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Association Study of Opioid Receptor Delta-Type 1 (OPRD1) Gene Variants with Nicotine Dependence in an Iranian Population

Alireza Sharafshah[1] · Bahram Soltani[1] · Parvaneh Keshavarz[1]

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Abstract
Twins studies indicate that many individual factors are associated with genetic polymorphisms in tobacco use, dependence vulnerability, and the ability to quit smoking. Opioid receptor delta-type 1 (OPRD1) is one of the most important genes in the opioid pathway. Therefore, the current study aimed to investigate the association of variants located in the intron 1 of the OPRD1 gene, including rs2236857, rs2236855, and rs760589, with susceptibility to nicotine dependence among northern Iranians. DNA of 426 individuals, including 224 smokers and 202 healthy people, were extracted with the salting-out standard technique, qualified with Agarose gel, then quantified with Nanodrop, and finally genotyped by Amplification Refractory Mutation System (ARMS) PCR. All statistical analyses were performed by SNPAlyze version 8.1 and SPSS version 20. Results revealed no significant association of all three studied variants with the susceptibility to nicotine dependence in any models of inheritance. However, there were five haplotypes with an overall frequency higher than 0.05; no significant impact of any of them on nicotine dependence was observed. Altogether, rs2236857, rs2236855, and rs760589 were not associated with nicotine dependence among northern Iranians.

Keywords Association · Nicotine dependence · OPRD1 · Variant · Haplotype

Introduction

Drug addiction and smoking are among the main health problems in the world. According to a World Health Organization report, there were 1.1 million smokers worldwide, of whom 1.3 were about 15 years old (Organization 2011). Nicotine, as an addictive substance, enters the bloodstream immediately after smoking tobacco and reaches the brain within just 2 s. There, by affecting the brain reward pathways, nicotine creates excitement and vitality; although such feelings last only a few minutes, the consumer has a great desire to consume it in order to continue enjoying the sensation and prevent hangover symptoms. In the long run, nicotine intake can make the body tolerance and resilience; thus, despite all the evidence on the harmful effects of tobacco, avoiding nicotine consumption becomes intolerable as its addiction is developed. Nicotine overdose occurs very gradually since its amount absorbed by the body does not cause an overdose (Fujioka and Shibamoto 2006; Holford et al. 2010; Kryger 2004; Lynch and Bonnie 1994; Rodgman and Green 2003; Rogge et al. 1994; Wolz et al. 2002). However, it is a toxic substance, and consuming too much of it can be dangerous. Excessive nicotine consumption can cause confusion, low blood pressure, and impaired respiration, leading to death in some cases (Brookman 1984; Holford et al. 2010).

Drug dependence has different causes, including genetic, environmental, and social factors. Since drug addiction is a complex disease, genetic results have been controversial in some studies; however, in some others, genetic changes have been proven to be directly associated with the disease (Albonaim et al. 2017; Dick and Foroud 2003; Rogge et al. 1994). Studies on twins have shown that many individual factors are associated with genetic polymorphisms in tobacco use, dependence vulnerability, and the ability to quit smoking. Smoking behavior is generally influenced by genetic factors. Since smoking is a polygenic phenotype, several genes are involved with (Heath et al. 1999; Li et al. 2003; Sullivan and Kendler 1999). The heritability to start smoking is estimated to be approximately 48–60%; a meta-analysis study reports

✉ Parvaneh Keshavarz
keshavarz@gums.ac.ir; alirezascharafschah@gmail.com

[1] Cellular and Molecular Research Center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

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higher heritability in women (55%) than men (37%). For nicotine dependence (ND), heritability is reported to be 55–67% (Haberstick et al. 2007; Heath et al. 1999; Kendler et al. 1999; Li et al. 2003; Maes et al. 2006, 2004; Rose et al. 2009; Sullivan and Kendler 1999; True et al. 1999; Vink et al. 2005). In addition to genes for the dopaminergic, serotonergic, and nicotine receptor pathways, other candidate genes for addictive behaviors and substance use disorders can be found in the endogenous opioid system (Thorgeirsson et al. 2010). For instance, an association between the most central OPRM1 gene polymorphism (A118G) and smoking addiction has been reported (Ray et al. 2011). Also, a recent study revealed that OPRK1 variants have noticeable associations with cigarette smoking among Iranians (Albonaim et al. 2020). According to the previous studies in different populations, it has been shown that intron 1 of the OPRD1 gene was significantly associated with heroin and cocaine addiction. rs2236857 was identified as a tag SNP, which is in a complete linkage disequilibrium (LD) with rs2236855, and the association of rs760589 with opium consumption in Iranian population has been demonstrated (Levran et al. 2008; Nelson et al. 2014; Sharafshah et al. 2017).

