Pharmacogenomic study of opioid addicts in methadone treatment

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“It's not the mountain we conquer, but ourselves"
Sir Edmund Percival Hillary

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ABSTRACT

Although the well established efficacy of methadone maintenance treatment (MMT) in the opioid dependence disorder, there is a group of patients that are poor responders. The study of the influence of methadone pharmacodynamics and pharmacokinetics in dose requirements and program outcome remains still controversial. The aim of this dissertation is to study the pharmacodynamic and pharmacokinetic factors involved in the methadone maintenance treatment efficacy.

The study recruited opioid dependence patients (DSM-IV criteria) from a MMT community program. Patients were clinically assessed and blood samples were obtained in order to evaluate methadone plasma concentrations of *(R,S)-, (R)* and *(S)*- methadone. Allelic variants of genes encoding the following proteins were assessed: BDNF, OPRM1, MYOCD, mGluR6, mGluR8, CRY1, NR4A2, 1q31.2 (rs965972), 2q21.2 (rs1867898), CYP3A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19 and P-glycoprotein. Responders and non-responders were defined by means of illicit opioid consumption detected in random urinalyses.

Differences in response status were found depending on different single nucleotide polymorphisms (SNPs of genes encoding for *BDNF*, *MYOCD* and *GRM6*. The CYP2D6 metabolizing phenotype was associated with response to MMT, and also with methadone dosage requirement and methadone plasma concentrations.

RESUM

Els programes de manteniment amb metadona (PMM) han demostrat eficàcia en el tractament del trastorn per dependència d'opiacis malgrat la persistència de pacients amb mala resposta al tractament. L'estudi dels factors farmacodinàmics i farmacocinètics implicats en la resposta terapèutica ofereix resultats controvertits. L'objectiu de la tesi doctoral que es presenta és estudiar els factors farmacodinàmics i farmacocinètics de la metadona que poden estar implicats en l'eficàcia del tractament.

S'han inclòs pacients ambulatoris diagnosticats de trastorn per dependència d'opiacis (segons criteris DSM-IV) en PMM. Els pacients s'han avaluat a nivell clínic i s'han obtingut mostres de sang per a l'estudi de les concentracions plasmàtiques de (*R,S)-,* (*R*) i *(S*)- metadona. S'han estudiat també les variants al·lèliques dels gens que codifiquen per: BDNF, OPRM1, MYOCD, mGluR6, mGluR8, CRY1, NR4A2, 1q31.2 (rs965972), 2q21.2 (rs1867898), CYP3A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19 i P-glicoproteïna. La mostra s'ha dividit en responedors i no responedors en funció del nombre de controls d'orina positius per a heroïna en analítiques realitzades de forma aleatòria.

Es van detectar diferències en resposta al tractament segons les variants dels gens codificants per a *BDNF*, *MYOCD* i *GRM6*. També es va detectar una associació entre el fenotip de CYP2D6, la resposta al tractament, la dosi requerida de metadona i les concentracions plasmàtiques.

PREFACE

Opioid dependence disorder is a chronic and relapsing disease causing severe impairment, medical complications and economical distress in patients and families. At this moment it's estimated that 0.4% of the world population is using opioids. As all substance abuse disorders, opioid dependence is the result of the interaction of genetic, environmental and drug-induced factors.

Therapeutic approach of opioid dependence have changed from abstinence oriented programs based in detoxification and opioid antagonist treatment (naltrexone) to substitution programs, which presented higher rates of treatment retention and decrease of illegal opioid use. Since its introduction in the 1960s by Dole & Nyswander, methadone maintenance treatment (MMT) programs have been the main therapeutical approach to opioid dependence disorder. Although MMT have demonstrated high rates of efficacy in the treatment of the opioid dependence disorder, there is still a non negligible proportion of patients that presents a poor response to MMT programs.

Methadone dose is the main factor involved in the MMT success, but there is a big interindividual variability in methadone dosage requirements, in part, due to the interindividual differences in methadone metabolism and frequent interactions with other pharmacological treatments and substances of abuse. There is a great interest in

describing the main factors associated with MMT outcome and dose requirements in patients.

Methadone is a semisynthetic opioid agonist, with high affinity for the mu-opioid receptors. Methadone is a chiral molecule, that is, it exists in two forms: *(R)*-methadone and *(S)*-methadone. It is administered as a racemic 50% mixture of both enantiomers, but only the *(R)*- methadone presents mu-opioid agonists effects, whereas the *(S)*-methadone has been associated with methadone side effects. Methadone's chirality, has also implications in differences in the metabolism and elimination of the enantiomers.

Pharmacogenetics refers to a blend of genetics and pharmacology concerned with genetically determined modifications of individual pharmacological responses. Studies on pharmacogenetics of MMT outcome present controversial results. Previous studies focused on the pharmacodynamics of MMT outcome have described an association of a single nucleotide polymorphism (SNP) of the D2 dopamine receptor (*DRD2*) and methadone maintenance outcome, and no association was found with polymorphisms at the gene encoding for the mu-opioid receptor (*OPRM1*).

Also, the studies on the pharmacokinetics of methadone showed contradictory results, and only genetic variability at the gene encoding for the P-glycoprotein (*ABCB1*) has been associated with differences in methadone dose requirements. The data concerning to genetic variability

of genes encoding for the cytochrome P450 offered negative results.

Taking all this data into account it seamed justified to perform a pharmacogenetic study on MMT efficacy and dose requirements, involving both, pharmacodynamic and pharmacokinetic factors.

CONTENTS

ABBREVIATIONS

ABCB1, multidrug resistance 1 gene

ACTH, adrenocorticotropic hormone

ARRB2, β-arrestin

ASI, Addiction Severity Index

BDNF, brain-derived neurotrophic factor

CE, capillary electrophoresis

COMT, Catechol-O-Methyltransferase

CpG, cytosine: guanine dinucleotides

CRY1, cryptochrome 1

CYP1A2, Cytochrome P450 1A2

CYP2B6, Cytochrome P450 2B6

CYP2C19, Cytochrome P450 2C19

CYP2C9, Cytochrome P450 2C9

CYP2D6, Cytochrome P450 2D6

CYP3A4, Cytochrome P450 3A4

CYP3A5, Cytochrome P450 3A5

D2A1, dopamine receptor 2, Taq1 polymorphism

DL, disequilibrium linkage

DNA, deoxyribonucleic Acid

DRD2, dopamine receptor 2

DRD4, dopamine receptor 4

DSM-IV, Diagnostic and Statistical Manual of Mental Disorders (4th Edition)

