Exp Biomed Res 2023; 6(4): 249-256 **DOI: 10.30714/j-ebr.2023.201**

Experimental Biomedical Research *Original article*

Evaluation of the predisposition of serotonin transporter polymorphisms (5-HTTLPR) and complement factor H (CFH) variants to smoking status

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ABSTRACT

Aim: To evaluate the susceptibility of the serotonin transporter-associated polymorphic region (5-HTTLPR) and complement factor H (CFH) gene variants to smoking status.

Method: Smokers and non-smokers were included in the study**.** The smoking amount was assessed based on the scores on the Fagerström Test for Nicotine Dependence (FTND). DNA is extracted from blood samples. 5-HTTLPR and CFH Y402H variants were analyzed by polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP) methods. The results were evaluated statistically.

Results: 5-HTTLPR and CFH Y402H genotype and allele distribution did not differ significantly between smokers and non-smokers ($p>0.05$). There was no deviation from Hardy-Weinberg equilibrium (HWE) for these variants in the groups.

Conclusion: Nicotine addiction is a complex phenomenon in which both genetic and environmental factors play a role. The identification of genes that play a role in addiction is important to elucidate the pathogenesis. The results of this study showed that 5-HTTLPR and CFH Y402H variants had no effect on nicotine addiction. However, these results need to be validated in larger sample groups and in different ethnic communities.

Key words: Nicotine, serotonin transporter gene, complement factor H, variant, PCR, RFLP.

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Received: 2023-04-04 / Revisisons: 2023-04-23
Accepted: 2023-05-01 / Published online: 2023-09-20

Introduction

Cigarette smoking is one of the most common addictive conditions. Smoking is an important public health problem as it is the leading cause of death from many diseases such as cancer, pulmonary disease, and heart disease. According to data from the World Health Organization, approximately six million people die annually from smoking worldwide [1]. Most of these individuals are physically dependent on nicotine, which is the main component of tobacco [2]. Nicotine addiction is a complex process consisting of biological, environmental, and behavioral factors. Many studies, such as genome-wide association analysis and candidate

gene association studies, have been conducted to elucidate the causes of nicotine addiction.

Serotonin (5-HT) plays a crucial role in regulating numerous brain functions, including cognition, sleep, motor activity, temperature regulation, sensory perception, mood, hormone synthesis, appetite, and sexual behavior [3]. Nicotine binds to nicotinic receptors in the brain, increasing the release of many neurotransmitters, including dopamine, serotonin, and norepinephrine [4]. Xu et al. reported that nicotine may be a factor in the onset of depression by suppressing 5-HT transporters [5]. The serotonin transporter gene (5-HTT) mainly regulates serotonergic neurotransmission in various regions of the brain. This gene is highly polymorphic, and these polymorphisms can affect mood disorders and smoking behavior. There is a 44-base pair deletion/insertion functional variant in the 5' promoter region of this gene. This variant is related to serotonin transporter gene-linked polymorphic region (5-HTTLPR). It contains two different types of alleles, short (S) and long (L), which affect the 5-HT transcription rate. The S allele has less transcriptional activity than the L allele [6].

Nicotine exerts immunosuppressive effects via central and peripheral mechanisms. It damages antigen and receptor-mediated signals transduction in the lymphoid system, resulting in a reduced immune response [7]. The complement system, which important part of the innate immune system, provides a strong defense against pathogens, and eliminates apoptotic cells, and immune complexes [8]. Complement factor H (CFH) regulates the complement system's alternative pathway, which bears anti-inflammatory actions, preventing harm to the host tissue. The CFH gene is located on 1q31.3. Its variations have been associated with an increased risk of inflammatory disease [9]. The CFH gene T1277C polymorphism (rs1061170) has a T to C transition at position 1277 in exon 9, resulting in the replacement of tyrosine with histidine at position 402 (Y402H) in the amino acid sequence [9]. Based on this information, the aim of this study was to investigate the effect of 5-HTTPLR and CFH Y402H variants on smoking status in the Turkish population.

Materials and methods

Subjects

Smokers and non-smokers were included in the study. All subjects were admitted to Yedikule Training and Research Hospital for Chest Diseases and Thoracic Surgery, Istanbul, Türkiye. Active smokers were included in the smoker group, defined as previous smokers of more than one cigarette per day. The smoking amount was assessed based on the scores on the Fagerstrom Test [10]. The control group was made up of "nonsmokers," or people who smoked less than one cigarette per day for less than a year of their lives. All groups included Turkish individuals over 18 years old. All subjects provided informed written consent before enrolling in the study. The Istanbul University, Medical Faculty Clinical Research Ethics Committee approved the protocol of the study (24.10.2014/18), and the study was carried out in accordance with the Declaration of Helsinki.

