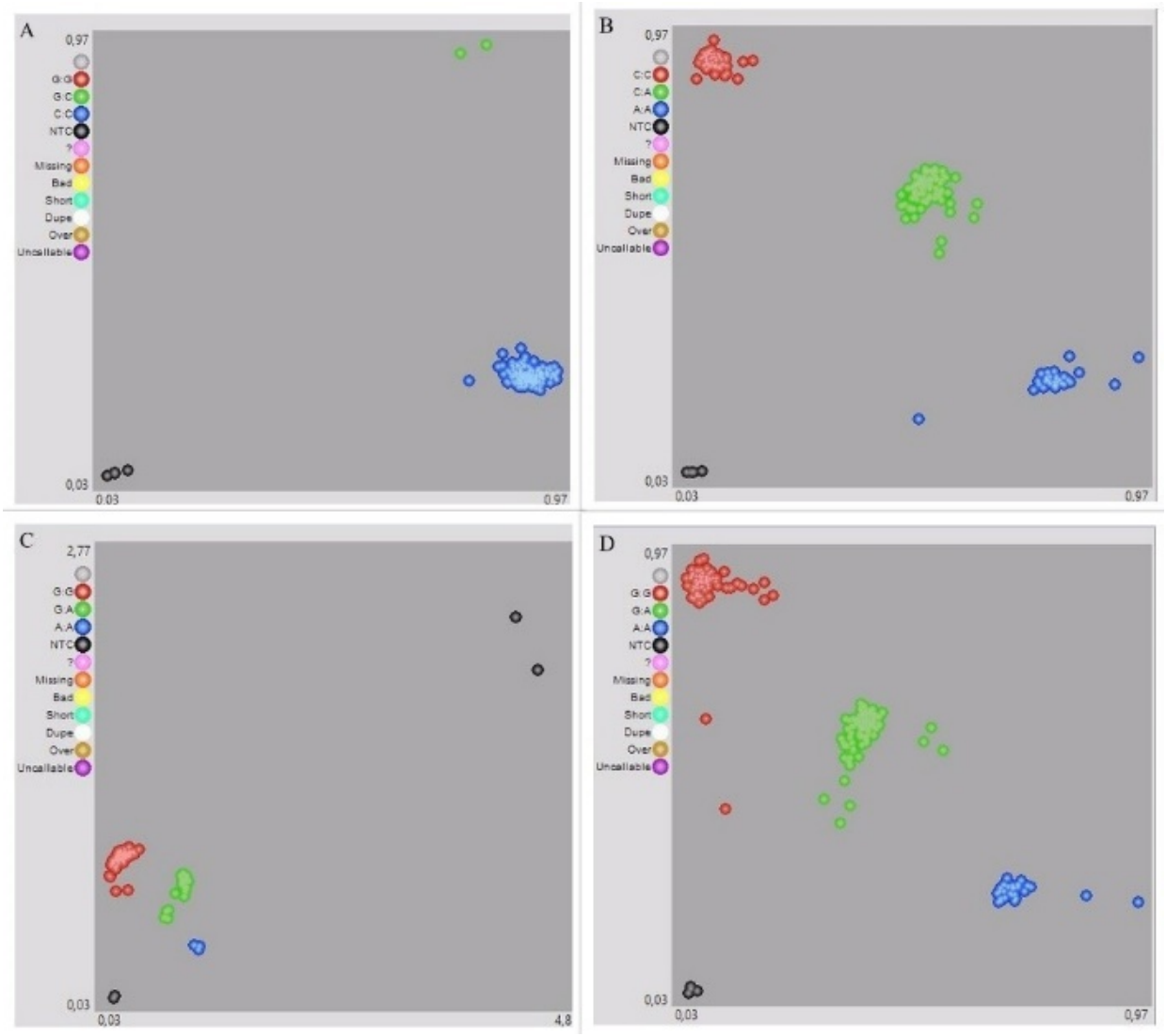

FIG. 1. Representative KASP - Cycle analysis photos for (A) rs6355, (B) rs1800532, (C) rs1386494, (D) rs7305115 SNPs.

(Table 1). The blood samples stored in EDTA tubes during routine autopsy in suicide cases and during blood donation in the control group were subjected to DNA isolation. Nucleospin Genomic DNA from Blood (Macherey-Nagel, Clontech, San Jose, CA, USA) kits were used for DNA isolation. Kit protocol standards were followed. A NanodropND-1000 device was used to measure the quantity and quality of the isolated DNA. Special attention was paid that the absorbance values of the DNA samples at 260/280 nm were close to the ideal value of 1.8 nm, and that the values at 260/230 nm, another nano drop parameter indicating the purity of DNA, were within the range of 1.8–2.2 nm.