DZ, dizygous

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine

EM, extensive metabolizer

EMCDDA, European Monitoring Centre for Drugs and Drug Addiction

EMDP, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine

FDA, U.S. Food and Drug Administration

GWAS, Genome-wide association study

HCV, hepatitis C virus

HIV, human immunodeficiency virus

HPA, hypothalamic-pituitary-adrenal axis

H_3PO_4, Phosphoric Acid

IM, intermediate metabolizer

LAAM, levacetylmethadol

LOD, limits of detection

LOQ, limits of quantization

MC2R, adrenocorticotropic hormone receptor

mGluR6, metabotropic glutamate receptor 6

mGluR8, metabotropic glutamate receptor 8

MMT, methadone maintenance treatment

mRNA, messenger ribonucleic acid

MYOCD, myocardin gene

MZ, monozygous

NMDA, N-methyl-D-aspartate receptor

NR4A2, Nuclear receptor subfamily 4, group A, member 2

OED, Observatorio Español sobre Drogas

OPRK1, kappa opioid receptor

OPRM1, mu-opioid receptor

PDYN, prodynorphin

PM, poor metabolizer

PRISM, Psychiatric Research Interview for Substance and Mental Disorders

SD, standard deviation

SNP, single nucleotide polymorphism

TCI, Cloninger's Temperament and Character Inventory

UM, ultrarapid metabolizer

UNODC, United Nations Office on Drugs and Crime

UV, diode-array detector

1. INTRODUCTION

1.INTRODUCTION

1.1. Epidemiology

Opioid dependence is a chronic and relapsing disorder with high costs to individuals, families, and society. The total number of opiate users at the global level is now estimated at 15.2-21.1million people, or 0.4% of the world's population aged 15-64 (United Nations Office on Drugs and Crime, 2009). Opiates, including the increasing abuse of prescription opioids, continue to be the main problem drug worldwide, accounting for some 60 per cent of treatment demand in Asia and in Europe; in the United States in 2007, a 1,5% among persons aged 12 or older have used heroin and 13.3% have used pain relievers which means a total of 3,4 million and 33 million people (Substance Abuse and Mental Health Services Administration, 2008). In the European Union, the Annual Report of the European Monitoring Centre for Drugs and Drug Addiction estimates of the prevalence of problem opioid use assuming the European Union as a whole, could imply some 1,4 million (1.2 million to 1.5 million) problem opioid users in the European Union in 2007 (European Monitoring Centre for Drugs and Drug Addiction [EMCDDA], 2009).

Although the general prevalence of opioid use has remained relatively stable during last years (United Nations Office on Drugs and Crime, 2009), the use of non-medical prescription opioid (as hydrocodone –Vicodin®- and oxycodone –OxyContin®-) are on rise (Johnston et al., 2009) mainly in adolescents.

In Spain, the majority of heroin users are males, over 25 years old, with an average initial age at first use of 20 years (Observatorio Español sobre Drogas [OED], 2009).

Opioid dependents usually present other substance use disorders (alcohol, cocaine, sedatives and cannabis) (Strain, 2002) and, the majority, are cigarette smokers (Strain, 2002). Also, there is a high rate of psychiatric comorbidity with non-substance abuse disorders, mainly affective disorders (Astals et al., 2008).

1.2. Neural mechanisms of opioid addiction.

The opioid dependence disorder, as all substance use disorders, is characterized by compulsion to seek and take the drug; loss of control in limiting intake; and emergence of a negative emotional state (i.e. dysphoria, anxiety, irritability) reflecting a motivational withdrawal syndrome when access to the drug is prevented (Koob & Volkow, 2010). Drug addiction involves elements of both impulsivity and compulsivity that yield a composite addiction cycle composed of three stages mediated by discrete neural circuits:

1- Binge/intoxication: ventral tegmental area and ventral striatum
2- Withdrawal/negative affect: extended amygdala
3- Preoccupation/anticipation' (craving): orbitofrontal cortex-dorsal striatum, prefrontal cortex, basolateral amygdala, hippocampus, and insula involved in craving and the

cingulate gyrus, dorsolateral prefrontal, and inferior frontal cortices in disrupted inhibitory control.

Opiates and opioids act on three opioid receptors (mu, kappa and delta) which are widely distributed in the brain and are associated with reward stimuli (Shippenberg, 2009; Shippenberg et al., 2009). Endogenous opioids, called endorphins, are the ligands of these receptors and play a central role in establishing habits and responses for survival and pain relief (Carvalho et al., 2006; Hayward & Low, 2007). Synthetic opioids (i.e. heroin) and natural opiates (i.e. morphine) act on the same receptors as endogenous opioids. Chronic use of opioids can result in brain adaptations associated with tolerance and dependence. Two neurotransmitters are associated in the establishment of reward in addiction: dopamine and norepinephrine (Weinshenker & Schroeder, 2007). Opioids increase dopamine concentrations at mesolimbic regions (specifically, the ventral tegmental area and the nucleus accumbens) by increasing the firing rate of dopamine neurons (Di Chiara & Imperato, 1988). Norepinephrine has been implicated in motivating drug-seeking behaviors (Weinshenker & Schroeder, 2007) and have been related with the clinics of the opioid withdrawal syndrome (Maldonado, 1997). With the chronic use of opioids, the brain's reward pathways express more receptors, requiring more neurotransmitter to obtain same effects.

1.3. Opioid addiction: a complex disease

Addiction is a complex disease with interacting factors, including environmental factors (cues, conditioning, stress, etc.), drug induced neurobiological changes and individual factors (genetics, comorbidity, personality traits and stress responsively) that ends in a compulsive behaviour of drug seeking (Kreek et al., 2005a; Sellman, 2010) (**Figure 1**).

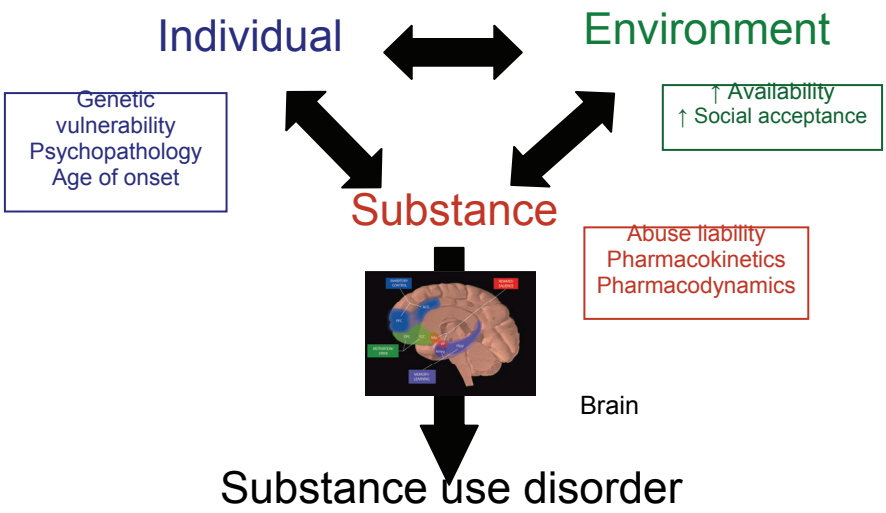

Fig.1 Factors contributing to develop a substance use disorder

Genetic advances have allowed improving the knowledge on individual characteristics that raise the vulnerability to develop a substance use disorder (Yuferov et al., 2010).

Family studies have demonstrated that opioid dependence disorder is a heritable illness (in fact, they are

among the most heritable of psychiatric disorders) and showed familiar aggregation in opioid consumption up to 10 times when compared to the general population (Maddux & Desmond, 1989; Merikangas et al., 1998). This familial concentration has been related to a larger severity of the disorder and polydrug use (Pickens et al., 2001). Adoption studies confirm a wider presence of drug dependence disorders in children of biological parents with alcohol or other substances use disorders even if adopted by non-drug user families. These studies set out three possible theories for transmission, first, the presence of antisocial personality disorder in the biological family, secondly, the presence of a drug dependence disorder in the biological family and lastly, some environmental factors (parents divorce, psychiatric disorders) in the adoption family (Cadoret et al., 1986; Cadoret et al., 1995; Grove et al., 1990).