Genotyping: Genomic DNA was extracted from whole blood according to the salting-out method [11]. The extracted DNA was stored at -20 °C until analysis. The 5-HTTLPR and CFH Y402H variants were analyzed with polymerase chain reaction (PCR) and/or restriction fragment length polymorphism (RFLP) methods, as described previously [6, 12]. The PCR method was used to analyze the 5-HTTLPR variant using the specified primers (F – TAGAGGGACTGAGCTGAGCTGGACAACC

Table 1. PCR conditions for variants.

Variants	Methods	Primer sequences	Annealing temperature / Cycle number
5-HTTLPR	PCR	F-TAGAGGGACTGAGCTGAGCTGGACAACCAC and R-GGTGTTGCCGCTCTGAATGC	59°C / 35 cycles
CFH Y402H	PCR-RFLP	F-ACTGTGGTCTGCGCTTTTG and R-TTTTTGGATGTTTATGCAATCTT	60°C / 34 cycles

AC; R-GGTGTTGCCGCTCTGAATGC). PCR products were run on a 2.5% agarose gel stained with ethidium bromide and visualized under UV light. 484 bp fragments were evaluated as short (S) alleles and 528 bp fragments as long (L) alleles.

CFH Y402H analysis was performed by the PCR-RFLP method using specific primers (F-ACTGTGGTCTGCGCTTTTG and R-TTTTTGGATGTTTATGCAATCTT).
Amplified products were cut with the NlaIII restriction enzyme. The cut products were run on a 3% agarose gel, stained with ethidium bromide, and visualized under UV. Fragments were displayed as the T allele, which produces a single fragment of 244 bp, and the H allele, which produces two fragments of 161 bp and 83 bp. PCR conditions for variants are shown in Table 1.

Statistical analysis

The software Statistical Package for the Social Sciences (IBM SPSS, version 21; SPSS Inc., Chicago, IL; USA) was used to analyze all of the data. The statistical significance of the difference between the smokers and the controls was calculated using logistic regression analysis. We also calculated the odds ratio (OR) and 95% confidence interval (CI). The chi-square test was performed to evaluate the differences between genotype and allele distribution in these variants, and Fisher's exact test was applied if required. We analyzed the data to see the appropriateness between the expected and observed genotypes and Hardy-Weinberg equilibrium (HWE). All two-tailed analyses were performed, and differences were considered statistically significant at $p< 0.05$.

Results

In our study, 5-HTTLPR and CFH Y402H variants were examined in smokers and non-smokers. The genotype distribution and allele frequencies of 5-HTTLPR and CFH Y402H variants are shown in Table 2.

5-HTTLPR variant

In the smoker group, the frequencies of S/S, S/L, and L/L genotypes were 30.4, 48.6%, and 21%, respectively. The S and L allele frequencies in the smokers were 54.7% and 45.3%, respectively. The frequencies of the S and L alleles in the non-smokers were 53.2% and 46.88%, respectively. In the non-smoker control group, the S/S, S/L, and L/L genotype frequencies were 30.9%, 44.5%, and 24.5 %, respectively.

There was no significant difference between smokers and non-smokers regarding 5-HTTLPR genotype and allele distribution ($p>0.05$). There was no deviation from HWE in groups for the 5-HTTLPR variant.

CFH Y402H variant

The Tyr/Tyr, Tyr/His, and His/His profiles for the *CFH* Y402H variant were 44.79%, 41.10%, and 14.11%, respectively, in the smoker group and 40.0%, 41.3%, and 18.7%, respectively, in the non-smokers. The Tyr and His allele frequencies in the smokers were 65.34% and 34.66%, respectively. CFH Y402H genotype and allele distribution were not different between the

Table 2. Genotype and allele distribution of variants in groups.

5-HTTLPR	**Smoker group n: 148 (%)**	**Non-smoker group n: 110 (%)**	***p***
Genotypes			
S/S	45 (30.4)	34 (30.9)	*>0.05*
S/L	72 (48.6)	49 (44.5)	
L/L	31 (21.0)	27 (24.5)	
Alleles			
S	162 (54.7)	117 (53.2)	*>0.05*
L	134 (45.3)	103 (46.8)	
HWE p	0.257	0.824	
CFH Y402H	**Smoker group n:163 (%)**	**Non-smoker group n:80 (%)**	***p***
Genotypes			
Tyr/Tyr	73 (44.79)	32 (40.0)	*>0.05*
Tyr/His	67 (41.10)	33 (41.3)	
His/His	23 (14.11)	15 (18.7)	
Alleles			
Tyr	213 (65.34)	97 (60.63)	*>0.05*
His	113 (34.66)	63 (39.37)	
HWE p	0.237	0.223	

HWE: Hardy-Weinberg Equilibrium

groups (*p>0.05*). There was no deviation from HWE for the CFH Y402H variant in the groups.

