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Haplotype-based association study of Opioid Receptor Kappa-type 1 (OPRK1) gene polymorphisms with nicotine dependence among male smokers

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The WHO estimates there are 1.3 billion smokers worldwide, 80% of whom are in low- and middle-income countries, with 8 million tobacco-related deaths and 1.2 million deaths due to second-hand smoke, many smokers doing so because of the highly addictive nature of nicotine [1]. With both biological and behavioural aspects, nicotine dependence is a complicated multi-dimensional multi-factorial disorder, and based on the literature of addiction biology, an addicted person smokes ≥10 cigarettes per day [2,3]. Animal studies have pointed to the importance of the Opioid Receptor Kappa-type 1 (*OPRK1*) gene on nicotine dependence [4]. Altering the function or regulation of *OPRK1* results in decreased or increased susceptibility of reward system to beta-endorphins and enkephalins, and gene–gene interactions through epistasis have plausible impacts on the molecular background of nicotine dependence. Variants within *OPRM1*, coding for the mu-opioid receptor, also have a place in susceptibility and dependence on smoking as a source of nicotine [5,6]. We hypothesised links between three single nucleotide polymorphisms (SNPs) in *OPRK1* (rs997917, rs6985606, and rs6473797) and their haplotypes with nicotine dependence.

We tested our hypothesis in a case–control study, recruiting 202 men with a mean [SD] age 39.9 [3.0] smoking at least 10 cigarettes a day and 202 healthy male non-smokers aged 38.7 [2.0] (p = 0/0001). The sample size was determined by PS (power and sample-size calculation) software (http://www.mc.vanderbilt.edu/prevmed/ps). Inclusion criteria of the controls were age >18 years old, and lack of nicotine consumption. Exclusion criteria for both groups were the use of any other addictive substance, alcohol dependence history, and psychotic complications. Urine toxicology tests were taken to check the absence of opiates or any other illicit drugs. All participants consented to participate in the study which was approved by the Ethics Committee for Human Genome/Gene Research [No. 1930400417].

Total DNA was extracted from the peripheral blood by Salting-Out standard technique [7]; the quality being checked by electrophoresis on 1% Agarose gel. The concentration and purification of DNA samples were quantified by Nanodrop (ND-1000). Three SNPs (rs997917, rs6985606, and rs6473797) were genotyped by ARMS-PCR (Amplification Refractory Mutation System) by a standard protocol (95°C for 5 minutes, 95°C for 30 sec, Annealing Time (58°C for rs997917, 57°C for rs6985606, and 56°C for rs6473797) for 30 seconds, 72°C for 40 seconds, and 4°C for 5 minutes as holding time in 30 total cycles). To measure the association of *OPRK1* SNPs with smoking, haplotype analyses were performed on rs997917, rs6985606, and rs6473797 for all genotypes. Haplotype analyses between the two study groups were implemented according to the maximum-likelihood method with an expectation-maximization algorithm. Notably, permutation *P*-values were calculated through comparing haplotype frequencies between the two study groups based on 10,000 replications. All statistical analyses were obtained from both SNPAlyze (ver.8.1, Dynacom, Japan) and SPSS (ver. 20, http://www.spss.com/) software. Allele and genotype frequencies of the OPRK1 SNPs in both study groups were compared and checked by a Pearson $\chi 2$ statistic. Hardy–Weinberg Equilibrium (HWE) was determined by a $\chi 2$ goodness-of-fit test. Genotype analyses were performed based on dominant, co-dominant, and recessive models of inheritance and odds ratio (OR), their 95% confidence interval (CI) ranges and their consistent *P*-values were computed by both SNPAlyze and the Web-Assotest program (http://www.ekstroem.com/). The level of significance for all statistical tests was considered to be $P < 0.05$. The present study with a total sample size of 404 participants had a statistical power of more than 90% to define an association with a *P*-value less than 0.05 for alleles with a frequency higher than 10%.

