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Original Article

The relationship between DRD2 TaqIA polymorphism and tobacco addiction

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ABSTRACT

Objectives: In this study, the relationship between tobacco addiction levels, early smoking, and DRD2 TaqIA polymorphism was investigated.

Materials and methods: The present study included 36 smokers and 12 non-smoking voluntary controls. Study groups were established as the non-smoking control group and addicted (less than 1 pack, between 1-2 packs, more than 2 packs per day) groups. Genotypes of the dopamine type-2 receptor (DRD2) gene TaqIA polymorphic region from genomic DNA isolated from oral swab samples were determined by PCR followed by Restriction Fragment Length Polymorphism (RFLP) analysis.

Results: Presence of the DRD2 TaqIA homozygous polymorphic genotype in addicted individuals who smoked two or more packs per day and the absence of non-smoker control individuals may indicate that the polymorphic TaqIA allele of the DRD2 gene is associated with smoking. It was found that individuals carrying the polymorphic TaqIA allele in homozygous (~13 years) and heterozygous forms (~16.3 years) had a lower age of starting smoking compared to homozygous wild types (~18.6 years). Mean BMI (body mass index) of the study participants was 24.66 kg/m2 (normal weight), waist/hip ratio 0.82 (no abdominal obesity).

Conclusion: The results of our study suggest that the TaqIA variant allele is related to early smoking condition. In addition, it was determined that there was a higher tendency of smoking addiction among children of addicted parents.

Keywords: Dopamine, DRD2 gene, polymorphism, TaqIA, tobacco addiction.

Today, with over 6 million people starting to smoke every year, smoking is reported as the cause of more than 10% of deaths worldwide.[1] According to the World Health Organization (WHO), 20% of the world population smokes and nearly 8 million people die each year due to diseases related to smoking and tobacco products.[1] It is known that the number of smokers worldwide exceeds 1.1 billion people.[2] Smoking is considered one of the most important causes of death due to lung cancer and is a major risk factor of various diseases such as cardiovascular disease, diabetes, and hypertension.[3]

High prevalence of tobacco addiction worldwide is one of the most serious global health problems. Smoking is a complex behavior affected by both genetic and environmental factors. The effect of genetic factors on tobacco addiction is 56%, followed by familial factors at 24%, and environmental factors at 29%.[4] Studies have shown that environmental factors start smoking; showed that genetic factors play a significant role in the transition from regular drinking to addiction. Studies have shown that environmental factors play a significant role in starting smoking, and genetic factors play a significant role in the transition from regular smoking to addiction.[5]

Dopamine (DA) was defined by Arvid Carlsson about 60 years ago.[6] While the DA subtypes of D1 and D2 were known until 1988, studies

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have defined the D3 and D4 receptors as "D2-like" and the D5 receptor as "D1-like" according to their pharmacological and primary mechanisms of action.[7] Dopamine is one of the neurotransmitters involved in signaling between neurons and functions as one of the power sources in all activities that require proper brain activity. Since DA is determined to be very effective in providing concentration in physical activities due to its function depending on the reward system, it has been shown that the activity can be performed more efficiently as a result of the increase in the amount of DA together with motivation while dealing with an activity. It is known that different effects and diseases occur according to increases and decreases in the regions where DA is secreted in the brain. While DA deficiency causes Parkinson's or attention deficit disorder, its excess causes addictions, hallucinations, bipolar disorder and many psychological disorders.[7] It is hypothesized that people with functional deficiencies in the DA reward pathway may be more prone to drug addiction, including nicotine addiction.[8]

The A1 allele of the dopamine D2 receptor gene (DRD2) is associated with a decreased number of DA binding sites in the brain, substance abuse, and addictive behavior.[9] Blum et al.[8] reported that reduced binding of DA to D2 receptors was associated with addiction. It is known that after inhalation, nicotine initiates a series of neurological events, also involving DA. Smoking-related nicotine causes DA to be released from the mesolimbic system, preventing withdrawal symptoms. Initial withdrawal symptoms can be controlled with about one cigarette per week. However, as the tolerance increases, the interval of relief provided by each cigarette gets shorter and shorter.[10]

There are hypotheses that the DRD2 gene in the 11q23 region is associated with the amount of smoking. It has been reported that there is greater prevalence of DRD2 gene TaqIA polymorphism among smokers compared to non-smokers.[11,12] According to the aforementioned studies, it is suggested that there is a lower number of D2 receptors in people with the DRD2 TaqIA variant allele, thus leading to a decrease in the rate of binding to the receptor. A 2011 study by Stapleton et al.[13] found that the DRD2 gene and TaqIA variant allele was associated with the response to quitting smoking and nicotine replacement therapy. In contrast to studies pointing to the relationship between DRD2 TaqIA polymorphism and nicotine addiction, Singleton et al.[14] did not find a significant relationship between the DRD2 allele and nicotine addiction. The differences can be deduced diversity of genetic background of populations, number or heterogeneity of the patients involved.