Discussion

Addiction is a chronic, recurrent disease that destroys the brain's reward circuitry, leading to seeking and other behavioral changes. Genetic variation and its downstream effects and differences in interindividual neurobiological circuits alter susceptibility to developing an addiction. Although addiction is multifactorial, heritability estimates that approximately 40-60% of population variability in becoming addicted to nicotine, alcohol, or illicit drugs can be attributed to genetic factors [13].

There are studies focused on searching for candidate genes to understand the molecular basis of smoking behavior.

5-HTT is a neuromodulator with widespread effects on the central nervous system. [14]. 5-HTT is localized in the presynaptic membrane of serotonergic neurons and plays a role in balancing serotonin transmission by taking the serotonin released from the synaptic cleft [15]. Its effect on nicotine addiction has been extensively researched. In addition, the role of 5-HT in alcohol consumption has been extensively evaluated in animal models. Several alcohol-preferring rat strains have been shown to have low 5-HT levels in limbic structures [16]. 5-HTT is a polymorphic gene. Polymorphisms affecting the serotonergic system, such as the 5-HTT system, have been associated with smoking-related behaviors. The L and short S alleles in the insertion or deletion polymorphism in this gene alter transcriptional efficiency. The S allele has been associated with reduced serotonin uptake,

leading to the hypothesis that individuals with the S allele are not prone to smoking [17]. Therefore, it has been postulated that variations in the serotonergic system may affect some aspects of smoking, such as mood changes during nicotine withdrawal [6]. Most of these studies have investigated the initiation and continuation of smoking. In addition, a clinical trial found that carriers of the L allele exhibit better dropout rates than carriers with the S/S genotype [18]. Kandemis et al. found that the allele and genotype distribution of 5-HTT VNTR was different between non-smokers and smokers in the Turkish Cypriot population [19]. Vaht et al. showed a relationship between the 5-HTTLPR genotype and alcohol use. However, they reported that the effect was dependent on gender [20]. In a meta-analysis, a significant correlation was found between the 5-HTTLPR genotype distribution and the amount of smoking. But, there was no significant relationship between genotype distribution and continuing or starting smoking [21]. However, there are studies showing that the 5-HTTLPR polymorphism does not affect smoking behavior [22-24]. Similarly, Lerman et al. evaluated the relationship between smoking practices and smoking cessation with 5-HTTLPR in their study. They found no significant difference in the distribution of 5-HTT genotypes between smokers and non-smokers Caucasians or African-Americans [25]. In this study, we found no association between 5-HTTLPR and smoking status in our population. Our study results are in line with those reported.

Immune mediators such as cytokines and chemokines may play a role in cognitive, behavioral, and brain structure abnormalities encountered in psychotic disorders, regulating neural development [26] and synapse plasticity [27]. The complement system is considered an important factor in innate immunity. Smokers have decreased functionality, increased hospitalization, more sedative use, a family history of mental disorders, more depressive disorders, and a lower quality of life than non-smokers [28]. Various studies have shown that smoking can lead to the activation of the complement system [29, 30]. Some polymorphisms have been identified in the CFH gene, but their possible effects on expression levels or the function of CFH are unclear [31]. Variant Y402H is assumed to be functional due to its location in the binding sites of CFH to heparin and C-reactive protein [32]. Substituting a histidine, which is positively charged, for a non-charged hydrophobic tyrosine in position 402 may modify the binding features and, subsequently, have functional effects. In different ethnic groups, it was found that the CFH Y402H variant was associated with macular degeneration related to age [33-35]. Considering that inflammation may play a role in the pathogenesis of smoking, we analyzed the CFH variant in our study groups. In this study, there was no significant difference between smokers and non-smokers in terms of CFH Y402H genotype and allele distribution.

Our study has some limitations. First, the age and duration of the onset of smoking were not questioned. Also, family characteristics were not evaluated. In addition, other variants that may have a role in these genes have not been examined.

Conclusions

Nicotine addiction is a complex phenomenon in which genetic and non-genetic factors interact. The identification of genes that play a role in addiction is important to elucidate the pathogenesis. In this study, we investigated the predisposition of 5-HTTLPR and CFH Y402H variants to smoking status in the Turkish population. Our findings supported the idea that

these variants did not play a role in smoking susceptibility in our population. However, these results need to be validated in larger samples and in different ethnic communities.

Funding: *This study was partly supported by Istanbul University, Istanbul Faculty of Medicine, ONAP 2186.47815.*

Conflict of interest: *The authors declare that they have no conflict of interest.*

Ethical statement: *The Istanbul University, Medical Faculty Clinical Research Ethics Committee approved the protocol of the study (24.10.2014/18).*

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