Table 1 shows genotype and allele distribution of OPRK1 SNPs among smokers and non-smokers. There were significant differences in the genotype and allele frequencies of the rs997917 and rs69859606 SNPs, with lower frequencies of the C allele and higher frequencies of the T allele in smokers. In both cases, the recessive

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Table 1. Genotype and allele distribution of OPRK1 SNPs among smoker and control subjects.

		rs997917 (C > T)			rs6985606 (C > T)			rs6473797 (T > C)	
		Cases	Controls		Cases	Controls		Cases	Controls
Genotype (%)	CC	30 (14.8)	30 (14.8)	CC	65 (32)	110 (54)	TT	73 (36)	92 (46)
	CT	18 (8.9)	62 (30)	CT	76 (38)	73 (36)	TC	80 (40)	66 (33)
	TT	154(76.3)	110 (55.2)	TT	61 (30)	19 (10)	CC	49 (24)	44 (22)
	P	<0.0001			<0.0001			0.15	
Allele (%)	C	78 (20)	122 (30.2)	C	103 (51)	183(72.5)	T	113 (56)	125 (61.9)
	T	326 (80)	282 (69.8)	T	99 (49)	92 (27.5)	C	89 (44)	77 (38.1)
	P	0.0003			<0.0001			0.09	
Dominant Model	OR	1 (0.58–1.73)			2.52 (1.68–3.78)			1.48 (0.99–2.2)	
	p	1			<0.0001			0.54	
Co-Dominant Model	OR	1.5 (1.14–1.9)			2.20 (1.67–2.90)			1.22 (0.95–1.57)	
	P	0.003			0.0001			0.13	
Recessive Model	OR	2.68 (1.75–4.11)			4.17 (2.38–7.29)			1.15 (0.58–1.83)	
	P	<0.0001			<0.0001			0.50	

OR = Odd Ratio with 95% confidence interval. CC, CT, and TT (for rs997917 and rs6985606) and TT, CT, and CC (for rs6473797) describe individuals with homozygous major alleles, heterozygous alleles, and homozygous minor alleles, respectively.

models provided the highest odd's ratios. SNPs that were at a Hardy–Weinberg equilibrium (HWE) and had minor allele frequency (MAF) higher than 0.05 were calculated by D' and r-square tests. Among the haplotypes including rs997917, rs6985606, and rs6473797, there were seven with overall frequencies >5% (Table 2). Of these, the C-T-C, C-T-T, C-C-T and T-T-C haplotypes were significantly different between smokers and non-smokers.

Opioid peptides and receptors play a role in various forms of addiction, including nicotine effects. In the opioid system, interactions of prodynorphin lead to nicotine aversive impacts that suppress the rewarding pathway of nicotine dependence through beta-endorphins and enkephalins [4]. Isola et al. showed that nicotine withdrawal causes an increase in prodynorphin expression in mice [8], whilst Ise et al. reported mecamylamine as a potent blocker of Cholinergic Receptor Nicotinic Alpha 2 Subunit in rats, suggesting that opioid receptor antagonists might be useful for the improvement of the negative impacts related to nicotine abstinence [9,10]. SNPs in *OPRM1, OPRD1*, and *OPRK*1 have been linked with a variety of addictions such as opium and alcohol. Ray et al. showed a significant association of the A118 G SNP in the mu-opioid receptor gene with subjective reward differences in cigarette smoking [11] whilst Levran et al. showed that rs6473797 in *OPRM1* was significantly associated with heroin dependence [12]. Ashenhurst et al. demonstrated the association of rs997917 in *OPRM1* with alcohol dependence [13]. The work of Albonaim et al. showed a parallel association of rs997917 and rs6985606 in *OPRK1* with heroin dependence [14]. Roche et al. examined variations in *OPRM1, OPRD1*, and *OPRK*1 in a smoking cessation trial, concluding that genetic variation in opioid receptors is unrelated to treatment responses to naltrexone [15]. This data is somewhat in a contrast to our own, but this may reflect the differences in the groups as the cohorts in the study of Roche et al. were of a different race (African-American) and heavy drinkers, whereas we studied Caucasian non-drinkers. The current study had some limitations; for instance, the sample size and the number of studied SNPs in *OPRK1* that should be considered in the future work.

Table 2. Haplotype analysis of OPRK1 SNPs among smokers and non-smokers.

