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A study on dopaminergic and serotonergic genes that have a role in smoking addiction at the DNA level

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ABSTRACT

Aim: To investigate *DRD2* (TaqIA and -141C Ins/Del), *DAT* (40-bp VNTR) and *MAO-A* (uVNTR) gene variants which have a role in dopaminergic and serotonergic systems within the frame of comparing them in smoker and non-smoker individuals; as well as to investigate them in case of clinical parameters such as their effects on age of starting to smoke, average number of cigarettes smoked each day and Fagerstrom Test for Nicotine Dependence score.

Methods: 164 smoker (90 male, 74 female) and 124 non-smoker (58 male, 66 female) individuals included in the study. Variants were analyzed by PCR or the PCR-RFLP method. Results were compared between groups and with clinical parameters.

Results: *DRD2*/-141C Ins/Del variant was found to be associated with smoking addiction (p<0.001) and clinical parameters (p=0.037), whereas *MAO-A*/uVNTR variant was associated with smoking addiction solely in male (p=0.003). No significant association was found in relation to smoking addiction and clinical parameters in *DRD2*/TaqIA and *DAT*/40 bp VNTR variants.

Conclusion: It was shown that *DRD2* Del/Del genotype, *MAO-A* 4R allele presence in males may contribute to the risk of smoking addiction; that *DRD2* Ins/Ins genotype, *MAO*-A 3.5R alleles in males may be linked to a protective effect. *DRD2* Ins/Del genotype was found to be associated with less smoking per day.

Key words: Genetics, nicotine dependence, dopamine, serotonin, variant.

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1. Introduction

Smoking addiction is a critical public health hazard in our country and in the whole world. According to data provided by World Health Organization (WHO), there are more than 1 billion smokers whose age is 15 and older globally and approximately 19 million cigarette smokers who are between 13 and 15 years old [1]. Cancer types caused by smoking, lung diseases, and cardiovascular diseases are among the leading reasons of death in Turkey as well as in many countries of the world. In addition, passive exposure to cigarette smoke also causes these diseases. This addiction behavior is responsible for around 7 million deaths

worldwide each year [2].

Nicotine is the main component in cigarette smoke that causes the addiction [3]. Nicotine has the potential to become as severely addictive as heroin and cocaine. The effect of nicotine, as well as other addictive substances, on dopaminergic and serotonergic systems in the brain is critical in case of addiction [4, 5]. Dopamine secretion from nucleus accumbens due to nicotine plays a crucial role in generating nicotine reward [6, 7]. Studies have shown that the nicotine susceptibility of variations in dopaminergic systems controls the smoking phenotypes such as starting to smoke early and desire to smoke under stress [8-10]. There are evidences demonstrating that the serotonergic mechanisms may mediate behavioral effects of nicotine besides dopamine system. Studies have shown that the presence of nicotine in the brain increases serotonin secretion, whereas nicotine withdrawal has quite the opposite effect on it [11, 12]. It is hypothesized that the smoking addiction may be related to decreased serotonin neurotransmission originating from genetic variant [13]. Parallel to these findings; dopamine receptor D2 (DRD2), monoamine oxidase-A (MAO-A) that plays a role in dopamine and serotonin breakdown, and genes that code dopamine transporter (DAT) proteins which regulate recovery of dopamine from synaptic gap are the suitable candidate genes that may be related to the genetic bases of smoking addiction. *DRD2* gene is located on chromosome 11q23.2 [14]. TaqIA polymorphism on this gene is a functional single-nucleotide polymorphism causing glutamic acid (A2 allele) and lysine (A1 allele) amino acid replacement on codon 713, and it is found to be related to DRD2 protein amount and change in substrate connection specificity [15, 16]. Other polymorphism in this gene is in gene's promotor region, is on -141 position and is either cytosine (C) insertion (Ins) or deletion (Del), hence named as -141C Ins/Del. This functional polymorphism alters *DRD2* expression, and Del allele is associated with low promotor activity [17].