Several studies have shown that people have variable tendencies to use drugs and nicotine. Understanding the molecular mechanisms of these substances affecting the human body seems to be an effective way to prevent the plausible damages and decrease the harmful consequences. Overall, this study investigates the association of *OPRD1* gene variants and their putative haplotypes with ND in smokers and healthy people of northern Iran since the previous studies have represented remarkable associations of these SNPs with opioid addiction.

Methods

Subjects

The sample size was computed by PS (power and sample-size calculation) software (http://www.mc.vanderbilt.edu/prevmed/ps). All participants consented to take part in a process approved by the Ethics Committee for Human Genome/Gene Research [IR.GUMS.REC.1396.532]. Sorting gender and age, a total of 224 male smokers were chosen. Inclusion criteria for the smokers were as follows: age over 18 years, not using any other drugs simultaneously, and not abusing substances except nicotine. Two hundred two healthy males were selected from people with no ND history by the time of the sampling with inclusion criteria, including age over 18 years, no history of nicotine and other addictive substance consumption, no history of drinking alcohol, and no history of psychotic problems. Urine toxicology tests were taken for confirming the absence of opiates or any other illicit drugs. Notably, all of the samples resided in Guilan province in the north of Iran and were selected randomly. Genomic DNA was extracted from the blood through salting-out standard protocol (Miller et al. 1988), and then extracted DNAs were examined by electrophoresis on 1% Agarose gel stained with Safe stain. Finally, DNAs were quantified by Nanodrop (ND-1000) for their concentration and purification levels.

Genotyping of *OPRD1* Variants

Three studied variants (rs2236857, rs2236855, and rs760589) were genotyped by the ARMS-PCR (Amplification Refractory Mutation System) standard protocol (95 ℃ for 5 min, 95 ℃ for 30 s, annealing time (56 °C for rs2236857, 57 °C for rs2236855, and 55.9 °C for rs760589) for 30 s, 72 ℃ for 40 s, and 4 ℃ for 5 min as holding time in 30 total cycles).

Haplotype Analysis

In order to assess the association of *OPRD1* gene variants with addiction susceptibility, haplotype analyses were accomplished on rs2236857, rs2236855, and rs760589 for all samples. The analyses between the two study groups were performed based on the maximum likelihood method with an expectation–maximization algorithm. Permutation *P* values were expected by equating haplotype frequencies between case and control subjects based on 10,000 replications.

Statistical Analysis

Statistical analyses were concluded from both SNPAlyze (ver.8.1, Dynacom, Japan) and SPSS (ver. 20, http://www.spss.com/) software. Allele and genotype frequencies of the *OPRD1* variants in the control and case groups were compared and checked by Pearson χ^2 statistic. Furthermore, deviations from Hardy–Weinberg equilibrium (HWE) were verified by a χ^2 goodness-of-fit test. Analyses were also done assuming dominant, co-dominant, and recessive models of inheritance and odds ratio (OR), their 95% confidence interval (CI) ranges. Additionally, their consistent *P* values were calculated by both SNPAlyze and the Web-Assotest program (http://www.ekstroem.com/). The significance level of all statistical tests was designated to be less than 0.05. The current study with a total sample size of 426 people had a statistical power of more than 90% to distinguish an association with $P < 0.05$ for alleles with a frequency higher than 10%.