Although studies have been conducted on DRD2 gene-related alcoholism[15] and schizophrenia[16] in Turkish population, there are currently no studies investigating the relationship between the amount of smoking and the TaqIA polymorphism of the DRD2 gene. Therefore, the primary objective of our study was to determine whether or not there was a relationship between the amount of smoking and DRD2 TaqIA polymorphism. Furthermore, another objective of our study was to determine whether or not there was relationship between age at onset of smoking and DRD2 TaqIA polymorphism. The current study also investigates the relationship between familial smoking and smoking addiction.

MATERIALS AND METHODS

Study sample

A total of 36 smokers (addicted group) and 12 volunteers who did chose not to smoke throughout their entire life (control group), with ages ranging between 18 and 55, at the Marmara University Göztepe campus between November-December 2018 were included in the study. The addicted group consisted of 16 women and 20 men, while the control consisted of 8 women and 4 men. Attention was paid to make sure there was no kinship between members of the addicted group and the control group. The criteria of age, sex, body mass index, and waist-hip measurements were taken into consideration when recruiting addicted and control subjects. Data regarding age, sex, height, weight, duration and amount of cigarette use, number of family members and which family members smoked were recorded. The addicted participants were grouped according to daily cigarette use (less than 1 pack, 1-2 packs, more than 2 packs a day). This study received approval from the Marmara University Local Ethics Committee (No. 09.2018.722).

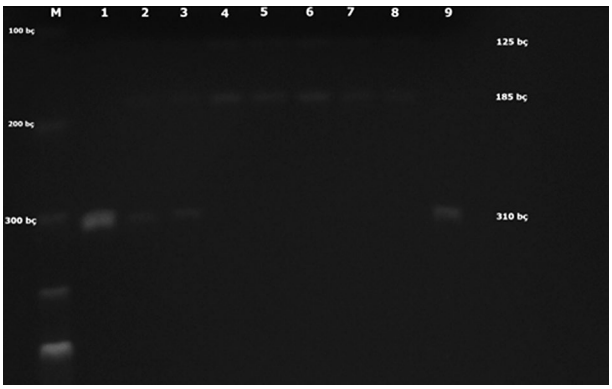

Figure 1. Appearance of DRD2 polymorphic genotypes in agarose gel electrophoresis after PCR-RFLP. M: Marker, Homozygous wild genotype (A2/A2) 125 bp and 185 bp (4, 5, 6, 7, 8); heterozygous genotype (A2/A1) 125 bp, 185 bp and 310 bp (2,3); homozygous mutant genotype (A1/A1) 310 bp (1,9).

Determining DRD2 gene TaqIA genotypes

The study was conducted at the Marmara University Molecular Metabolism Research Laboratory. Genomic DNAs were isolated, according to the Quick-gDNA Miniprep Kit (Zymo Research Corp.) protocol, from intraoral swab samples taken from volunteers who agreed to participate in the study. 2X AmpMaster™ Taq (No-dye), GeneAll® Kit was used for PCR. The primer sequence used in the reaction was 5′GCCACCACGGCTGGCCAAG3′ for Primer A, and 5′CCTTCCTGAGTGTCATCAACC3′ for Primer B. For amplification, PCR steps were as follows: initial denaturation at 95°C/2 min, followed by 34 cycles of denaturation at 95°C/25 s, annealing at 62°C/10 s and extension at 72°C/90 s. The final extension was carried out at 72°C/2 min. BioRad T100 was used for thermal cycling. Amplicons yielded from the PCR process were cleaved with the Restriction Fragment Length Polymorphism (RFLP) method using Taq I restriction enzyme (5U). The PCR product, enzyme buffer, and dH_2O mixture that were prepared for cleavage were first incubated at 65°C for 4 hours, then at 80°C for 20 minutes. Samples obtained after cleavage were run through 1.5% agarose gel at 120 mV and observed under UV light to determine genotypes.

RESULTS

Mean age of the study group was 33.56 years, mean BMI was 24.66 (normal weight), and mean waist/hip ratio was 0.82. Appearance of the homozygous wild, heterozygous, and homozygous variants of DRD2 TaqI genotypes in agarose gel are displayed in Figure 1. Homozygous wild genotype (A2/A2) were determined as 125 and 185 bp, heterozygous genotype (A2/A1) as 125, 185, and 310 bp, and homozygous mutant genotype (A1/A1) as 310 bp long fragments in agarose gel (Figure 1).

According to the results, DRD2 Taq I A homozygous mutant genotype was found in 3 participants who smoked 2 or more packs a day, indicating that DRD2 A1 genotype was significantly associated with the amount of smoking compared to the control group (Table 1). Polymorphic genotype was not observed in any of the individuals who smoked 1 pack a day or 1-2 packs a day, and was not found to be associated with amount of smoking. Thus, it was determined that the presence of polymorphic allele of the DRD2 gene has a significant effect when the amount of smoking exceeds two packs per day.