Twin studies try to distinguish between general vulnerability to any substance or specific vulnerability to one kind of drug (Kendler et al., 2003; Tsuang et al., 1998). In **Table 1**, are presented the most representative twin studies published until now: the Vietnam veterans (males) (Tsuang et al., 1998), the Virginia twin register (both genders) (Kendler, et al., 1999; Kendler et al., 2003), and opioid dependent patients twin register (van den Bree et al., 1998). Tsuang et al (1998), in a register of Vietnam veterans studied in 3.372 pair of twins, by a semi structured interview, the use of different substances in an individual and the abuse/dependence

disorders of different substances in the individuals. The investigators concluded that there were a shared vulnerability for all substances and a specific vulnerability for specific substances. Shared vulnerability could be influenced by genetic factors (43%) and environmental factors (31%). Specific vulnerability for any substance (26%) had specific genetic influences. Opioid dependence disorders displayed a bigger specific vulnerability level (50%) and a lower genetic shared vulnerability (30%) in relation to other substances.

Table 1. Twin studies in opioid abuse/dependence disorders

Author	**Register**	**Diagnosis**	**Concord.**		Variance			
Tsuang et al., 1998,	Vietnam veterans	DSM-III-R (opioid abuse/dep abuse/ dep N=3371	MZ 0.67	DZ 0.29	H2 0.43	C2 __	N2 0.31	M2 0.27
Kendler et al., 1999 Kendler et al., 2003	Virginia Twins register (general population)	DSM N=1198 pairs of male twins - Opioid abuse - Opioid dependence N=1934 pairs of female twins - Opioid abuse/dependence	 0.55 __ 0.33 0.50	 0.03 __ 0 0	 0.52 __ __ __	 __ __ __ __	 0.48 __ __ __	 __ __ __ __
Van den Bree et al., 1998	Patients in treatment (Minnesota, USA)	DSM-II-R (opioid abuse/dependence) N=188 pairs of male/female twins -Male/Female gender twins	 0.87 0.34	 0.65 0.95	 0.57 0	 0.30 0.60	 0.13 0.40	

MZ= monozygous; DZ: dizygous; Concord: Concordance level
H2=heritability; C2=shared environmental component; N2=specific environmental component; M2= shared genetic component

On the other hand, the expression of a genetic predisposition may be, in part, conditional on exposure to environmental determinants. In twin studies, environmental factors, including family environment, influence the

development of alcohol and other substance abuse disorders in subjects with a relatively high genetic risk (Kendler et al., 2003; Tsuang et al., 1998).

So, identifying gene-environment interactions will be a crucial issue in the study of addictions, which by definition depend on exposure to an addictive agent and are strongly modulated by other environmental factors.

1.4.- Genetic factors in opioid addiction

During the last years, a substantial amount of scientific reports have been published on molecular genetics in opioid use disorders both, in animal models and humans. In humans, association and linkage studies are the two main experimental approaches for the investigation of genetic polymorphisms and their contribution to drug addiction vulnerability (Kreek et al., 2005a; Yuferov et al., 2010). Linkage studies use families to provide evidence of how close a genetic marker is to an allele causing the studied phenotype. Association studies may be performed with unrelated individuals or with parent-offspring trios. Linkage studies are hard to perform due to the difficulties on finding families with more than one member affected.

In the last years, a novel technique has been introduced in the study of genetics and addiction due to the development of high-density microarray technology, being possible to assess many single-nucleotide genetic variants in one individual: genomewide association studies (GWAS)

compare groups of individuals analyzed using high-density microarrays (Nielsen et al., 2008; Yuferov et al., 2010).

The study of epigenetic modifications and their implication in addiction has become a recent subject of research. The transmission of information not encoded in the DNA sequence is termed epigenetic inheritance. DNA methylation and covalent histone modifications are the primary sources of epigenetic inheritances. DNA methylation of cytosine residues in genomic DNA is a common epigenetic mechanism controlling gene expression and occurs trough the addition of a methyl group to cytosine residues in cytosine: guanine (CpG) dinucleotides by DNA methylation enzymes. CPG dinucleotides are often clustered in “CpG islands”. CpG islands are at least 200 base pairs with a CpG percentage that is greater than 50% and CpG content of at least 60% of that which would be expected. These CpG islands are found in the promoter region of the gene and located upstream of the transcription start site to within the first exon. This kind of approach has been recently applied to the study of the genetic risk factors in the development of opioid dependence disorder (Nielsen et al., 2009).

1.4.1. Opioid endogenous system

The opioid endogenous system plays an important role in opioid addiction (Mayer & Hollt, 2006) and also it has been implicated in the pathophysiology of dependence of alcohol and cocaine (Kreek et al., 2009).

The **μ-opioid receptor** belongs to G protein receptors family and its structure has 7 transmembrane domains (see **Figure 2**).

Human Mu Opioid Receptor

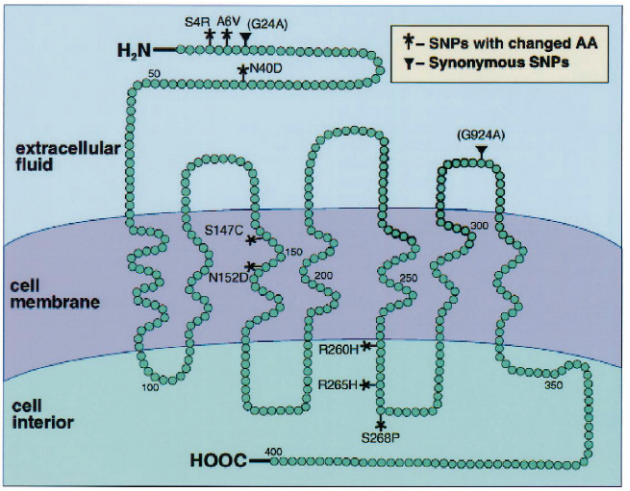

Fig. 2. The human mu-opioid receptor and positions of identified coding region single nucleotide polymorphisms. From LaForge et al., 2000.

Heroin is transformed in 6-monoacetyl morphine by deacetylation and later on, in morphine, both compounds are strong μ receptors agonists, as the endogenous opioid β-endorphin. Once the μ-opioid receptor is activated reinforcing effects of opioids as euphoria, appear, contributing to the development of dependence. The gene encoding the receptor is located in chromosome 6 -6q24-q25- (Wang et al., 1994).