The gene that codes DAT (*SLC6A3*, *DAT1*) is located on chromosome 5p15.33. *DAT* variant, which is the most studied variant, is a 40-bp VNTR located on 3'-untranslated region (3'-UTR) and may repeat between 3 and 13 times. It is proclaimed that this polymorphism may affect *DAT* expression [18].

MAO-A gene is located on chromosome X [19]. A 30-bp VNTR polymorphism is spotted on its promotor region of its gene that affects transcription level [20]. Studies shown that transcription efficiency is two to three times more in long alleles (3.5R, 4R and 5R) compared to short alleles (3R and 2R) and that 4R variant is transcribed more efficiently than other variants [20, 21].

The objective of this study is to evaluate DRD2 (TaqIA and -141C Ins/Del), *DAT* (40-bp VNTR), *MAO-A* (uVNTR) functional gene variants between smokers and non-smokers. It also aims to explore the association of these genetic variants with various clinical factors, such as the age of smoking initiation, the average number of cigarettes smoked each day and the scores obtained from the Fagerström Test for Nicotine Dependence (FTND).

2. Materials and metods

In this case-control study, 164 smoker individuals (90 males, 74 females) who applied to Yedikule Chest Diseases and Thoracic Surgery Training and Research Hospital, Smoking Cessation Polyclinic between ages of 18 and 80 are included in patient group. 124 non-smoker healthy individuals (58 males, 66 females) with no addictions between ages of 18 and 67 in control group. Our research has begun after acquiring the permission of Istanbul

University Istanbul Faculty of Medicine Clinic Studies Ethics Committee (2014/1196). Each voluntary participant is informed regarding the study and signed forms of consents are received.

Demographic data of the individuals in non-smoker group and demographic and clinical data and data regarding smoking addiction levels are recorded of the individuals in smoker group. Smoking addiction levels of individuals have been determined by using Fagerstrom Test for Nicotine Dependence (FTND) that consists of six items which evaluate the smoking behavior in different aspects [22, 23].

DNA is extracted from the peripheral blood samples of participants. Genetic variants of DRD2 (-141C Ins/Del) and DRD2 (TaqIA) are analyzed by PCR, while genetic variants of DAT (40bç VNTR) and MAO-A (uVNTR) are assessed by PCR-RFLP, according to previously established protocols [17, 24, 25, 26]. Details regarding the primers, annealing temperatures, and restriction enzymes utilized are provided in Table 1. PCR products are separated on 2.5% agarose gel, and digestion products are separated on 3.5% agarose gel, subjected to 80V for a minimum duration of 1 hour. The gels are subsequently visualized under UV light using a gel imaging system and individual genotypes are determined.

The results are compared both between the groups and with clinical parameters. Data analyses are performed with IBM SPSS Statistics for Windows, Version 21.0 (Armonk, NY: IBM Corp.). The genotype and allele distributions between smokers and non-smokers are compared by chi-square test or when appropriate by Fisher's exact test. Additionally, deviations from Hardy-Weinberg equilibrium are evaluated in both groups. The significance tests are two-tailed, and the results are considered statistically significant when p is less than 0.05.

3. Results

164 smokers and 124 non-smokers are included into our study. The age average of smoker group is 42.87 ± 13.24, whereas non-smoker group's age average is 33.95 ± 12.48. Gender distribution between smoker and non-smoker groups are determined to be similar (p=0.173).

Age of starting to smoke in individuals in the smoker group ranges between 8 and 44 and calculated to be 19.22 ± 6.09 age on average. Duration of smoking varies between 1 and 60 years, and average duration is calculated to be 23.87 ± 13.66 years. The number of cigarettes smoked per day by individuals in smoker group

Table 1. Primer sequences and methods used in analysis of studied region.