Table 1 Genotype and allele distribution of OPRD1 gene variants among smokers and control subjects

SNP	Subjects	Genotype (%)			Allele (%)		*p*- value		Dominant model		Co-dominant model		Recessive model	
		MM	Mm	mm	Major	Minor	Genotype	Allele	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
rs2236857 (T>C)	Case	104 (46.4)	78 (34.8)	42 (18.8)	286 (63.8)	162 (36.2)	0.19	0.17	0.91 (0.62–1.32)	0.59	1.18 (0.91–1.54)	0.21	0.61 (0.36–1.05)	0.07
	Control	99 (49)	78 (38.6)	25 (12.4)	276 (68.3)	128 (31.7)								
rs2236855 (C>A)	Case	170 (75.9)	38 (16.9)	16 (7.2)	378 (84.4)	70 (15.6)	0.74	0.68	1.02 (0.65–1.58)	0.95	0.95 (0.70–1.29)	0.75	1.28 (0.63–2.58)	0.49
	Control	153 (75.7)	31 (15.3)	18 (9)	335 (82.9)	67 (17.1)								
rs760589 (G>A)	Case	98 (43.8)	88 (39.3)	38 (13.1)	284 (63.5)	164 (36.5)	0.39	0.45	1.01 (0.69–1.48)	0.97	0.92 (0.71–1.18)	0.49	1.36 (0.84–2.21)	0.21
	Control	88 (43.6)	70 (34.7)	44 (21.7)	246 (61)	158 (39)								

MM, Mm, and mm describe individuals with homozygous major alleles, heterozygous alleles, and homozygous minor alleles

Table 2 Haplotype analysis of oprd1 gene variants between smokers and healthy individuals

	Haplotype	Overall	Case	Control	Permutation *P*
1	T-C-G	0.44	0.44	0.44	0.96
2	C-C-A	0.14	0.14	0.14	0.92
3	T-C-A	0.14	0.13	0.14	0.7
4	C-C-G	0.12	0.13	0.11	0.3
5	C-A-A	0.06	0.06	0.05	0.86

Three SNPs (including rs2236857, rs2236855, and rs760589) were analyzed for haplotype associations. Five haplotypes with a frequency of more than 0.05% was found. *P* means *p*-value

Results

Comparison of the Allelic and Genotypic Frequency of *OPRD1* Variants

In the present research, the DNAs of 224 smokers (mean age = 39.9 ± 3) and 202 healthy controls (mean age = 38.7 ± 2) were analyzed to achieve the association of *OPRD1* variants with the susceptibility to ND. Three variants of *OPRD1* were examined in 426 study samples. Allele and genotype distribution frequencies of each variant in both study groups are represented in Table 1.

The genotype frequencies of rs2236857 in smokers and healthy people were as follows: T/T 46.4 and 49%, T/C 34.8 and 38.6%, and C/C 18.8 and 12.4%. The results show no significant difference in genotype frequencies of the two groups under any models of inheritance ($P = 0.07$, OR = 0.61, 95% CI = 0.61 [0.36–1.05] under the recessive model). The genotype frequencies of rs2236855 in the smokers and healthy controls were as follows: C/C 75.9 and 75.7%, C/A 16.9 and 15.3%, and A/A 7.2 and 9%, respectively. The frequency of this variant had no significant difference in the two study groups ($P = 0.49$, OR = 1.28, 95% CI [0.63–2.58] under the recessive model). For the other studied SNP (rs760589), the genotype frequencies in the two study groups, smokers (G/G 43.8%, G/A 39.3%, and A/A 13.1%), and healthy individuals (G/G 43.6%, G/A 34.7%, and A/A 21.7%) indicated no significant difference under any models of inheritance ($P = 0.21$, OR = 1.36, 95%CI [0.84–2.21] under the recessive model).

Comparison of Haplotype Frequencies in Control and Case Groups

Haplotype analyses were performed with SNPAlyze software. Among the haplotypes, including rs2236857, rs2236855, and rs760589, five haplotypes had overall frequencies higher than 5%. Further, among these haplotypes, no one has a *P* value lower than 0.05, showing significant differences between the two study groups (Table 2).

Discussion

The present study investigated the association of *OPRD1* variants (rs2236857, rs2236855, and rs760589) with ND among 224 smokers and 204 healthy people in a northern Iranian population. Due to the strong associations of these SNPs with opioid addiction in the prior studies, this study was designed to test the plausible association of these SNPs with nicotine dependence. All three variants were genotyped by the ARMS-PCR technique, and the final data were assessed via SNPAlyze and SPSS software. Results showed no significant association between the three studied SNPs and opioid dependence; also, no significant haplotype was found.