Multiple polymorphisms of the *OPRM1* gene have been identified, some of them located in the coding region with changes in receptor final structure. The most widely studied polymorphism in *OPRM1* is a SNP in exon1, involving a substitution A→G (A118G), which encodes an Asn40Asp amino acid substitution. The Asp40 residue results in a three-fold increase in β-endorphin binding compared with the Asn40-containing protein (Bond et al., 1998) probably related with a suppression of a potential site of glycosilation in the N-terminal region. This SNP reduces the agonist-induced receptor signaling efficacy, but not the binding, in postmortem brain (Oertel et al., 2009). Its allelic frequency differs among different ethnic populations: 2% in Afro-Americans, 8-14% in Caucasians and 30-49% in Asians (Bond et al., 1998; Gelernter et al., 1999; Szeto et al., 2001). *In vitro* studies (Kroslak et al., 2007) showed differences in gene expression and in signal response to agonists. Post-mortem studies show an allelic expression imbalance, where the 118G allele displays a lower expression than the 118A allele in autopsy brain samples, indicating loss of OPRM1 function (Drakenberg et al., 2006; Zhang et al., 2005). Association studies report contradictory data in the implication of A118G polymorphism in opioid dependence (Arias et al., 2006; Kreek et al., 2005; Yuferov et al., 2010). Finally, in an epigenetic study (Nielsen et al., 2009) it was reported that in DNA obtained form peripheral lymphocytes, 2 of 16 CpG sites in a

region of the *OPRM1* gene promoter had significantly higher methylation in former heroin addicts than in controls.

The human **kappa opioid receptor** (*OPRK1)* gene is located in chromosome 8q11.2, its structure has been reported by Yuferov et al. (2004), and it consists in 4 major exons and 3 introns (**Figure 3**).

Human Kappa Opioid Receptor

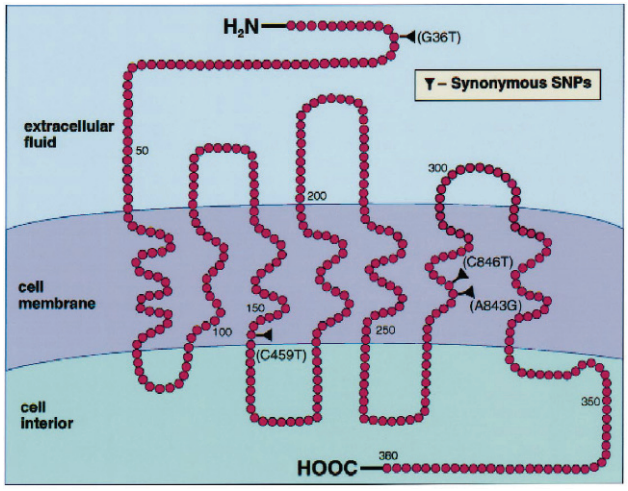

Fig.3. The human kappa-opioid receptor and positions of identified coding region single nucleotide polymorphisms. From LaForge et al., 2000.

This receptor is located in several areas of the dopaminergic nigrostriatal and mesolimbic-mesocortical systems, and plays an important role (associated with the dynorphin receptor) in the modulation of opioid, cocaine and

other rewarding stimuli, modulating the dopaminergic tone. The stimulation of the kappa opioid receptor by dynorphin peptides decreases basal and drug-induced dopamine concentrations in several areas of the dopaminergic nigrostriatal and mesolimbic-mesocortical system as a countermodulatory mechanism of the brain (Kreek et al., 2005b). The exons and intron 1 were sequenced and evaluated polymorphisms in 291 subjects (Yuferov et al., 2004). Twelve SNPs were identified, nine novel variants and three previously reported SNPs. The 36G>T SNP exhibited a point-wise significant association with disease status. Other studies have replicated these findings (Gerra et al., 2007; Zhang et al., 2008).

Prodynorphin is the precursor for the opioid peptides dynorphin A and B and α-neoendorphin. It has been shown to inhibit neurotransmission by acting through kappa receptors. Dynorphin reduces dopaminergic tone in the mesocortical-limbic projections. The prodynorphin gene (*PDYN*) promoter has a 68-basepair repeat polymorphism that is functionally important in association with substance abuse (Dahl et al., 2005). Ray et al. (2005) examined this polymorphism for association in a sample of 168 opioid-dependent patients and 122 ethnically and geographically matched controls. Authors found a significant difference in genotype and allele frequencies between African American and European American populations. They did not detect significant

differences in genotype or allele frequencies between the patients and controls within the European American ethnic group. However, they detected a weak association when compared allele frequencies of patients and controls in the African American population.

1.4.2. Dopaminergic system

The dopaminergic system is involved in the rewarding effects of opioids and drugs of abuse. Heroin raises dopamine concentrations in the reward areas of the brain (nucleus accumbens, caudate putamen). Many polymorphisms have been identified in genes of the dopaminergic system, but results concerning association studies with opioid addiction are contradictory.

The **D2 receptor gene** (*DRD2*) is located in chromosome 11 (11q22-23) and contains 8 exons. The first study with this gene was done in an alcohol dependent population (Blum et al., 1990); there was a significant increase in the frequency of TaqI A1 allele. This allele corresponds with a restriction fragment located 10kb from the 3'region of the gene. Although D2A1 allele is outside from the coding region, it is in a disequilibrium linkage (DL) with a DRD2 gene variant that results in receptor low density (Noble et al., 1991).

The DRD2 gene has not only been associated with substance abuse disorders, but also with other illnesses as attention deficit disorder, Gilles de la Tourette syndrome,

autism and post-traumatic stress disorder (Comings et al., 1991). Lawford et al. (2000) studied the prevalence of *TaqIA1* in 95 Caucasian patients in methadone maintenance treatment. They observed that the prevalence of *TaqIA1* was higher in patients compared to healthy controls.

In Chinese opioid dependent population, three polymorphisms have been studied *(-141ΔC*, *Ser311Cys* and *TaqI*). A significant association with the *-141ΔC* polymorphism was found (Li et al., 2002). Also, a haplotype analysis has been done in two populations (Chinese and German) (Xu et al., 2004). Whereas in Chinese population one haplotype has been related to heroin consumption, in the German population two different polymorphisms have been described as protective (Xu et al., 2004). Finally, a recent study also identified a SNP in the DRD2 gene modulating the risk of opiate addiction (Doehring et al., 2009).

The **D4 dopamine receptor gene** (*DRD4*) has three polymorphic variations in the coding sequence. A 48-base-pair sequence in the putative third cytoplasmic loop of this receptor exists either as a direct-repeat sequence (D4.2), as a fourfold repeat (D4.4) or as a sevenfold repeat (D4.7) (Van Tol et al., 1992). The presence of a larger or a lower number of duplications and changes in protein length imply physiological differences in the ligand binding capacity with the receptor “in vitro” (Asghari et al., 1995). Association studies have been performed to investigate the implication of this receptor in

substance abuse and opioid dependence. Three studies have observed that long alleles in exon 3 are present in patients with substance dependence disorder (Kotler et al., 1997; T. Li et al., 1997; Vandenbergh et al., 2000). Nevertheless, other studies could not replicate these results (Franke et al., 2000; Gelernter et al., 1997).

1.4.3. Hy*pothalamic-pituitary-adrenal (HPA) axis*

Addictive diseases are associated with dysregulation of the **hypothalamic-pituitary-adrenal** (HPA) axis: hypoactivity was found in medication-free illicit drug-free former heroin addicts (Kreek et al., 1983). Also, a significant association was found between one minor allele (184G>A) of the gene encoding for the ACTH receptor (*MC2R*) with a protective effect from heroin addiction in Hispanics (Proudnikov et al., 2008).