Studied Region	**Method (Restriction Enzyme)**	**Primer Sequence**	**Annealing Temperature /Number of Cycles**	**[Ref]**
DRD2 (-141C Ins/Del)	PCR-RFLP (BstNI*)	ACTGGCGAGCAGACGGTGAGGACCC TGCGCGCGTGAGGCTGCCGGTTCGG	63°C / 35 cycles	[17]
DRD2 (TaqIA)	PCR-RFLP (TaqI**)	GCACGTGCCACCATACCC TGCAGAGCAGTCAGGCTG	57°C / 30 cycles	[24]
DAT (40bç VNTR)	PCR	TGTGGTGTAGGGAACGGCCTGAG CTTCCTGGAGGTCACGGCTCAAGG	65°C / 42 cycles	[25]
MAO-A (uVNTR)	PCR	ACAGCCTGACCGTGGAGAAG GAACGGACGCTCCATTCGGA	62°C / 35 cycles	[26]

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varies between 1 and 60. Average number of cigarettes smoked in a day is 20.34 ± 11.92.

74.8% of the individuals in smoker group (N=116) tried to cease smoking at least once, and 25.2% of them (N=39) has never tried to cease smoking. After following for six months, it is determined that the ratio of the people who ceased smoking is 15.5% (N=23), and the ratio of the people who continued to smoke is 84.5% (N=125). The distribution of the answers given by individuals in the smoker group to Fagerstrom Test for Nicotine Dependence is demonstrated in Table 2.

3.1. DRD2 TaqIA

DRD2 TaqIA variant genotype and allele frequencies have been compared between smoker/non-smoker groups and no significant difference is observed (Table 3). It is determined that in smoker group, genotype distribution deviates from Hardy-Weinberg equilibrium (p=0.045) (Table 3). Genotype and allele distribution in groups who ceased/not ceased smoking after treatment is also compared, and no significant relationship is found (p>0.05 for each) (data is not shown). *DRD2* TaqIA genotypes have been compared in terms of

Table 2. Patient results for fagerstrom test for nicotine dependence.

Fagerstrom Test for Nicotine Dependence Questions	Answers	N (%)
How soon after you wake up do you smoke your first cigarette?	After 1 hour	36 (%22.9)
	30-60 minutes	41 (%26.1)
	6-9 minutes	26 (%16.6)
	Within 5 minutes	54 (%34.4)
Do you find it difficult to refrain from smoking in places where it is forbidden?	No	68 (%43.3)
	Yes	89 (%56.7)
Which cigarette would you hate most to give up?	All others	64 (%40.8)
	The first one in the morning	93 (%59.2)
How many cigarettes/day do you smoke?	10 or less	37 (%23.6)
	11-20	62 (%39.5)
	21-30	40 (%25.5)
	31 or more	18 (%11.5)
Do you smoke more frequently during the first hours after waking than during the rest of the day?	No	63 (%40.1)
	Yes	94 (%59.9)
Do you smoke if you are so ill that you are in bed most of the day?	No	68 (%43.6)
	Yes	88 (%56.4)

Table 3. DRD2 TaqIA genotype and allele distribution in smoker/non-smoker groups.

Variables		Smoker	Non-smoker	*p*
		N (%)	N (%)	
***DRD2* TaqIA Genotype**	A1/A1	8 (%5.1)	5 (%5.8)	*0.147*
	A1/A2	37 (%23.6)	30 (%34.9)	
	A2/A2	112 (%71.3)	51 (%59.3)	
	Total	157 (%100.0)	86 (%100.0)	
	HWEp	0.045	0.833	
***DRD2* TaqIA Allele**	A1	53 (%16.9)	40 (%23.3)	*0.087*
	A2	261 (%83.1)	132 (%76.7)	
	Total	314 (%100.0)	172 (%100.0)	

number of cigarettes smoked each day, Fagerstrom nicotine dependence score and age of starting to smoke, and no significant difference has been observed (p>0.05 for each) (data is not shown).

3.2. DRD2 -141C Ins/Del

Upon comparing genotype frequencies of this region between smoker/non-smoker groups, it is determined that in non-smoker group Ins/Ins genotype is significantly increased, whereas Del/Del genotype is significantly high in smoker group (p<0.001) (Table 4). In addition, allele distribution in smoker and non-smoker groups is found to be significantly different (p<0.001). Ins allele is higher in non-smoker group, whereas Del allele is high in smoker group (Table 4). Genotype distributions both in smoker group and non-smoker group are determined to be deviating from Hardy-Weinberg equilibrium (respectively; p=0.000 and p=0.027) (Table 4). Genotype and allele distribution in groups who ceased/not ceased smoking after treatment is also compared and no significant relationship is found (p>0.05 for each) (data is not shown).

