Association between opioid receptor mu 1 (*OPRM1*) gene polymorphisms and tobacco and alcohol consumption in a Spanish population

Francesc Francés*, Olga Portolés, Ana Castelló, José Antonio Costa, Fernando Verdú

Department of Preventive and Legal Medicine, School of Medicine, University of Valencia, Valencia Spain., Atenea Health Center, Aldaia, Spain

ABSTRACT

Evidence gained from animals and humans suggests that the encephalic opioid system might be involved in the development of drug addiction through its role in reward. Our aim is to assess the influence of genetic variations in the opioid receptor mu 1 on alcohol and tobacco consumption in a Spanish population. 763 unrelated individuals (465 women, 298 men) aged 18-85 years were recruited between October 2011 and April 2012. Participants were requested to answer a 35-item questionnaire on tobacco and alcohol consumption, as well as to complete the AUDIT and Fagerström tests. Individuals were genotyped for three polymorphisms in the opioid receptor mu 1 (OPRM1) gene, using a TaqMan® protocol. In males, the rs10485057 polymorphism was associated with total pure ethanol intake and with the risk of being an alcohol consumer. Also, this polymorphism was significantly associated with higher Fagerström scores. Rs1799971 had a different influence on adaptive and maladaptive patterns of alcohol use. Despite the limited sample size, our study might enrich current knowledge on patterns of alcohol use, because it encompasses both extreme and adaptive phenotypes, providing thus a wider perspective on this subject.

KEYWORDS: Tobacco; alcohol; drug abuse; genetic polymorphism
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INTRODUCTION

Opioid receptors are predominantly expressed in the brain cortex, limbic system, and brain stem. Mu receptor is the most expressed opioid receptor in the amygdala, thalamus, and mesencephalon. The opioid mu receptor is encoded by the *OPRM1* gene, located on chromosome 6q24–q25, spanning over 200 kb and containing at least nine exons [1]. This receptor can bind to several different endogenous ligands, such as b-endorphin, enkephalin, and exogenous opioids. The encephalic opioid system is involved in the development of drug addiction through its role in reward [2]. Knockout mice lacking mu receptors showed less reward and reinforcement manifestations after repeated exposure to several abuse drugs [3]. Furthermore, exposure to non-opioid abuse drugs, such as alcohol, cocaine and tobacco, is also associated with up- or down-regulation of the encephalic opioid receptor levels. In particular, nicotine enhances the endorphinic mu receptor mRNA and protein expression in brain regions important for drug reward in rodents [4,5] and stimulates endogenous opioid release [6], resulting in mu receptor activation. Also, Positron Emission Tomography (PET) studies have demonstrated increased levels of OPRM1 in the ventral striatum in alcohol-dependent patients and alcohol-craving states [7]. Furthermore, antagonists of mu receptors such as naltrexone have been shown to reduce alcohol consumption [8].

Several polymorphisms in the *OPRM1* gene have been reported to be associated with alcohol use and dependence, *A118G* (Asn40Asp, rs1799971) being the most commonly reported. The lower expression of the receptor observed in G carriers [9-11], together with the results of studies suggesting increasing consumption rates in G carriers have led to the so-called "opioid receptor deficiency theory". This theory proposes that a lower amount of receptor would lead to increases in alcohol intake with compensative ends. However, this theoretical construct does not consider the receptor affinity. An initial study found that the G allele was associated with higher affinity receptors [12], thus potentially diluting the strength of the defective OPRM1 proposed in this theory. More recent works suggested a lowering effect of the G allele on

Corresponding Author: Francesc Francés,
Department of Preventive and Legal Medicine. School of Medicine,
University of València, Avda Blasco Ibáñez 15, 46010 València, Spain
E-mail: ffrrances@uv.es, Phone: +34-963864655; Fax: +34-963864166

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receptor binding potential [10,13], whereas other works failed to demonstrate any effect on receptor affinity [14].