1.4.4. *Neurotrophins*

Brain-derived neurotrophic factor (BDNF), a member of the nerve growth factor-related family of neurotrophins, is widely expressed in the adult mammalian brain and accumulating evidences indicate that may be involved in the mechanisms underlying substance abuse. Data derived from animal studies, showed that it modulates dopaminergic and serotonergic functions; dopamine circuitry has been linked to substance abuse (Dluzen et al., 1999). Murine models have shown that BDNF in the ventral tegmental area (VTA) induces a transition to a drug-dependent motivational state (Vargas-

Pérez et al., 2009), and intra-VTA BDNF infusion produces long-lasting enhancement of cocaine seeking (Grimm et al., 2003) and it increases the rewarding effects of cocaine (Horger et al., 1999).

The human BDNF gene has been mapped to chromosome 11. A common single-nucleotide polymorphism (G196A) in the BDNF gene that results in a valine to methionine substitution in the prodomain has been shown to affect intracellular trafficking and activity-dependent secretion of BDNF (Chen et al., 2004; Egan et al., 2003). One study (Cheng et al., 2005) tested the hypothesis that the *BDNF*-gene Val66Met polymorphism is associated with substance abuse. Authors studied this polymorphism in 103 methamphetamine and 200 heroin-dependent cases and 122 healthy controls. Significant differences in *BDNF* Val66Met genotype distribution were found between subjects associated with substance abuse; analysis of the allele frequency showed that the 66Met allele was less common in the methamphetamine- and heroin-dependent groups than in the control group. This polymorphism may have a direct effect on the susceptibility to substance abuse, probably affecting other neurotransmitter systems, such as the dopaminergic system.

1.4.5. Other genes

Other genes that have been related to opioid dependence are: the catechol-O-methyltransferase (*COMT*) gene, the serotonin transporter gene, the tryptophan hydroxylase gene,

and the hydroxytryptamine (serotonin)-1B-receptor gene (Kreek et al., 2005b; Yuferov et al., 2010).

1.5.- Treatment of opioid addiction

1.5.1. Treatment strategies in opioid addiction

The two main treatment strategies in opioid dependence are: abstinence-oriented treatments and maintenance treatments (Veilleux et al., 2010).

In the **abstinence-oriented treatments**, the goal is to remove the opioid in a controlled fashion, with a complete eradication of the opioid-dependent life-style. Abstinence is usually achieved in two stages: detoxification and relapse prevention. Detoxification involves the substitution of the abused opioid by other long half-life opioid agonist (methadone or buprenorphine) or an alpha2 adrenergic agonist (clonidine or lofexidine) and a progressive reduction in order to reduce the intensity of the withdrawal syndrome (Amato, et al., 2005a; Gowing et al., 2009a; Gowing et al., 2009b). When comparing methadone and alpha2 adrenergic agonist treatments, studies showed similar results by means of withdrawal intensity and treatment completion (Gowing et al., 2009b). However, participants stayed in treatment for longer and experienced fewer adverse side effects on methadone and buprenorphine (Ziedonis et al., 2009). Also, clonidine, the most frequently tested alpha2 adrenergic

agonist, has been associated with potentially hazardous side effects as sedation and hypotension (Gowing et al., 2009b).

Naltrexone is a full opioid antagonist used in the relapse prevention phase. The advantage of naltrexone includes decrease in opioid craving, can be administered in a standard outpatient office setting and no abuse potential. Despite this, naltrexone has shown low rates of efficacy, with poor retention and high relapse rates (Minozzi et al., 2006; Sullivan et al., 2006).

Opioid agonist maintenance treatment stabilizes brain neurochemistry by replacing short-acting opioids with a long-acting opioid that has relative steady-state pharmacokinetics, such as methadone or buprenorphine. Opioid agonist maintenance treatment is designed to have a minimal euphoric effect, blocks the euphoria associated with the administration of exogenous opioids and eliminates the phenomenon of opioid withdrawal (Fiellin et al., 2006). The four most frequently studied medications for maintenance treatment are methadone, levacetylmethadol (LAAM), buprenorphine and diacetylmorphine (heroin). The role of methadone maintenance treatments will be explained in the next section.

Buprenorphine is a partial opioid agonist, with a ceiling effect for respiratory depression and with reduced abuse potential (Veilleux et al., 2010). Studies found buprenorphine therapeutically superior to naltrexone (Schottenfeld et al.,

2008). When compared to placebo, buprenorphine at medium and high doses improved treatment retention and opioid use (verified by urinalysis) (Mattick et al., 2008). However, when compared to methadone maintenance, the evidence is less conclusive in terms of treatment retention (Mattick et al., 2008).

LAAM, another full-agonist, is associated with suppression of heroin use; however, present more drop outs of treatment, likely due to increased adverse events (Clark et al., 2002). Moreover, LAAM has been excluded from treatments due to the possibility of a fatal ventricular arrhythmia ("torsade des pointes") (Clark et al., 2002; Wedam et al., 2007).

Diacetylmorphine has also been studied in patients with a history of unsuccessful agonist treatment. Heroin increased treatment retention and reduced engagement in illegal activities in patients who previously failed in other maintenance programs (Haasen et al., 2007; Oviedo-Joekes et al., 2009; Strang et al., 2010).

1.6. Methadone maintenance treatment

Methadone has been used in the treatment of opioid dependence since the 1960s (Dole & Nyswander, 1965) and is the most well-studied and utilized drug. It was approved in 1972 by the U.S. Food and Drug Administration (FDA) as a treatment for opioid addiction. In general, methadone

maintenance treatment (MMT) is considered the first line treatment for opioid dependence

1.6.1. Pharmacology of methadone

1.6.1.1. Methadone Pharmacodynamics

Methadone (**Figure 4**) is a semisynthetic opioid agonist that is used in the chronic treatment of pain and in the opioid dependence disorder. Its mechanism of action is mediated by the activation of the opioid receptors, principally the mu type. At higher doses it can effectively block the euphoric effects of exogenous opioids (Dole et al., 1966). Methadone is an agonist at mu, delta, and to a lesser extent at kappa opioid receptors (Kristensen et al., 1995).

Methadone also displays N-methyl-D-aspartate (NMDA) receptor antagonist properties, which affect the development of tolerance (Davis & Inturrisi, 1999).

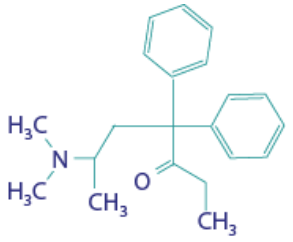

Fig. 4. Methadone chemical structure

Methadone and its main metabolites, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine (EMDP) are chiral molecules, which means that methadone has an

asymmetrical carbon atom in its structure implying that it exists in two enantiomeric forms, having the same chemical structure but different spatial arrangements, with one enantiomer being the mirror image of the other. Methadone is usually administered as a racemate, a 50:50 mixture of two enantiomers called *(R)*- or levo- or l- methadone, and *(S)*- or dextro- or d-methadone. *(R)*-methadone has a higher affinity for opioid receptors and an increased analgesic potency than the *(S)*-enantiomer (Eap et al., 2002). Despite lacking strong opioid effects, *(S)*-methadone may be a clinically important determinant of *(R,S)*-methadone therapeutic and adverse responses (negative mood effects –tension, fatigue, confusion…) (Elkader et al., 2009a; Mitchell et al., 2004). Although *(R)*-methadone is believed to account for most if not all the therapeutic effects of methadone maintenance treatment, *(R,S)*-methadone is normally used in therapeutics due to its lower production costs and evidence that it produces similar therapeutic outcomes when compared with *(R)*-methadone alone (de Vos et al., 1998; Eap et al., 1996).