DRD2 -141C Ins/Del genotypes have been compared in terms of number of cigarettes smoked per day, Fagerstrom nicotine dependence score and age of starting to smoke; and the difference between genotypes is statistically significant in case of number of cigarettes smoked per day (p=0.037) (Table 5). The differences are examined in detail, and it is found that the individuals having Ins/Del genotype smoke significantly less than the

Table 4. DRD2 -141C Ins/Del genotype and allele distribution in smoker/non-smoker groups.

Variables		**Smoker**	**Non-smoker**	***p***
		N (%)	**N (%)**	
***DRD2* -141C Ins/Del Genotype**	Ins/Ins	72 (%49.3)	**64 (%81.0)**	***<0.001***
	Ins/Del	32 (%21.9)	12 (%15.2)	
	Del/Del	**42 (%28.8)**	3 (%3.8)	
	Total	146 (%100.0)	79 (%100.0)	
	HWEp	0.000	0.027	
***DRD2* -141C Ins/Del Allele**	Ins	176 (%60.3)	**140 (%88.6)**	***<0.001***
	Del	**116 (%39.7)**	18 (%11.4)	
	Total	292 (%100.0)	158 (%100.0)	

Table 5. Comparison of DRD2 -141C Ins/Del genotypes in terms of various parameters.

Variables		**N**	**Mean**	**Median**	**Minimum**	**Maximum**	***p***
Number of cigarettes smoked per day	Ins/Ins	70	23.43	20	2	60	***0.037***
	Ins/Del	30	**16.80**	20	5	35	
	Del/Del	42	18.76	20	1	60	
Fagerstrom nicotine dependence score	Ins/Ins	72	5.39	6	0	10	*0.824*
	Ins/Del	28	4.96	5.5	0	10	
	Del/Del	41	5.37	6	0	10	
Age of starting to smoke	Ins/Ins	70	18.77	18	10	40	*0.084*
	Ins/Del	31	21.55	20	10	44	
	Del/Del	41	18.00	17	8	37	

individuals having Ins/Ins genotype (heterozygote advantage) (p=0.022). There is no significant difference between genotypes in terms of other parameters (p>0.05 for each) (Table 5).

3.3. DAT 40-bp VNTR

Distribution of genotypes and alleles of *DAT* 40-bp VNTR variant in smoker/non-smoker groups and also distribution of it in groups who cease/not cease smoking after treatment is determined. Genotypes determined in order for making statistical evaluation, divided into two groups as 10R/10R genotype and other genotypes [27]. When genotype groups are compared in smoker/non-smoker groups, no significant difference is determined (p>0.05) (data is not shown). Distribution of the genotype groups in groups who ceased/not ceased smoking is also compared, and no significant relationship is observed (p>0.05 for each) (data is not shown).

3.4. MAO-A uVNTR

As *MAO-A* is a gene located on X chromosome, genotype frequencies of *MAO-A* uVNTR variant are separately handled for men and women groups. There is no statistically significant difference observed in women in smoker/non-smoker groups (p>0.005) (Table 6). In men, it is determined that 3.5R variant is significantly high in non-smoker group than smoker group and 4R variant is significantly high in smoker group (p=0.003) (Table 6). There is no significant difference observed in genotype distribution of people who ceased/not ceased smoking after treatment (p>0.05) (data is not shown).

4. Discussion

Nicotine, one of the important components of cigarette smoke, elevates synaptic concentrations of dopamine and serotonin more than natural rewards do. This is regarded as one of the factors as to why smoking habit is maintained. It is assumed that the genes regulating extracellular dopamine and serotonin concentrations are related to developing smoking addiction risk and cessation of smoking. *DRD2* (TaqIA and -141C Ins/Del), *DAT* (40-bp VNTR), *MAO-A* (uVNTR) gene variants in dopaminergic and serotonergic pathways of smoker and non-smoker individuals have been compared in this study and the relations of these gene variants regarding the clinical parameters such as age of starting to smoke, number of cigarettes smoked per day, and

Table 6. MAO-A uVNTR genotype and allele distribution in smoker/non-smoker groups.