2.3 Genotyping

A competitive Allele Specific polymerase chain reaction (PCR) (KASP) method was used in the study. KASP is a fluorescence-based endpoint genotyping technology of SNPs used to identify an allele at a locus in DNA [56].

The following protocol was used for KASP trials: DNA samples were diluted to 15 ng/μL and 2.5 μL of each sample was individually added in 384-well sample plates. A negative control was added to the final wells. Before starting genotyping, the master mix was prepared using the KASP mix in the KASP kit (LGC Genomics, Bellshill, United Kingdom) and the primary mix. A master mix of 2.5 μL was added to each well. The KASP protocol was followed after transferring the samples and master mixes to the plates. According to the KASP protocol, a water bath-based hydrocycle device was used, and the protocol named Touch Down 61–55 was selected. The most important feature of this protocol is that the temperature is reduced from the 61 °C annealing phase to the final 55 °C by 0.6 °C after each cycle.

After the amplification, data analysis of the plates was performed with the Omega device that analyzes the FAM-HEX probes. During the initial analyses following amplification, additional cycles were performed, as the glows may be in pink color and scattered owing to incomplete primer binding. Following the initial analysis, 12 recycles were performed for 4 plates. These 12 recycles were performed in 4 stages, with $\times$3, $\times$6, $\times$9, and $\times$12 at each stage. TD Recycle Program was selected on the hydrocycle device while recycling. The plates prepared for each SNP selected in our study were analyzed

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TABLE 2. Comparison of the demographic characteristics of the study group.

Characteristics	Study group		Test value; p
	Suicide group (n = 100)	Control group (n = 100)	
Sex (Men/Women)	85/15	88/12	Chi square = 0.171; 0.679
Mean Age (SD)	38.8 (13.2)	35.6 (10.4)	z = 1.610; p = 0.107

with the Omega device after each recycling stage, and the most suitable cycle was subsequently selected.

2.4 Statistical analysis

Data obtained in the study group were evaluated using the IBM SPSS (version 23.0, IBM Corp., Chicago, IL, USA) software. Sociodemographic characteristics of the study group were presented with descriptive statistical data such as number, percentage, and standard deviation (SD). The normality of distribution of data that were obtained was evaluated by Shapiro–Wilk test. Chi-square (χ^2) and Mann–Whitney U analyses.

3. Results

3.1 Characteristics of the study population

In the present study, the suicide cases were aged 18–65 years, their mean age (±SD) was 38.8 (±13.2) and median age 40 years. The control group were aged 18–60 years, their mean (±SD) age was 35.6 (±10.4) and median age 34 years. There was no difference between the suicide and control groups in terms of mean age (z = 1.610; p = 0.107). 85% of the suicide cases and 88% of the control group were men, and there was no difference between the study groups in terms of sex (χ^2 = 0.171; 0.679). The comparison of the demographic characteristics of the study groups is presented in Table 2.

The most common method used in the suicide group was hanging (89%). There was no difference between men and women in terms of suicide methods (χ^2 = 7.469, p = 0.280). The comparison of sex and suicide methods is shown in Table 3.

TABLE 3. Comparison of gender and suicide methods in the study group.

Suicide methods	Sex		Test value; p
	Men	Women	
Hanging	75 (88.2)	14 (93.3)	2.240; 0.692
Firearm	5 (5.9)	0 (0.0)	
Carbon monoxide poisoning	1 (1.2)	0 (0.0)	
Sharp object injury	2 (2.4)	0 (0.0)	
Falling from a height	2 (2.4)	1 (6.7)	
Total	85 (85.0)	15 (15.0)	

3.2 Evaluation of KASP results

The plates read with the Omega Device appeared to be suitable for ×9 cycle analysis for all four polymorphisms (Fig. 1). The TPH-1 gene contained the rs1800532 polymorphism alleles A and C. Six (6%) samples in suicide cases and seven (7%) in the control group were not included due to the absence of proper bonding during KASP analyses. In the study group, for the TPH-1 gene rs1800532 polymorphism, 80 A alleles (42.6%) and 108 C alleles (57.4%) were identified in suicide cases in a total of 94 individuals and 188 alleles. A total of 77 A alleles (41.4) and 109 C alleles (58.6%) were identified in 93 individuals and 186 alleles. In both the suicide group and the control group, there was no difference between men and women in terms of TPH-1 gene rs1800532 polymorphism ($p > 0.05$ for each). The comparison of the study groups in terms of TPH-1 gene rs1800532 polymorphism is presented in Table 4, and the comparison of gender and presence of rs1800532 polymorphism in study group is included in Table 5.