Studies conducted on the effect of this Single Nucleotide Polymorphism (SNP) on alcohol consumption also have contradictory results [15]. In a recent meta-analysis that included 28 different studies with a total of over 8,000 subjects, authors suggested that the OPRM1 rs1799971 variant not appear to influence risk for substance dependence [16]. Among those studies that have found significant association, major evidence points to a link between the 118G allele and alcohol dependence [17], alcohol craving [13,18,19], the presence of alcohol use disorder [20] and total alcohol intake [19]. In contrast to these findings, other researchers have not been able to show any association between this genetic polymorphism, alcohol consumption, and states of alcohol dependence [2,21,22]. Most studies finding associations between this SNP and alcohol consumption and/or dependence were undertaken on Asian populations, whereas those conducted on Caucasians mostly fail to demonstrate any association [23]. An ethnic-selective modulation on the effect of this SNP is a possible explanation of these results.

Likewise, in the studies on tobacco dependence, the rs1799971 variant is the most widely studied one, and results are equally discrepant [24,25].

The information regarding the relationship between other *OPRM1* gene polymorphisms and tobacco and alcohol consumption in humans is scarce. To date, no studies have been undertaken in a Spanish population.

Therefore, the aim of our study is to assess the possible association between polymorphisms in the *OPRM1* gene and alcohol and tobacco consumption in a Spanish population as well as to assess the possible modulating effects of environmental factors.

MATERIALS AND METHODS

Subjects

763 unrelated subjects (465 women, 298 men) participated in the study. This sample was obtained from a Caucasian non-isolated urban population and is representative of the Spanish-Mediterranean general population.

Participants (aged 18-85 years) were recruited through social, cultural and primary health care centers of the Valencian Community on the east Mediterranean coast of Spain. Individuals suffering from transmissible pathologies, physical or psychic decapacitating diseases were excluded. All the participants were informed about the aims of the study, its rules, and its procedures. After ensuring that they had understood the purpose and the implications of the study, they were requested to sign an informed consent form. The study protocol was approved by the Ethics Committee of the School of Medicine, University of Valencia, Spain.

DNA extraction and genotyping of selected polymorphisms

A whole saliva sample was obtained from each participant, and genomic DNA was isolated employing the standard methods.

PCR was performed in an Eppendorf DNA thermal cycler. Samples were genotyped for three *OPRM1* gene Single Nucleotide Polymorphisms (SNPs): rs1799971, rs510769, and rs10485057. For this purpose, we used a TaqMan® genotyping protocol.

The description of this protocol is as following: 20 ng of DNA was poured into each well of the reaction plate. 2.50 µL of TaqMan® Universal PCR Master Mix and 0.25 µL of the corresponding TaqMan SNP genotyping assay, containing VIC and FAM probes, were then added to each well.

The plate was then run on an Applied Biosystems 7900HT Fast Real-Time PCR under the following thermal cycling conditions: After an initial denaturation at 95ºC for 10 minutes, 40 cycles of denaturation at 92ºC for 15 seconds and annealing at 60ºC for 60 seconds were performed. Finally, genotype assignation was undertaken by registering the fluorescence emissions from each well at the corresponding VIC and FAM dye wavelengths.

Tobacco/Alcohol consumption and addiction clinical and lifestyle variables

A number of socio-demographic and behavioral variables such as gender, age, educational level, marital status, the amount of stress in everyday life and working place were assessed in order to appraise their potential confounding effect on the association study.

Alcohol consumption was carefully evaluated by a set of 22 questions on the use of alcoholic beverages during workdays and weekends, described in a previous article [26]. In the questionnaire, occasional or regular consumption of alcoholic beverages (including beer, white wine, red wine, champagne, brandy, whisky, vodka, anisette, martini, etc.) on working days and weekends was assessed. The mean ethanol consumption (in grams) was calculated by multiplying the amount consumed (in milliliters) by the percentage of ethanol supplied by each specific beverage according to the alcoholic graduation equivalence table [27]. Alcohol consumption was considered as a continuous variable expressed in grams per day. In addition, alcohol consumption was categorized as a "drinker" variable: "teetotaler" (consuming no alcohol) and "alcohol consumers" (any amount of alcohol consumed). In order to assess the level of ethanol dependence among alcohol drinkers,

participants were requested to answer an AUDIT questionnaire [28]. Alcohol use disorder (AUD) was considered to exist when the AUDIT test score was ≥8 [28].