Variables		**Smoker**	**Non-smoker**	***p***
		N (%)	**N (%)**	
Females *MAO-A* uVNTR Genotype	3.5R/3.5R	17 (%23.6)	6 (%9.7)	*0.081*
	3.5R/4R	30 (%41.7)	29 (%46.8)	
	3.5R/5R	0 (%0.0)	3 (%4.8)	
	4R/4R	23 (%31.9)	23 (%37.1)	
	4R/5R	2 (%2.8)	1 (%1.6)	
	Total	72(%100.0)	62 (%100.0)	
Males *MAO-A* uVNTR Genotype	3.5R	26 (%30.2)	**29 (%56.9)**	***0.003***
	4R	**58 (%67.4)**	20 (%39.2)	
	5R	2 (%2.3)	2 (%3.9)	
	Total	86 (%100.0)	51 (%100.0)	

Fagerstrom Test for Nicotine Dependence (FTND) score have been studied. There is no comprehensive study in the literature that examine the relationship between these genetic factors, smoking status, and clinical parameters within our population. Our research fills this gap by identifying genetic factors specific to our population. This research will enhance our understanding of the causes of smoking addiction and provide insights into the effectiveness of various treatment approaches.

DRD2 is located in dopaminergic neurons which are centrally present in brain's reward pathways and serves a function in dopamine secretion as an auto receptor [28]. The substantial place of it in regulating the dopaminergic system turned DRD2 into the focus of many genetic studies regarding the addictions. *DRD2* TaqIA variant is one of the most studied variants in terms of addiction. It is asserted that A1 allele is related to DRD2 receptor intensity and function decrease [16, 29]. Various studies propose that A1 allele is correlated with alcoholism [30], substance use disorder [31] and obesity [32]. Some studies which study relationship between *DRD2* TaqIA variant and smoking addiction correlates A1 allele with smoking addiction tendency [33-36]. In a research containing Malay male population it is found that A1/A2 genotype is related to smoking [37], whereas in researches in Japan population, A2/A2 genotype is correlated with high smoking addiction risk [38, 39]. Styn et al. alleged that the individuals who have A2/A2 genotype have more tendency to cease smoking compared to the individuals who have at least one A1 allele [40]. There are also some studies in literature that compared smoking and non-smoking individuals in terms of *DRD2* TaqIA variant and found no significant difference in allele distribution [41-43]. However, in various studies, it is asserted that there is no significant relationship between TaqIA variant and smoking cessation [44, 45]. Liu et al. determined a correlation between *DRD2* TaqIA variation and number of cigarettes smoked per day in a study they performed on Chinese males *DRD2* TaqIA [46]. On the contrary, in various studies that evaluate TaqIA genotypes in terms of age of starting to smoke, number of cigarettes smoked per day and FTND score, there is no relationship is found [42, 47, 48]. Whereas in our study; when TaqIA variant is examined in terms of smoking and smoking cessation, there is no significant difference is observed between groups. Suchlike, there is no significant correlation is spotted between TaqIA genotypes and number of cigarettes smoked per day, FTND score and age of starting to smoke.

-141C Ins/Del functional variant in *DRD2* gene changes the *DRD2* expression, Del allele is correlated with low promotor activity and Del allele is determined to cause 21-43% less *DRD2* expression (17). It is seen that in the studies in literature, there is no significant relationship between -141C Ins/Del variant and smoking status, smoking cessation, age of starting to smoke and number of cigarettes smoked per day [36, 38, 40, 48]. In a study consisting of electronic cigarette smokers, there is no significant relationship is found between -141C Ins/Del variation and smoking status again [49]. In our study, -141C Ins/Del variant is determined to be significantly correlated with smoking both on genotype and allele level (Table 4). Ins/Ins genotype exhibits a significantly higher frequency among non-smokers, while Del/Del genotype is predominantly found in the smoker group. Similarly, the presence of Ins allele increases among non-smokers, while Del allele is more prevalent in smokers. These findings suggest that Ins allele may be associated with a protective effect against smoking addiction, whereas Del allele appears to be linked to a predisposition for smoking addiction.