TABLE 4. Comparison of study groups in terms of TPH-1 gene rs1800532 polymorphism.

Polymorphism		Study group		Test value; p
		Suicide group	Control group	
		n (%)	n (%)	
Genotype	CC	32 (34.0)	32 (34.4)	0.124; 0.940
	CA	44 (46.8)	45 (48.4)	
	AA	18 (19.2)	16 (17.2)	
Allele	C	108 (57.4)	109 (58.6)	0.051; 0.821
	A	80 (42.6)	77 (41.4)	

TABLE 5. Comparison of sex and presence of TPH-1 gene rs1800532 polymorphism in study group.

Polymorphism		Study group			
		Suicide group		Control group	
		Men	Women	Men	Women
		n (%)	n (%)	n (%)	n (%)
Genotype	CC	24 (30.4)	8 (53.3)	30 (37.0)	2 (16.7)
	CA	37 (46.8)	7 (46.7)	36 (44.5)	9 (75.0)
	AA	18 (22.8)	0 (0.0)	15 (18.5)	1 (8.3)
Test Value; p		5.369; 0.068		3.907; 0.142	

The TPH-2 gene contains the rs7305115 polymorphism alleles A and G. Due to the lack of proper bonding during the KASP analyses, 3 (3%) samples in suicide cases and 5 (5%) samples from the control group could not be included. In the *TPH-2* gene rs7305115 polymorphism, 76 A alleles (39.1%) and 118 G alleles (60.8%) were found in suicide cases in a total of 97 individuals and 194 alleles. In the control groups, 68 A alleles (35.7%) and 122 G alleles (64.2%) were found in 95 individuals and 190 alleles. In both suicide and control groups, there was no difference between men and women in terms of TPH-2 gene rs7305115 polymorphism ($p > 0.05$ for

each). The comparison of the study groups in terms ofTPH-2 gene rs7305115 polymorphism is included in Table 6, the comparison of gender and presence of TPH-2 gene rs7305115 polymorphism among study groups is shown in Table 7.

TABLE 6. Comparison of study groups in terms of TPH-2 gene rs7305115 polymorphism in study group.

Polymorphism		Study group		Test value; p
		Suicide group	Control group	
		n (%)	n (%)	
Genotype	GG	34 (35.1)	41 (43.2)	1.781; 0.410
	GA	50 (51.5)	40 (42.1)	
	AA	13 (13.4)	14 (14.7)	
Allele	G	118 (60.8)	122 (64.2)	0.469; 0.493
	A	76 (39.1)	68 (35.7)	

TABLE 7. Comparison of sex and presence of TPH-2 gene rs7305115 polymorphism in suicide cases.

Polymorphism		Study group			
		Suicide group		Control group	
		Men	Women	Men	Women
		n (%)	n (%)	n (%)	n (%)
Genotype	GG	27 (32.9)	7 (46.7)	13 (15.7)	1 (8.3)
	CA	42 (51.2)	8 (53.3)	35 (42.2)	5 (41.7)
	AA	13 (15.9)	0 (0.0)	35 (42.2)	6 (14.6)
Test Value; p		3.072; 0.215		0.532; 0.767	

The SLC6A4 gene contains the rs6355 polymorphism alleles C and G. One (1%) sample from suicide cases and five (5%) samples from the control group were not included due to the fact that proper binding was not achieved during the KASP analyses. For the SLC6A4 gene rs6355 polymorphism, 197 C alleles (99.4%) and 1 G allele (0.5%) were found in suicide cases in 99 individuals and 198 alleles. In the control groups, 189 C alleles (99.4%) and 1 G allele (0.52%) were observed in 95 individuals and 190 alleles. Among suicide cases, there were 98 (99%) individuals with CC genotype, 1 individual (1%) with GC genotype, and no individuals with GG genotype. In the control group, there were 94 individuals (98.9%) with CC genotype, 1 individual (1.1%) with GC genotype, and no individuals with GG genotype. The comparison of the study groups in terms of SLC6A4 gene rs6355 polymorphism is shown in Table 8.

TABLE 8. Comparison of study groups in terms of SLC6A4 gene rs6355 polymorphism.

Polymorphism		Study group		Test value; p
		Suicide group	Control group	
		n (%)	n (%)	
Genotype	GG	-	-	1.000 [b]
	GC	1 (1.0)	1 (1.1)	
	CC	98 (99.0)	94 (98.9)	
Allele	G	1 (0.6)	1 (0.6)	1.000 [b]
	C	197 (99.4)	189 (99.4)	

[b]: Fisher's Exact.