The adverse events of methadone are similar to those of morphine. In general are mild and tolerable and the most common are constipation, sweating and insomnia which tend to lessen due to the development of tolerance (Kreek, 1973). There are two important adverse events related to methadone: the risk of respiratory depression and the risk of ventricular arrhythmia (Eap et al., 2002). The prolongation of the QTc interval on electrocardiogram and cardiac

arrhythmias have been described since 2002 (Krantz et al., 2002). Usually, patients with QTc prolongation present other risk factors (Martell et al., 2003) and exposed to higher doses of methadone (Fonseca et al., 2009. **Appendix 1**).

1.6.1.2. Methadone pharmacokinetics

Methadone is rapidly absorbed after an oral dose, it can be detected in the blood at 15-45 minutes after oral administration and peak plasma concentrations occur at 2-4 hours after administration (Eap et al., 2002). The oral bioavailability of methadone was found to be around 70-80% in a range of doses of 10 to 60 mg with large intersubject variations. Methadone is highly bound to plasma proteins, including albumin, lipoproteins, and mainly to alpha-1-glycoprotein. Enantiomeric differences are also relevant in protein binding (the unbound fraction for *(S)*-methadone is 10% while for the *(R)*-enantiomer is 14%) and its metabolic disposition

Although administered as a racemic mixture that contains the same amounts of *(R)*-methadone and *(S)*-methadone, the *(R)-/(S)*-methadone ratio varies significantly over the 24-hour administration interval in steady-state conditions (Beck et al., 1991; Foster et al., 2004); and also, large interindividual differences can be seen in the *(R)-/(S)*-methadone ratio (Eap et al., 1996). All these variables might contribute to inter-individual response differences to methadone treatment.

Methadone is extensively metabolized in the body, mainly by CYP3A4 in liver, and in the intestinal epithelium. The main metabolite (2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine; EDDP) is inactive: it is formed by N-demethylation and spontaneous cyclisation (**Figure 5**). Methadone half-life is about 28 hours with a large interindividual variability (between 4 and 91 hours). *(R)*-methadone has a longer half-life than *(S)*-methadone (38 vs. 29 hours, respectively). After chronic administration it has been detected a reduction in half-life (from 55 to 22 hours) because it induces its own metabolism regulated by cytochrome P450 CYP3A4 (Wolff et al., 2000).

The elimination of methadone is mostly due to metabolic clearance. The limited amounts of circulating drug that undergo glomerular filtration are partially reabsorbed by the kidney tubules, and this reabsortion is pH-dependent. Methadone clearance varies from 23 to 210 ml/min and there are significant differences between both enantiomers (158 ml/min vs. 129 ml/min for *(R)*- and *(S)*- respectively). Urinary pH, has profound effects on methadone excretion, and on the volume of distribution of the drug. By keeping the urinary pH constant, the inter-individual differences of methadone elimination and plasma concentrations are considerably reduced.

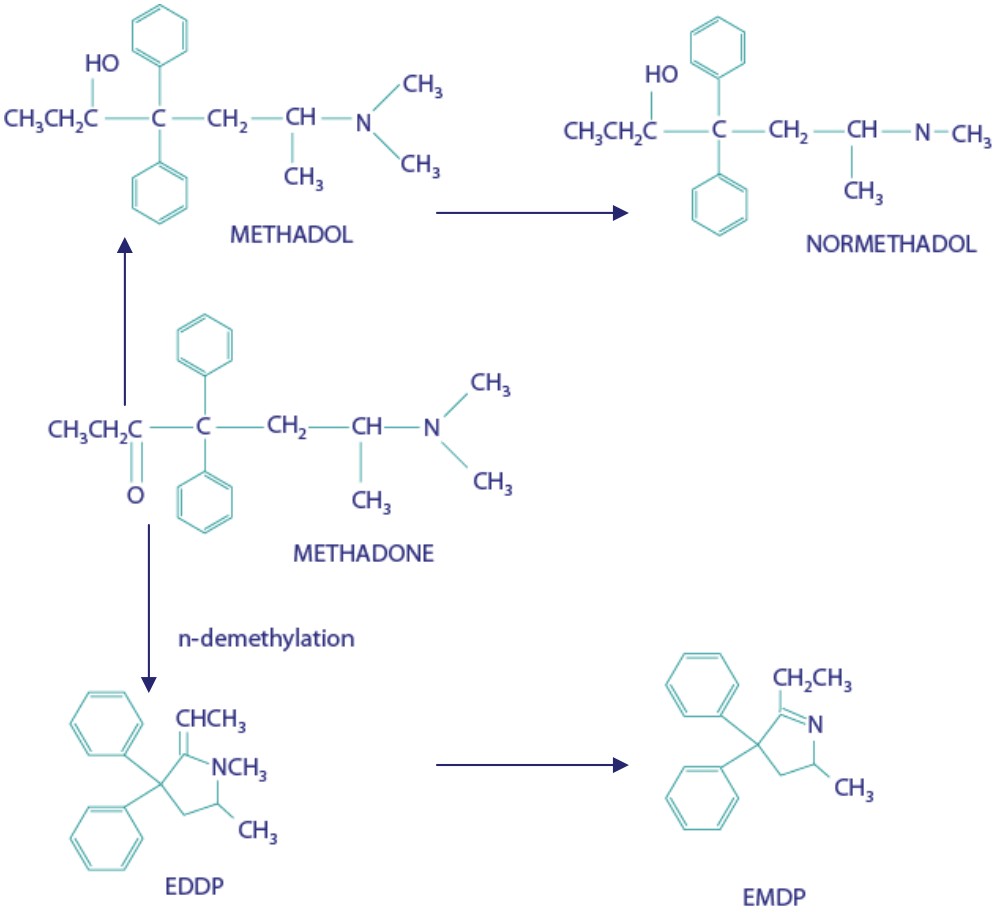

Figure 5. Metabolism of methadone.

1.6.1.3. Metabolism by cytochrome P450

The involvement of different isoenzymes of cytochrome P450 in methadone metabolism is important to understand the clinical pharmacology of this substance.

In vitro and in vivo studies have shown that the isoenzymes of cytochrome P450, CYP3A4 and to a lesser extent, CYP2D6 are involved in methadone metabolism. Other isoenzymes as CYP1A2, CYP2C9 and CYP2C19 might also contribute but their relevance remains to be proved.

CYP3A4

CYP3A4 is the major isoenzyme of cytochrome P450 involved EDDP formation from methadone as described in *in vitro* (Foster et al., 1999) and *in vivo* studies (interaction studies) (Eap et al., 2002; Shinderman et al., 2003; Shiran et al., 2009). The activity of this enzyme is highly variable among individuals, and can be affected by health status, environmental aspects and genetic factors. This metabolic pathway is apparently not enantioselective (Foster et al., 1999; Totah et al., 2007; Totah et al., 2008).