Genotypes, when investigated in case of number of cigarettes smoked per day, it is observed that the number of the cigarettes smoked per day by the individuals that have Ins/Del genotype is significantly lower than those who have Ins/Ins genotype (Table 5). This circumstance can be interpreted as the individuals having Ins/Del genotype have the heterozygote advantage. There is no relationship observed between -141C Ins/Del variant and smoking cessation in our study. When genotypes are compared in terms of age of starting to smoke and FTND score, there is no significant difference observed as well.

DAT1 gene codes dopamine transporter (DAT) which is an important part of dopaminergic system and regulator of extracellular dopamine intensity [50-52]. It is proposed that the 40-bp VNTR variant may affect *DAT* expression [18]. 9R and 10R alleles are the most common ones, and allele frequencies differ among populations. Uzun et al. [53] found mostly 10R and 11R variants in the study they performed on Turkish population regarding the relation between obesity and *DAT1* VNTR gene variant. The results of the studies that examine the correlation between DAT protein level and 10R allele are contradictory. DAT intensity in individuals having homozygote 10R genotype is observed to be significantly increased via some of the brain imaging studies [54]. In various studies, 10R allele is correlated with abnormally active DAT level, correspondingly increasing the dopamine retrieval [55]. It is assumed that individuals having 10R allele may receive higher rewards from nicotine [56].

There are also some studies that correlate 10R allele with decreased DAT intensity [57, 58]. Ohmoto et al. [27] expressed that 10R/10R genotype may decrease *DAT* expression, causing high synaptic dopamine level; and this situation may prevent heavy smoking. Some reports show that there are no functional differences in this variant [59-61]. There are some studies that correlate *DAT1* gene with addiction behavior [40, 62-65]. Studies that investigate the relationship between smoking addiction and 40-bp VNTR variant brought a wide variety of results. Various studies correlate *DAT1* 9R allele with low smoking addiction risk and increase in smoking cessation [62, 66-68]. Laucht et al. [69] found out that *DAT1* 10R/10R genotype is related to early smoking and decrease in smoking cessation. On contrary of these studies, Vandenbergh et al. [70] suggested that 10R allele is observed more frequently in adults who never smoked, whereas Ohmoto et al. [27] observed in the wake of a study they performed in 2014 that 10R/10R genotype is correlated with low nicotine addiction. There are studies that do not discover a relation between *DAT* 40-bp VNTR variant and smoking addiction. Sieminska et al. [56] observed in the wake of a study they performed in 2009 that there is no significant difference in *DAT1* gene variants distribution in smokers and non-smokers. Yet in the same study, when they compared 10R/10R variant to other genotypes, they could not determine a significant difference in terms of number of cigarette smoked each day and the age of smoking initiation. Jorm et al. [71] did not determine a significant relation between *DAT1* gene and starting and ceasing to smoke. In other studies, no relationship is observed between smoking cessation and *DAT1* gene variant [44, 72]. In our study; genotypes belonging to *DAT* 40-bp VNTR variant are split into two groups for enabling statistical evaluation: 10R/10R genotype and other genotypes [27]. Genotype groups have been compared between smoker/non-smoker groups and no significant difference is determined. There is no significant difference observed in distribution of people who ceased/not ceased smoking after treatment.