There are few studies with a focus on candidate variants in the opioid pathway that possibly affect the ND; however, there are several many on opioid dependence. Levran et al. showed that rs2236857 polymorphism in the American population is significantly associated with heroin use (Levran et al. 2008). Nelson et al. demonstrated that rs2236857 and rs2236855 are both in a LD and strongly correlated with substance use (Nelson et al. 2014). Moreover, Sharafshah et al. reported a significant relationship between the three variants, including rs2236857, rs2236855, and rs760589, with opioid use in the methadone-addicted Iranian population (Sharafshah et al. 2017). Berrendero et al. provided evidence for the distinct roles of different endogenous opioid components (OPRD1, OPRM1, OPRK1) in nicotine addictive properties (Berrendero et al. 2010). Studies by Berrendo et al., Walters et al., Göktalay et al., Ismayilova and Shoaib, Liu and Jernigan, and Trigo et al. indicated that activation of mu-opioid receptors (MORs) by the intrinsic ligand β-endorphin is implicated in the effects of the nicotine reward pathway (Berrendero et al. 2002; Göktalay et al. 2006; Ismayilova and Shoaib 2010; Liu and Jernigan 2011; Trigo et al. 2009; Walters et al. 2005). Endogenous enkephalins have also been shown to bind to the nicotine reward pathway (MOR and delta opioid receptors) (Berrendero et al. 2005). Galeote et al. stated that prodynorphin-derived opioid peptides, which are key endogenous ligands for the kappa opioid receptor (KOR), appear to participate in nicotine-induced reactions since arbitrary consumption of nicotine improved after deletion of the prodynorphin gene (Galeote et al. 2009). Besides, McCarthy et al. reported a decrease in DOR sensitivity in the nucleus accumbens during the early discontinuation of nicotine in mice, indicating a potential role of this receptor as an effective component of nicotine abstinence (McCarthy et al. 2011).

Among opioid receptor genes (OPRD1, OPRK1, and OPRM1), OPRM1 gene variants with ND have only been studied. Indeed, most of the reports focus on the A118G structural variant of OPRM1, representing controversial results on the association of this variant with ND. To the best of the authors' knowledge, there is no exact investigation on the association of rs2236857, rs2236855, and rs760589 with susceptibility to ND. Thus, the results of the present research are reported for the first time; these data cannot be compared directly with another study with a different population. It seems that the variants of the OPRD1 gene in the intron 1 involved with opioid addiction may not be involved with ND; however, as a plausible suggestion, the three studied and other variants of the OPRD1 gene should be genotyped in the other population, focusing on ND.

This study had some limitations concerning its sample size and the number of studied variants in the OPRD1 gene, which is better to be noticed in future studies.

In conclusion, variants in the intron 1 of the OPRD1 gene and their involving haplotypes have not a key role in ND. Thus, it is highly recommended to investigate the other regions of the OPRD1 gene. Altogether, the studied variants in the intron 1 of the OPRD1 gene (rs2236857, rs2236855, and rs760589) are not associated with ND among northern Iranians. However, further studies on the association of genetic and epigenetic parameters with cigarette smoking seem to be needed to definitively address the factors affecting this disease as one of the major issues at the social and individual levels in the world, especially in Iran.

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Author Contributions Mr. Alireza Sharafshah did all of experimental section of this research; also, he wrote some section of the paper including "Methods" and "Discussion" sections. Dr. Bahram Soltani had a full-time supervision on the experiment parts of the research and was being involved with the design of the research. Dr. Parvaneh Keshavarz was the main designer of the research, with full supervision on both experimental and writing parts of the paper; also, she wrote the majority of the paper.

Data Availability The authors have complete access to the data and material, and the data and materials reported in the paper are completely reliable.

Compliance with Ethical Standards

Ethical Approval All participants consented to take part in a process approved by the Ethics Committee for Human Genome/Gene Research [IR.GUMS.REC.1396.532], and it is certified that the study is performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent for Publication The authors affirm that human research participants provided informed consent for publication of their genomic data from the blood samples.

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