CYP3A4 has a larger expression in liver, but it is also expressed in bowel. It is implicated in the metabolism of different drugs such as benzodiazepines (i.e. alprazolam), antidepressants (sertraline) and other that constitute about 30% of those used in therapeutics. This metabolic pathway is inducible by other drugs such as rifampicine, phenobarbital, phenytoin and carbamazepine. As a result of metabolic induction these drugs may provoke a withdrawal syndrome in patients in MMT. Also drugs such as dexametasone, spironolactone and HIV treatments (protease inhibitors) could decrease plasma concentrations about 40%-50% (McCance-

Katz & Mandell, 2010). Induction of CYP3A4 at the beginning of MMT probably explains, at least in part, the increased EDDP/methadone ratio, the increased clearance, the decreased elimination half-life and the decreased methadone steady-state plasma concentrations measured in patients at the first month of treatment (Wolff et al., 2000) and justify the need of dosage adaptation.

CYP2B6

In vitro and in vivo studies have shown that CYP2B6 is a contributor to methadone metabolism (Li et al., 2008) with an observed stereoselectivity towards de (*S*)-enantiomer (Crettol et al., 2005; Totah et al., 2007; Totah et al., 2008).

CYP2D6

CYP2D6 is expressed in the liver and is subject to genetic polymorphism. *In vitro* studies show a minor role of CYP2D6 in the formation of EDDP from methadone (Foster et al., 1999) with a stereoselectivity towards *(R)*-methadone (Wang & DeVane, 2003). However, observed interactions between methadone and CYP2D6 inhibitors seemed to indicate a more relevant contribution to methadone metabolic disposition (Begre et al., 2002; Eap et al., 1997).

CYP1A2

Available in vivo and in vitro data suggest that CYP1A2 is not involved in methadone metabolism (Li et al., 2008) and

studies on plasma concentrations didn't find influence (Crettol et al., 2006).

Other cytochromes

Other enzymes recently have been evaluated to investigate its relationship with methadone metabolism: CYP2C19 and CYP2C9. Whereas some authors describe an influence in methadone metabolic disposition (Gerber et al., 2004; Totah et al., 2007), other authors (Crettol et al., 2005) haven't found an influence on enantiomer methadone plasma concentrations.

1.6.2. MMT outcome

MMT is the most widely used treatment for heroin dependence and a substantial body of research exists that supports its effectiveness in the treatment of opioid dependence disorder. MMT has demonstrated its efficacy in retaining patients in treatment and decreasing illicit opioid use (Amato et al., 2005b; Farre et al., 2002); decreasing risk behaviors related to the HIV/sexually transmitted diseases (as hepatitis C infection) (Gibson et al., 1999; Marsch, 1998; Novick et al., 1986); decreasing criminal behavior related with drug use (Dolan et al., 2005; Dole et al., 1968); reducing the risk of fatal overdose (Brugal et al., 2005); and improving health related quality of life (Torrens et al., 1997; Torrens et al., 1999). For a revision of MMT programs efficacy see (Torrens & Fonseca, 2009).

In spite of its well-established therapeutic efficacy, there is a large inter-individual variability in outcome; between the 30-80% of patients treated are poor responders to methadone treatment when retention in program and/or illicit opioid use are considered as the main measures of outcome (Bell et al., 2006; Goldstein et al., 2002; Johnson et al., 2000; Termorshuizen et al., 2005; United States General Accounting Office, 1990). Classically, the main strategies used to optimize the MMT programs have been focused in the characteristics of the MMT program, mainly in relation to the methadone provided: high vs. low methadone doses; abstinence oriented programs vs. maintenance-oriented programs, etc) and psychosocial programs offered (Ward et al., 1998).

Several studies discarded the implication of variables as gender, ethnicity, education level, working status, years of consumption and previous treatments in the poor response to MMT (Torrens et al., 1996). However, the role of other subjects' characteristics like psychiatric comorbidity disorders non-drug related, mainly depression in axis I and, antisocial personality disorder in axis II, the most prevalent disorders (Astals et al., 2008; Astals et al., 2009) among treated subjects, as predictors of poor response to MMT remain controversial (Alterman & Cacciola, 1991; Rounsaville et al., 1982; Rounsaville et al., 1986).

Recently, the study of therapeutic response to MMT has been focused in biological variables of subjects, as

genetics, and pharmacodynamics and pharmacokinetics of methadone.

1.6.2.1. MMT outcome: the role of dosage and plasma concentrations

One of the major sources of disagreement among prescribers is the optimal methadone dosage for MMT. In their early work, (Dole & Nyswander, 1965) recommended methadone maintenance dosages of 80-120 mg/day, and several recent studies have consistently shown that an adequate methadone dosage, at least 50 mg/day (but typically 80-100 mg/day), is a major factor in the success of MMT (Epstein et al., 2009; Farre et al., 2002; Preston et al., 2000; Strain et al., 1993; Strain et al., 1999; Termorshuizen et al., 2005). However, many MMT programs prescribe low dosages of methadone, for political, philosophical and moral reasons.

Although a strong correlation between methadone dose and concentrations in plasma has been noted (Wolff & Hay, 1992), the relationship may not be linear. Moreover, there is a need to account for the high degree of inter-individual variability (Eap et al., 2002; Trafton et al., 2006).

Because of the large variability of methadone concentrations, several studies were focused in finding optimal methadone blood concentrations for effective MMT. In some studies, such threshold concentrations could not be found, (Bell et al., 1990; Borg et al., 1995; Dyer et al., 1999; Loimer et al., 1991; Torrens et al., 1998), whereas other

studies have found a relationship between methadone plasma concentration and outcome, measured by the presence of withdrawal symptoms (Bell et al., 1988; Tennant, 1987), or illegal drugs consumption (Crettol et al., 2005; Eap et al., 2000). Values ranging from 50 to 600 µg/l of *(R)/(S)*-methadone have been proposed by several investigators (Bell et al., 1988; Dole & Nyswander, 1965; Holmstrand et al., 1978; Kell, 1995; Loimer & Schmid, 1992; Tennant, 1987; Wolff & Hay, 1992). A concentration of 400µg/L is now often necessary to provide stabilized maintenance, and is used as reference value. Usually, the measurement of methadone plasma concentrations has been recommended for confirming inadequate dosage rather than for determining the optimum dose. Such concentrations should be interpreted within the context of the overall clinical presentation.

As the opioid effect of *(R,S)*-methadone resides mainly in the *(R)*-enantiomer, and considering the wide interindividual variability of the *(R)/(S)*-methadone ratio it could be more reliable to measure the concentration of *(R)*-methadone than *(R,S)*-methadone to correlate blood concentrations with therapeutic outcome. Some studies have worked on this issue (Eap et al., 2000; Eap et al., 2001; Hiltunen et al., 1999). Hiltunen et al. (1999) evaluated 50 patients (25 satisfied with its MMT and 25 unsatisfied with its MMT); although (*R*)-methadone and (*R,S*)-methadone plasma concentrations were similar in the two groups of patients, associations with well-being and methadone plasma

concentrations were stronger for (*R*)-methadone than (*R,S*)-methadone, so, the author recommended the use of (*R*)-methadone plasma concentrations and rating scales when monitoring patients in MMT programs.