MAO enzyme facilitates oxidative deamination of biogenic amines, including dopamine and serotonin [73, 74]. The gene that codes MAO-A protein, one of the two subtypes of MAO, is located on the short arm of chromosome X [75]. This gene is an important candidate in terms of smoking addiction genetics. Some evidence shows that the individual differences in MAO enzyme amount and activity may affect smoking or smoking cessation behaviors [76]. Fowler et al. [77, 78] demonstrated in a study in which positron emission tomography method is used that smokers have less active MAO-A isoenzyme compared to that of non-smokers. It is asserted that *MAO-A* (uVNTR) variant in this gene causes differences in enzyme level and activity. It is shown in studies that *MAO-A* 4R variant is transcribed more efficiently than other variants and that transcription efficiency is up to two to three times higher in long alleles (3.5R, 4R and 5R) than short alleles (3R and 2R) [21]. Ito et al. [79] have not determined a significant distribution difference between smoker and non-smokers in males in terms of *MAO-A* genotype, whereas males that have 4R allele have a higher FTND score compared to those that do not. In females, having 4R allele is correlated with lower smoking risk. Jin et al. (2006) [80] designated that males that have 3R genotype, which causes low MAO-A enzyme activity, have a higher risk of smoking compared to the males that have 4R genotype. *MAO* gene variants that have a low enzyme activity are stated to be protective against heavy smoking, in a meta-analysis study, comprising 11 articles, made in order to evaluate the relationship between *MAO* variants and smoking habits. In addition, *MAO-A* uVNTR active variant is specified to lower the success rate of smoking cessation in males. In the study of Tochigi et al. [81], there is no significant relation is found between *MAO-A* uVNTR and smoking habit. Alike, in the study of Huang et al. [82], 1518 persons were genotyped in terms of *MAO-A* uVNTR variant and no significant correlation is spotted between *MAO-A* uVNTR variant and smoking habit. In a different study, it is ascertained that females having a short allele have a lower risk of starting to smoke. In the same study, compared to non-smoker females, homozygote short allele is observed more frequently in smoker females [83]. Kõks et al. [84] correlated 3R allele with a stronger smoking addiction in a study performed on Vietnamese male population. In our study; genotype distributions of *MAO-A* uVNTR variant is compared between smoker/non-smoker and groups that ceased/not ceased smoking after treatment (separately for males and females). No statistically significant difference is observed in females, whereas in males 3.5R variant is found to be higher in non-smoker group and 4R variant is found to be higher in smoker group (Table 6). Nevertheless, it is observed that there is no statistically significant difference between the groups that ceased/not ceased smoking after treatment.

4.1. Conclusions

Consequently, both genotype and allele distributions of *DRD2*/-141C Ins/Del variants are determined to be significantly different between smoker and non-smoker groups. In non-smoker group, Ins/Ins genotype and Ins allele is significantly high, whereas in smoker group Del/Del genotype and Del allele is significantly high. No significant difference is observed in case of *MAO-A*/uVNTR variant genotype distribution in females between smoker and non-smoker groups; whereas in males 3.5R variant is observed significantly more frequently in non-smoker group, and 4R variant is observed significantly more frequently in smoker. No significant difference is determined between smoker and non-smoker groups in terms of

DRD2/TaqIA and *DAT*/40-bp VNTR variants' genotype and allele distribution. Also, when the studied variants are investigated in cases of clinical parameters such as age of starting to smoke, Fagerstrom Test for Nicotine Dependence score and number of cigarettes smoked per day; a significant correlation is determined between *DRD2*/-141C Ins/Del variant and number of the cigarettes smoked per day only. The number of cigarettes smoked per day by individuals having Ins/Del genotype is significantly lower than that of individuals having Ins/Ins genotype.

This study has several limitations, including its relatively small sample size from a single center and its focus solely on the Turkish population. Despite these limitations, it is the first research to examine *DRD2* (TaqIA and -141C Ins/Del), *DAT* (40-bp VNTR), and *MAO-A* (uVNTR) gene variants in relation to smoking behavior and associated clinical parameters in the Turkish population. Our findings suggest that the *DRD2* Del/Del genotype and presence of the *MAO-A* 4R allele in males may contribute to the tendency for smoking addiction; and that the *DRD2* Ins/Ins genotype and presence of the *MAO-A* 3.5R allele in males may be correlated with protection. These results contribute valuable insights into the genetic underpinnings of smoking addiction in the Turkish population. Future research should involve larger sample sizes and diverse populations, and should also consider epigenetic factors. Addressing these limitations could contribute to the development of more effective strategies for preventing and treating nicotine addiction.

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