Association of *MYOCD*, *GRM6* and *CRY* genes with response to methadone maintenance treatment

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BACKGROUND

Opioid dependence is a chronic and relapsing disorder with high costs to individuals, families, and society. Genetic predisposition is a major factor contributing to the development of heroin addiction. Methadone maintenance treatment (MMT) is the most widely used treatment for heroin dependence; however, there is a large inter-individual variability in outcome[1]. The study of patients' genetic backgrounds in the therapeutic response to MMT has become an issue of increasing interest: recent studies have focused in genes coding for methadone-metabolizing enzymes, transporter proteins, dopaminergic receptors, and brain-derived neurotrophic factor[2-5].
A previous genome-wide association study[6] identified some single nucleotide polymorphisms (SNPs) and genotype patterns modulating risk for heroin dependence.
This study aims to replicate the results by narrowing down the phenotype to methadone maintenance treatment (MMT) response.

METHODS

A sample of 116 Opioid dependent subjects (DSM-IV), in MMT were recruited and classified as Responders and Nonresponders according to illicit opioid consumption detected in randomised urinalysis. To be eligible for the study, patients had to be Caucasian, enrolled in MMT for at least six months and receiving a stable methadone dose for the last two months. The following data were obtained: socio-demographic characteristics, serological status (HIV, HCV), history of substance use, and previous psychiatric treatment, substance and non-substance use psychiatric disorders (PRISM-IV) and the degree of addiction-related impairment (ASI). Written informed consent was obtained from each subject and the study was approved by the Ethical and Clinical Research Committee of our institution (IMAS-Hospital del Mar).
We selected the most significantly associated variants that were part of a genotype pattern: rs1714984 (*MYOCD*), rs965972 (1q31.2) and rs1867898 (2q21.2). Five SNPs in candidate genes with the lowest P-values were also selected: rs1034576 (*GRM8*), rs1861591 (*CRY1*), rs953741 (*GRM6*), rs1074287 (*OPRM1*), and rs1405735 (*NR4A2*).

RESULTS

We included 116 subjects (83 responders; 33 nonresponders). Groups were similar with regard to methadone dose and other substance use disorders and psychiatric comorbidity (table 1). SNPs were in Hardy-Weinberg equilibrium. Significant differences in genotype distributions between groups were found for rs1861591. Subjects carrying the AA genotype had higher risk of being nonresponders (OR=2.99; 95%CI 1.14-7.85; P=0.035), although this difference disappeared with correction for multiple testing. There was an epistatic effect between rs1714984 and rs953741 (P=0.008). Patients carrying the A allele at rs1714984 had an increased risk of being nonresponders only when they were carriers of the AG genotype at rs953741 (OR=10.83, 95%CI 2.52-46.66) [table 2].

Table 1. Mean Characteristics of Study Groups

	Responders N= 83	Nonresponders N= 33	P*
Male (%)	60 (72)	24 (73)	1.000
Age, mean (SD)	38 (7)	35 (9)	0.075
Years at school, mean (SD)	9 (3)	8 (3)	0.165
Single (%)	38 (46)	18 (55)	0.581
Legal background (%)	42 (51)	13 (42)	0.406
Live with family (%)	58 (71)	23 (51)	0.981
Offspring, mean (SD)	1 (1)	1 (1)	0.529
Employed (%)	28 (34)	13 (42)	0.304
HIV + (%)	32 (39)	9 (27)	0.288
HCV + (%)	61 (74)	22 (67)	0.498
Illicit opioid consumption in months, mean (SD)	135 (86)	97 (61)	**0.010**
Age of onset of heroin use (SD)	20 (6)	22 (7)	0.214
Other substances dependence disorder – lifetime prevalence (%)			
Alcohol	16 (26)	5 (17)	1.000
Sedatives	16 (26)	6 (21)	1.000
Stimulants	2 (2)	0 (0)	1.000
Cannabis	10 (13)	2 (7)	0.508
Cocaine	38 (41)	15(52)	0.829
Days of heroin consumption in the last 30 days, mean(SD)	0.3 (1)	16 (11)	**<0.001**
Days of cocaine consumption in the last 30 days, mean(SD)	2 (6)	9 (12)	0.056
Psychiatric comorbidity (lifetime prevalence) (%)	57 (72)	19 (66)	0.635
Months in MMT, mean (SD)	42 (46)	24 (36)	0.023
Methadone dosage, mean (SD)	100 (68)	78 (43)	0.082
ASI scores, mean (SD)			
General health status	3 (2)	4 (2)	0.155
Working problems	4 (3)	4 (3)	0.612
Alcohol use	1 (2)	1 (1)	0.785
Substance use	5 (2)	6 (2)	**<0.001**
Legal problems	1 (2)	2 (2)	**0.048**
Social relationships	3 (3)	3 (2)	0.648
Psychological status	3 (3)	2 (2)	0.701

**Bold numbers represent significant P-values, after Bonferroni correction*
SD: standard deviation

Table 2. Interaction results for SNPs rs1714984 and rs953741

rs1714984 (*MYOCD*)	rs953741 (*GRM6*) AA R N	AA NR N	AA OR (95%CI)	AG R N	AG NR N	AG OR (95%CI)	GG R N	GG NR N	GG OR (95%CI)
GG	18	10	1.00	26	4	1.00	2	1	1.00
AG-AA	19	5	0.5 (0.14-1.66)	6	10	10.83 (2.52-46.66)	3	2	1.33 (0.07-26.62)

R: Responders; NR: Nonresponders

CONCLUSIONS

We detected an association between MMT response and different variants in genes *CRY1*, *MYOCD* and *GRM6*. Those genes have been involved respectively in the molecular regulation of the clock machinery, in the pathophysiology of neurological and mood disorders and cell proliferation, migration and control of smooth muscle gene expression. There was a pharmacogenetic epistatic effect between SNPs in *MYOCD* and *GRM6* that appear to be modulating the MMT response.

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