Tobacco smoking habits were assessed by inviting the participants to complete a 13-item questionnaire – the questionnaire specified the amount and type of consumed tobacco, as well as the temporal evolution of habit, familiar co-morbidity and attempts at relinquishing the smoking habit.

Participants who admitted being habitual smokers were invited to answer the Fagerström questionnaire in order to quantify their level of nicotine addiction.

Statistical analysis

All statistical analyzes were performed using SPSS software package (SPSS Inc, Chicago, Illinois). To assess the associations between tobacco and alcohol consumption, codominants, dominants, and recessive models were fitted. To estimate associations between genotypes and the quantitative intake (grams of alcohol per day, number of cigarettes per day), ANOVA, t-Student and logistic regression models were performed. In the case of variables with non-parametric distribution, mean differences were assessed using Kruskal-Wallis tests. Tobacco and alcohol consumption risk was estimated employing logistic regression models, calculating the Odds Ratio (OR) and its confidence interval (CI). Crude and covariate-adjusted models were carried out. Hardy-Weinberg equilibrium was assessed using the Chi-Square test. The level of bilateral p significance was considered statistically significant when under $p = 0.05$.

RESULTS

The general socio-demographic characteristics of our participants are presented in Table 1. The alcohol consumption habit is very common in the study sample (82.6%). In contrast, tobacco smoking is less frequent (22.9%). Men generally consumed higher amounts of alcohol and tobacco than women – however, the difference was statistically significant only in the case of AUDIT score.

The genotype distribution of the three studied SNPs was: 526 AA, 220 A/G and 17 GG for the rs179971, 477 CC, 249 CT and 37 TT for the rs510769 polymorphism, and 632 AA, 126 AG and 5 GG for the 10485057 polymorphism, respectively. These genotype distributions were in line with Hardy-Weinberg equilibrium ($p = 0.280$, $p = 0.541$ and $p = 0.636$, respectively).

Table 2 shows the genotype frequencies of the three polymorphisms among tobacco and alcohol consumers and non-consumers.

Assessing the relationship between the rs1799971 and alcohol consumption, we found that (after adjusting for age, marital status and educational level, tobacco smoking and stress level) women carriers of the G allele had less risk of being alcohol consumers (OR=0.55; 95% CI: 0.304-0.998; $p = 0.049$).

However, when we assessed the associations of this polymorphism with the presence or absence of alcohol use disorder through the AUDIT score, we found that, after covariate adjustment, male carriers of the G allele had an increased risk of presenting an alcohol pattern consumption compatible with AUD (OR=2.52; 95% CI: 1.02-6.24; $p = 0.046$).

No association between the rs1799971 polymorphism and the number of cigarettes or the Fagerström test scores was found.

The rs510769 polymorphism was not associated with alcohol or tobacco consumption. Furthermore, we did not find any association either with quantitative variables of alcohol and cigarette consumption or with addiction test scores.

The third polymorphism studied, rs10485057, was associated with total pure alcohol intake in male participants (7.73±8.55 mg/day in AA as opposed to 4.93±5.80 mg/day in AG; $p = 0.026$). When covariates were taken into account in the analysis, the results remained significant ($p = 0.043$). Moreover, AA individuals were associated with an increased risk of being alcohol consumers (OR=2.40; 95%CI: 1.40-4.12; $p = 0.001$). The AA genotype was also associated with the increased AUDIT scores, but this value did not reach statistical significance.

When tobacco smoking was assessed, after adjusting for covariates we found that male carriers of the AA variant in the rs10485057 locus had higher Fagerström scores than G carriers (3.16±2.93 in AA in contrast to 2.83±2.59 in G carriers; $p = 0.037$). AA male participants also showed increased tobacco consumption expressed in number of cigarettes/day, but this value did not reach statistical significance.