The TPH-2 gene contains the alleles A and G of the rs1386494 polymorphism. During the KASP analyses, 12 (12%) samples from suicide cases and 24 (24%) samples from the control group were not included, due to the fact that the primers did not bind satisfactorily. In the TPH-2 gene rs1386494 polymorphism, 16 A (9.1%) and 160 G alleles (90.9%) were observed in suicide cases in 88 individuals and 176 alleles. In the control groups, 15 A alleles (9.9%) and 137 G alleles (90.1%) were found in 76 individuals and 152 alleles. No difference was found between men and women in terms of TPH-2 gene rs1386494 polymorphism ($p > 0.05$ for each). The comparison of the study groups in terms of TPH-2 gene rs1386494 is shown in Table 9, and the comparison of sex and presence of TPH-2 gene rs1386494 among study groups is shown in Table 10.

TABLE 9. Comparison of the study groups in terms of TPH-2 gene rs1386494 polymorphism.

Polymorphism		Study group		Test value; p
		Suicide group	Control group	
		n (%)	n (%)	
Genotype	GG	74 (84.1)	62 (81.6)	0.557; 0.757
	GA	12 (13.6)	13 (17.1)	
	AA	2 (% 2.3)	1 (1.3)	
Allele	G	160 (90.9)	137 (90.1)	0.003; 0.960
	A	16 (9.1)	15 (9.9)	

TABLE 10. Comparison of sex and presence of TPH-2 gene rs1386494 polymorphism in study group.

Polymorphism		Study group			
		Suicide group		Control group	
		Men	Women	Men	Women
		n (%)	n (%)	n (%)	n (%)
Genotype	GG	60 (81.1)	14 (100.0)	1 (1.5)	0 (0.0)
	CA	12 (16.2)	0 (0.0)	11 (16.9)	2 (18.2)
	AA	2 (2.7)	0 (0.0)	53 (81.5)	9 (81.8)
Test Value; p		3.150; 0.207		0.178; 0.915	

4. Discussion

In this study, polymorphic variants rs1800532, rs7305115, rs6355, and rs1386494 were evaluated among 100 women and men cases of completed suicide and 100 healthy women and men controls.

In a study conducted with 2587 suicide-related deaths in Finland, it was found that men typically died through suicide by hanging and women through self-poisoning [57]. Another study on 181 cases that underwent post-suicide forensic autopsy in Iran reported that the most common suicide method was hanging in men, and consuming drugs and toxic substances in women [58]. In our study, it was found in 100 suicide cases that resulted in death that the most common method of suicide was by hanging in men, consistent with the literature, and the most common suicide method was also by hanging in women, which was contrary to other studies. The

incidence of suicide methods varies from country to country and region to region [59].

Despite the fact that TPH polymorphisms in suicides have been studied in different populations, no consensus has been reached regarding their significance [51, 52, 60]. Studies conducted in the Turkish population are very limited in number [61], and have been inconclusive. In a study by Aydın et al., it was concluded that the AA genotype of the rs1800532 polymorphism of the TPH-1 gene may be relevant in suicidal behavior [21]. Another study performed in the Turkish population also concluded that the AA genotype of the rs1800532 polymorphism of the TPH-1 gene may be relevant in suicidal behavior [21]. Contrary to that study, no significant relationship was found between the two groups in our study. In another study conducted with 109 individuals with suicidal behavior and 98 healthy individuals, it was observed that the A allele was at a higher frequency in individuals with suicidal behavior than those in the control groups. Furthermore, the frequencies of the CC genotype and C allele were significantly higher among men, but there was no statistically significant difference between men and women [61]. Our study also supports this result, as no significant difference was found between men and women. Another study has demonstrated that the rs1800532 minor allele has a protective role for men younger than 35 years and women older than 50 years, whereas it tends to be a risk factor in older men ($\geq$50 years) [62]. In a study conducted in China, no statistically significant relationship was found in terms of rs1800532 polymorphism between suicidal and non-suicidal individuals, similar to the results of our study [63]. In studies investigating the relationship of rs1800532 polymorphism genotypes of the TPH-1 gene with suicide, it was observed that variable results were obtained across different populations.