	Haplotype	Overall	Cases	Controls	*P*	PP
1	C-T-C	0.26	0.20	0.31	0.0007	0.0001
2	C-C-C	0.21	0.22	0.21	0.51	0.59
3	C-T-T	0.17	0.23	0.10	<0.001	<0.001
4	C-C-T	0.12	0.15	0.08	0.002	0.008
5	T-T-C	0.09	0.03	0.14	<0.001	<0.001
6	T-T-T	0.08	0.09	0.07	0.25	0.36
7	T-C-C	0.06	0.05	0.08	0.1	0.23

Three SNPs (including rs997917, rs6473797, and rs6985606) were examined for haplotype associations. PP refers to the Permutation *P*-value.

The literature regarding the association of opioid system with nicotine dependence is small compared with other types of addictions that include far fewer people, and consequently more investigations into the genetics of nicotine addiction are needed. This work represents an advance in biomedical science because it shows strong links between *OPRK1* SNPs and their haplotypes with nicotine dependence.

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Disclosure statement

The authors of the present study have no conflict of interest to disclose.

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References

[1] World Health Organisation. [cited 2020 Jun 3]. https://www.who.int/health-topics/tobacco#tab=tab_1.

[2] Sullivan PF, Kendler KS. The genetic epidemiology of smoking. Nicotine Tobacco Res. 1999;1:S51–S57.

[3] Chenoweth MJ, Novalen M, Hawk LW, et al. Known and novel sources of variability in the nicotine metabolite ratio in a large sample of treatment-seeking smokers. Cancer Epidemiol Preventi Biomarkers. 2014; 23:1773–1782.

[4] Berrendero F, Robledo P, Trigo MT, et al. Neurobiological mechanisms involved in nicotine dependence and reward: participation of the endogenous opioid system. Neurosci Biobehav Rev. 2010;35:220–231.

[5] True WR, Xian H, Scherrer JF, et al. Common genetic vulnerability for nicotine and alcohol dependence in men. Archives Gen Psychiatry. 1999;56:655–661.

[6] Verhagen M, Kleinjan M, Engels R. A systematic review of the A118G (Asn40Asp) variant of OPRM1 in relation to smoking initiation, nicotine dependence and smoking cessation. Pharmacogenomics. 2012;13:917–933.

[7] Miller S, Dykes D, Polesky H. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988;16:12–15.

[8] Isola R, Zhang H, Tejwani GA, et al. Dynorphin and prodynorphin mRNA changes in the striatum during nicotine withdrawal. Synapse. 2008;62:448–455.

[9] Ise Y, Narita M, Nagase H, et al. Modulation of opioidergic system on mecamylamine-precipitated nicotine-withdrawal aversion in rats. Psychopharmacol. 2000;151:49–54.

[10] Ise Y, Narita M, Nagase H, et al. Modulation of κ-opioidergic systems on mecamylamine-precipitated nicotine-withdrawal aversion in rats. Neurosci Lets. 2002;323:164–166.

[11] Ray R, Ruparel K, Newberg A, et al. Human Mu Opioid Receptor (OPRM1 A118G) polymorphism is associated with brain mu-opioid receptor binding potential in smokers. Proc Natl Acad Sci USA. 2011;108:9268–9273.

[12] Levran O, Londono D, O'hara K, et al. Genetic susceptibility to heroin addiction: a candidate gene association study. Genes Brain Behav. 2008;7:720–729.

[13] Ashenhurst JR, Bujarski S, Ray LA. Delta and kappa opioid receptor polymorphisms influence the effects of naltrexone on subjective responses to alcohol. Pharmacol Biochem Behav. 2012;103:253–259.

[14] Albonaim A, Fazel H, Sharafshah A, et al. Association of OPRK1 gene polymorphisms with opioid dependence in addicted men undergoing methadone treatment in an Iranian population. J Addict Dis. 2017;36:227–235.

[15] Roche D, Trela CJ, Argos M, et al. Lack of association between opioid-receptor genotypes and smoking cessation outcomes in a randomized, controlled naltrexone trial. Alcohol and Alcoholism. 2019; 54:559–565.