DISCUSSION

The endorphin pathway seems to play a crucial role in the initiation and perpetuation of addictive behaviors. However, published evidence on the relationship between human genetic variations at the *OPRM1* locus and drug consumption is contradictory. In this study, we performed the association analyzes in order to test whether *OPRM1* polymorphisms were associated with consumption and addiction to legal toxic substances, such as alcohol and tobacco. We found an association between the rs1797779 G allele and a decreased risk of being an alcohol consumer in women. Moreover, when assessed the risk of presenting with AUD, we found that male carriers of the G allele had a significantly higher risk of presenting this pathologic consumption pattern. In our study, although the A-allele was associated with being an alcohol consumer in

TABLE 1. Means (SD) and proportions of socio-demographic variables in the study sample

	Teetotalers n=133		*p**	Alcohol consumers n=630		*p**
	Men (25)	Women (108)		Men (273)	Women (357)	
Age (yrs)	38.6±15.4	41.0±13.6	0.449	39.9±14.7	36.7±14.8	0.062
Education level n (%)			0.412			0.910
Illiterate	-	-		2 (0.7)	2 (0.6)	
Primary	9 (36)	30 (27.8)		44 (16.1)	63 (17.6)	
Secondary	11 (44)	42 (38.9)		136 (49.9)	169 (47.3)	
University	5 (20)	36 (33.3)		91 (33.3)	123 (34.5)	
Marital status n (%)			0.602			<0.001
Unmarried	10 (40)	31 (28.7)		108 (39.6)	176 (49.3)	
Married	15 (60)	69 (63.9)		158 (57.9)	148 (41.6)	
Unmarried couple	-	2 (1.8)		2 (0.7)	4 (1.1)	
Divorced	-	3 (2.8)		3 (1.1)	17 (4.8)	
Widow (er)	-	3 (2.8)		2 (0.7)	12 (3.4)	
Stress at work (1-10)	6.54±2.87	5.70±2.75	0.187	5.85±2.54	5.39±2.65	0.039
Life stress (1-10)	5.28±2.32	6.33±2.42	0.052	4.94±2.17	5.17±2.29	0.207
Alcohol intake (g/d)	-	-	-	7.50±8.78	4.75±6.50	0.004
AUDIT score	-	-	-	4.26±2.90	3.54±2.53	<0.001
Alcohol use disorder (AUDIT≥8) n (%)	-	-	-	29 (10.6)	26 (7.3)	0.110
	Non smokers n=588		*p**	Tobacco Smokers n=175		*p**
	Men (230)	Women (358)		Men (68)	Women (107)	
Age (yrs)	39.0±15.3	37.2±15.3	0.163	42.3±12.7	39.6±12.7	0.172
Education level n (%)			0.959			0.832
Illiterate	2 (0.9)	2 (0.6)		-	-	
Primary	48 (20.9)	77 (21.5)		21 (30.9)	30 (28)	
Secondary	119 (51.7)	188 (52.5)		30 (44.1)	46 (43)	
University	61 (26.5)	91 (25.4)		17 (25)	31 (29)	
Marital status n (%)			<0.001			0.456
Unmarried	88 (38.3)	158 (44.1)		22 (32.4)	41 (38.3)	
Married	138 (59.8)	167 (46.7)		43 (63.2)	58 (54.3)	
Unmarried Couple	1 (0.5)	5 (1.4)		1 (1.5)	1 (0.9)	
Divorced	1 (0.5)	17 (4.7)		2 (2.9)	3 (2.8)	
Widow (er)	2 (0.9)	11 (3.1)		-	4 (3.7)	
Stress at work (1-10)	5.79±2.55	5.34±2.56	0.055	6.30±2.62	5.90±2.93	0.375
Life stress (1-10)	4.92±2.18	5.23±2.32	0.100	5.13±2.21	6.05±2.44	0.013
Number of cigarettes (n/day)	-	-	-	12.9±10.3	11.2±8.7	0.248
Fageström score	-	-	-	3.38±2.96	2.89±2.09	0.322

* ***P*** values obtained from comparing the gender of alcohol consumers and teetotalers, and tobacco smokers and non smokers, respectively