In order to understand the relationship with suicide, these studies should be conducted more frequently in different societies. The rs7305115 polymorphism is an important TPH-2 gene polymorphism in studies on the genetics of suicide. It has been investigated in various societies and variable results have been obtained [64–68]. Similar to the current study, a study on adolescents with suicidal behavior in France revealed no relationship between rs7305115 polymorphism and suicidal behavior [69]. In a study conducted in the Mexican population, a positive correlation was found between the AA genotype of rs7305115 polymorphism and suicidal behavior [54]. In our study, no relationship was found in rs7305115 polymorphism between the selected groups and between sexes. Another key TPH-2 polymorphism is rs1386494. There are limited studies on this polymorphism. In a study investigating suicidal behavior in individuals with alcohol addiction, no significant relationship was found between this polymorphism and individuals with alcohol addiction who exhibit suicidal behavior [70].

The SLC6A4 gene, which is located on chromosome 17 and plays a role in the reuptake of serotonin released from the synaptic gap, is one of the prominent genes in studies on suicidal behavior [29]. The 5HTTLPR polymorphism has been studied in the literature [41, 71, 72], and a study reported that this polymorphism was associated with suicidal behavior [29].

In another study, changes in SLC6A4 mRNA expression in patients with severe depression with suicidal behavior were investigated, and it was concluded that the SLC6A4 gene could be used as a biomarker in suicidal behavior [73]. In our study, rs6355 polymorphism, which has not been previously studied in the Turkish population; no statistically significant difference was found between the groups.

One reason for the variable results obtained in the studies is that suicidal behavior has a complex origin. Environmental and psychiatric components are also risk factors along with genetic factors. Groups comprised of individuals diagnosed with schizophrenia, bipolar disorder, alcohol and/or substance addiction, or severe depression that exhibit suicidal behavior were selected in the majority of the studies. It is known that selected polymorphisms may be associated with psychiatric diseases [50, 51]. Therefore, it is imperative to consider psychiatric and physiological factors. In the present study, patients without a history of psychiatric illness, schizophrenia, bipolar mood disorder, mental retardation, history of chronic disease, and substance and alcohol addiction were selected to minimize this effect.

The KASP system used in the genotyping phase of the study is a new PCR technology. To the best of our knowledge, this method has never been used in other studies. In the KASP analysis, amplification occurs in a hydrocycle device which is operated based on the principle of a hot water bath. It differs from other PCR methods because of this feature. Compared to other genotyping systems used in studies, this method is both economical and less time-consuming. Another advantage of this method is that more than one SNP can be analyzed at the same time and samples of 384 individuals can be placed on a black-and-white plate. The fact that the hydrocycle device has a feature that can simultaneously read four plates is advantageous for concurrently analyzing more than one SNP.

This study has some limitations. Usually 80% (0.8) power is regarded as a goal in a replication study. This study had 80% power to detect an effect with OR = 2 with SNP MAF = 0.15, or OR = 1.8 with SNP MAF = 0.47. But such higher effect levels are not expected for single SNPs. The study has limited power to detect the expected effect sizes of these SNPs. Therefore, the negative results are expected due to the insufficient power, which is a major reason for the negative statistical results ($p > 0.05$).

There is a study on mice in the literature [74]. There are also studies on urine and saliva samples [75]. These studies are important for understanding brain functions and diseases. Therefore, studies on suicide should also be developed.

In our results, although no statistically significant differences were found; we observed non-significant tendencies of increased minor alleles in suicidal men for all TPH SNPs. Our study is one of a limited number of studies on suicide in the Turkish population. It is an important preliminary study for future studies on the genetic component of suicidal behavior

and determination of candidate genes.

5. Conclusions

Our study is a preliminary study. The serotonergic system plays a role in our behaviors. Our study has limited power to detect the expected effect sizes of these SNPs. Therefore, it would be valuable to study the serotonergic system as a complex.

Abbreviations

DNA, Deoxyribonucleic Acid; EDTA, Ethylenediaminetetraacetic Acid; CASP, Competitive allele specific PCR; MAF, Minor Alele Frequency; PCR, Polymerase Chain Reaction; TPH, Tryptophan Hydroxylase; TPH-1, Tryptophan Hydroxylase 1; TPH-2, Tryptophan Hydroxylase 2.

Author contributions

NC and NLST designed the research study. DYK performed the research. HO, OC, NT Collection and analysis of samples. EE provided help on analyzing the data. DYK, EE, NC and NLST wrote the manuscript. All authors contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Ethics approval and consent to participate

Ethics Committee of Ankara University Faculty of Medicine for this study (Decision No: 09-387-15, May 25, 2015) and approved by Ministry of Justice (Decision No: 21589509/694, Aug 6, 2015).

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Conflict of interest

The authors declare no conflict of interest.

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