When the age at onset of smoking of homozygous polymorphic individuals (mean age 13 years) was compared to individuals with

Table 1. Distribution of DRD2 genotypes among the study groups

	Homozygous wild (A2/A2)		Heterozygous (A1/A2)		Homozygous mutant (A1/A1)	
Daily tobacco use (packs)	n	%	n	%	n	%
Non-smoker	5	41.7	7	58.3	0	0.0
<1 pack	7	58.3	5	41.7	0	0.0
1-2 packs	4	33.3	8	66.7	0	0.0
≥2 packs	3	25.0	6	50.0	3	25.0

DRD2: Dopamine D2 receptor gene.

Table 2. Familial smoking behavior among the study groups

	No familial smoking		Familial smoking	
Daily tobacco use (packs)	n	%	n	%
Non-smoker	5	41.7	7	58.3
<1 pack	1	8.3	11	91.7
1-2 packs	4	33.3	8	66.7
≥2 packs	4	33.3	8	66.7

DRD2: Dopamine D2 receptor gene.

heterozygous genotypes (mean age 16.3 years) and wild genotypes (mean 18.6 years), it was determined that individuals with mutant and heterozygous genotypes started smoking at a younger age.

When the familial smoking behaviors of the study groups were examined, the most familial smoking behavior was observed as 91.7% in the group that smoked 1 pack a day. As for the other two groups, the same rate of familial smoking behavior was observed as 66.7%, and no significant relationship was established between familial smoking behavior and amount of smoking (Table 2).

DISCUSSION

In addition to being involved in several neurobiological processes, dopamine plays an important role in the development of addiction, as it acts as a key element for the balance between reward and reluctance. Dopamine D2 (DRD2) is the most studied gene in the research of genetic causes of addiction. According to the results of different meta-analysis studies, it was determined that the A1 allele of DRD2 gene (TaqIA polymorphism) can increase the risk of alcohol use (31% increase, p=4.5×10-8). Although its relationship with substance abuse has been investigated, as in DRD1 and DRD3, a convincing association has not been found. Although there are some reports that DRD4 and DRD5 genes are associated with specific addictive disorders, none of them have been proven to be associated with addiction.[17]

Taq I A polymorphism is one of the most studied gene regions in DA signaling and obesity. In addition to obesity, the A1 allele is associated with attention deficit hyperactivity disorder (ADHD),[9] addiction,[8] and alcoholism.[18] The A1 allele has also been associated with greater impulsivity, bad timing, and impaired memory and learning.[19] While there are studies that have found a positive association between DRD2 TaqIA allele and substance addiction,[11,13,14] there are also studies that have not found such an association.[12,15,16] In accordance with these results, in our study we found that the individuals with DRD2 Taq I A homozygous polymorphic genotype were comprised of smokers who smoked 2 or more packs a day. We believe that the absence of the DRD2 TaqIA homozygous polymorphic genotype among non-smoking controls may indicate that the mutant TaqIA allele of the DRD2 gene is associated with the amount of smoking. In addition, age at onset of smoking was much younger among participants who carried homozygous and heterozygous form of TaqIA polymorphism compared to those who carried the wild homozygous genotype, indicating that the TaqIA polymorphic allele may be related to the age at onset of smoking.

No relationship was observed between smoking addiction and obesity (eating addiction). Studies based on ethnic origin have shown that about 30% of Europeans, 60% of Asians, and 41% of the African populations carried one or two copies of the A1 allele. Although many studies have investigated the relationship between DRD2 TaqIA polymorphism and tobacco use and smoking behavior, they have yielded conflicting results.[19] Munafò et al.[20] conducted a study to consider whether or not this relationship varied according to gender, but did not find a significant relationship. However, an association was found between early age at onset and continuing this behavior among men. In another study investigating the relationship between the DRD2 gene and the amount of smoking, DRD2 gene polymorphism

was not found to be associated with starting and continuing smoking, but when the relationship between smoking and ethnicity was investigated, it was determined that Caucasians, especially males started smoking earlier and continued smoking at greater rates compared to Asians.[12] Since conflicting results of studies may be attributed to genetic differences between populations, the current study conducted among our society will contribute new data to the literature.

A study by Kleinjan et al.[21] included 1,399 adolescents with mean age of 12.3 years and reported high rates of tobacco and drug use among adolescents with DRD2 TaqIA polymorphic and heterozygous forms, and that familial tobacco use was present in most of their families. In addition, it has been previously determined that individuals whose families smoked had higher rates of starting smoking at an early age and smoked in high quantities,[4,22] which was consistent with our results. Ultimately, we believe that preventing smoking among DRD2 TaqIA polymorphic and heterozygous individuals with family histories of smoking is especially important in terms of protecting public health.

In conclusion, in order to raise awareness of the significance of DRD2 genotypes in tobacco addiction, identifying children with heterozygous and polymorphic DRD2 genotypes through genetic screening as in our study will greatly benefit public health services, as it may allow families of these children to understand the importance of keeping these children away from substance exposure (due to high tendency to addiction) and encourage them towards different hobbies, thus we believe this can reduce tobacco addiction. In addition, presence of participants in the control group who carried DRD2 TaqAI polymorphic allele and who have family members that smoke suggests that education and awareness may succeed in preventing smoking. The fact that it is possible to control negative consequences that may arise from the genetic information that we carry, such as the tendency towards addiction, through conscious choices will lead to healthier societies.

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