TABLE 2. Genotype frequencies distribution in the study sample

	Teetotalers (133)			Alcohol consumers (630)		Non-smokers (588)			Tobacco smokers (175)	
Rs1799971 (n (%))										
A/A	101	(75.9%)		425	(67.5%)	408	(69.4)		118	(67.3)
A/G	30	(22.6%)	p=0.211	190	(30.1%)	166	(28.2)	***p***=0.716	54	(31)
G/G	2	(1.5%)		15	(2.4%)	14	(2.4)		3	(1.7)
Rs510769 (n (%))										
C/C	81	(60.9)		396	(62.9%)	371	(63.1)		106	(60.6%)
C/T	45	(33.8)	p=0.880	204	(32.4%)	188	(32)	***p***=0.772	61	(34.8%)
T/T	7	(5.3)		30	(4.7%)	29	(4.9)		8	(4.6%)
Rs10485057 (n (%))										
A/A	99	(74.4)		533	(84.6)	485	(82.5)		147	(84.0)
A/G	32	(24.0)	p=0.016	94	(15.0)	98	(16.7)	***p***=0.458	28	(16.0)
G/G	2	(1.5)		3	(0.4)	5	(0.8)		0	(0)

*P value obtained employing the χ^2 test by comparing genotype frequencies between alcohol consumers and teetotalers, and tobacco smokers and nonsmokers

women, men carrying the G-allele showed a higher risk of having AUD.

It is important to emphasize that most of the published studies had a case-control design, and associations were

assessed by comparisons with Alcohol Use Disorder patients or equivalents – although they may share biological and psychological characteristics, they cannot represent the general population.

Important evidence on the possible effects of this SNP on the general population has been published before, in Ehlers CL et al. (2008) [29] for example [29]. In that study, the selected sample was recruited from a general population of Native American origin. Results confirmed that the G-allele was associated with the unpleasant alcohol effects, resulting thus in lower alcohol intake. It seems that non-dysfunctional alcohol consumption is not comparable with pathological drinking patterns such as AUD, and these two might also differ in its genetic origin. However, other studies have found evidence that there might exist an association between A allele and AUD. The recent study by Koller G et al. (2012) [22], conducted on alcohol-dependent Caucasian subjects, demonstrated an increased frequency of the A allele in these patients.

Although interesting, the "opioid receptor deficiency" theory has several weak points. If G forms are associated with less amount of ***OPRM1,*** and higher alcohol consumption are justified by compensatory mechanisms, it is difficult to understand the lower reinforcement observed in knockout mice for the OPRM1. Also, the reduction of alcohol consumption following naltrexone (blocking of the OPRM1), especially strong in G forms, cannot be explained [30].

Two molecular factors are suggested to be important in determining the effect on rs1799971 genotype-related alcohol consumption: the number of the receptors, but also their affinity. However, it is reasonable to think that genetic (multilocus effects and interlocus correlations) and environmental factors could modulate the specific effect of the SNP on these two parameters. This phenomenon could be responsible for the contradictory findings reported in the case of the rs1799971 polymorphism [22], also called flip-flop results [31]. Supporting this hypothesis are recent works that suggest several other non-genetic factors influencing ***OPRM1*** expression [32,33].

Regarding nicotine dependence, the lack of an association between this SNP and tobacco consumption observed in our study is in concordance with the results of the only work undertaken on a Spanish population on this issue [34].

The rs10485057 is associated with alcohol intake and consumption in our male subsample. There is no further evidence in the available literature on this issue, and additional studies will help to explain the specific effect of this polymorphism on alcohol intake and addiction. Regarding tobacco smoking, we found only a marginal association between the rs10485057 polymorphism and the Fageström test outcome, showing that males homozygote carriers of the A variant have higher test scores. No association was found between this and other polymorphisms studied and the amount of cigarettes consumed. Bearing in mind the limitations of our study in terms of the low prevalence of tobacco smokers, we need to refer to the research published by Zhang et al. (2006), stating that A and the G alleles in the rs10485057 locus were present in two haplotypes: one associated with tobacco consumption and another with nicotine dependence.

Our study has several weaknesses that must be taken into account. The first and most important is the limited sample size, together with the low frequency of both rare alleles and prevalence of smoking habit. Both of them could influence the association analysis.

CONCLUSION

Our results suggest that ***OPRM1*** gene polymorphisms are associated with tobacco and alcohol consumption in a Spanish population, and this association could be modulated by genetic and environmental factors.

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DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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