Eap et al. (2000) reported in a study measuring the steady-state plasma concentrations of methadone enantiomers and its relationship with illegal opioid consumption. The ratios of (*R*)-, *(S)-,* and (*R,S*)-concentrations-to-dose corrected by weights were significantly and inversely correlated with methadone doses. They recommend a plasma concentration threshold of 400 µg/L for (*R,S*)-methadone and 250 µg/L for (*R*)-methadone. No association was found between therapeutic response and enantiomer or racemic concentrations.

1.6.2.2. Pharmacogenomics in MMT outcome

The study of the relationship between patient's genetic background and therapeutic response to MMT has become an issue of increasing interest (Haile et al., 2008; Li et al., 2008). Most of the studies have focused on genetic polymorphisms in genes coding for methadone-metabolizing enzymes and transporter proteins like p-glycoprotein (Crettol et al., 2006; Crettol et al., 2008a; Levran et al., 2008); also, polymorphisms associated to pharmacodynamic factors as dopamine receptors, mu-opioid receptor (Barratt et al., 2006; Crettol et al., 2008b; Lawford et al., 2000) have been studied.

1.6.2.2.1. Pharmacodynamics of MMT response

Interindividual differences in MMT outcome, could also due to its pharmacological effects such as pain relief, mood states, withdrawal symptoms and satisfaction with treatment.

Opioid endogenous system

The **μ-opioid receptor** has been described previously according to its association with opioid dependence. Other studies have evaluated the functional consequences of *OPRM1* variability. For example, subjects expressing the protein variant Asp40 present a stronger cortisol response to receptor blockade by naloxone and a reduced agonist effect of morphine-6-glucoronide (Hernandez-Avila et al., 2003), also, there have been described interindividual differences in pain scores and self-administered intravenous morphine (Sia et al., 2008) and differences in the doses of analgesics in pain treatment (Wu et al., 2009). A previous study has reported an association of the *OPRM1* rs1799971 (A118G) SNP with a decreased potency of methadone (Lötsch et al., 2006). Crettol et al. (2008b) didn't found any association between *OPRM1* polymorphisms and MMT outcome, nor with methadone doses. Although evidence seems that A118G polymorphism is important for opioid therapy, there are not conclusive results in the implications on MMT response.

Dopaminergic system

As described previously, dopamine receptors play an important role in the rewarding effects of drugs of abuse. Some studies have also investigated the implications of ***DRD2*** **polymorphisms** in MMT outcome. Lawford et al. (2000) reported a significant association of the *DRD2* TaqI A_1 allele with both, opioid dependence compared to a healthy control group, and also with response to MMT. Lately, this finding was not confirmed in another study (Barratt et al., 2006).

Crettol et al. (2008b) analyzed in the same gene a synonymous polymorphism (DRD2 957C>T) which was shown to be in linkage disequilibrium with the TaqI A SNP in a group of 238 heroin dependent subjects in a MMT program. The carriers of the 957CC were more frequently non-responders to treatment with a shorter period of negative results in urine tests.

Finally, in a recent study (Doehring et al., 2009), in a sample of 85 MMT patients , average and maximum daily methadone doses were significantly associated with the DRD2 rs6275C>T SNP; the carriers of the T allele needed higher methadone doses than noncarriers, and also it was associated with longer time to reach the maximum methadone dose.

Recently, an association between β-arrestin 2 (*ARRB2*) and methadone maintenance treatment has been described (Oneda et al., 2010). β-arrestin 2 is a component of

the G-protein-coupled receptor complex and is involved in mu opioid and D2 receptor signaling.

Nevertheless, few studies have been published abordando the genetics of pharmacodynamics in MMT response.

1.6.2.2.2. Pharmacokinetics in MMT response

CYP3A4

As explained previously, CYP3A4 has been demonstrated in vitro studies to be the main CYP isoform implicated in methadone metabolism in a non stereoselective way. Many SNPs have been described but most of them occur with low allelic frequencies (below 5%) (Li et al., 2008). The most studied variant is the *CYP3A4*1B*. In an in vitro study, the *CYP3A4*1B* variant allele was associated with a 1.5-fold increase in transcription, whereas other report showed that there was no change in enzyme activity (Li et al., 2008).

In an *in vivo* study by Crettol et al. (2006) the carriers of the *CYP3A4*1B* variant presented a 1.4-fold increase for *(S)*-methadone and 1.1-fold increase for *(R)*-methadone; also, the CYP3A4*1B variant carriers have more probability to be in the low-dose group, suggesting that they have higher methadone plasma concentrations and require lower methadone doses.

CYP2B6

In vivo studies also demonstrated that CYP2B6 genotype influences methadone plasma concentrations, mainly *(S)*-methadone. Multiple SNPs within the CYP2B6 gene, located on chromosome 19q13.2, have been described. The 1459C>T genetic polymorphism (Arg487Cys), present in CYP2B6*5 and *7 alleles, corresponds to lower CYP2B6 protein levels, in both, homozygous and heterozygous individuals compared to wild types (Lang et al., 2001).

Other authors showed that CYP2B6*6/*6 homozygous individuals (Gln172His, Lys262Arg) have low CYP2B6 protein levels. In a study in opioid dependent subjects under MMT, CYP2B6 genotype influenced *(S)*-methadone plasma concentrations and only weakly *(R)*-methadone plasma concentrations, and no differences were found in terms of MMT response nor methadone dose requirements. This stereoselectivity towards the non active enantiomer could explain this results (Crettol et al., 2006).

CYP2D6

CYP2D6 displays a genetic polymorphism with a number of allelic variants. One hundred allelic variant of the *CYP2D6* have been identified; of these, *3 to *8 are nonfunctional, *9, *10, *41 have reduced function, and *1, *2, *35, *4 and *41 can be duplicated, resulting in an increased expression of functional (or non-functional) CYP2D6 protein. Allele combinations determine *CYP2D6* phenotype, which includes

poor metabolizer (PM; two non functional alleles), extensive metabolizer (EM; at least one functional allele) and ultra-rapid metabolizer (UM; a multiple copies of a functional allele and/or allele with promoter mutation). These phenotypes have been related with methadone plasma concentrations (Eap et al., 2001), being apparently higher those seen in PM subjects.

In the study of Crettol et al. (2006), the authors found that UM presented lower trough *(R,S)*-methadone plasma concentrations compared to the EM or IM whereas *CYP2D6* PM status showed no influence. The authors suggested that the compensatory activity of other CYP could explain this, and also the fact that a high number of patients were taken other medications may also have transformed the activity status.

A study conducted by Perez de los Cobos et al. (2007), showed that, those heroin dependent subjects under MMT and UM phenotype reported less satisfaction with their methadone dose, whereas PM didn't complain about their methadone treatment.

Discrepancies between genotype and *in vivo* CYP2D6 activity in MMT patients have been described (Shiran et al., 2003); the authors postulated that the finding was consistent with inhibition of CYP2D6 activity by methadone (Wu et al., 1993).

CYP2C9

Some studies have described an involvement of CYP2C9 in methadone metabolism (Li et al., 2008) but results are controversial. There is an important interindividual variability in the activity of CYP2C9 which is genetically determined. There are two variant alleles (*2 [430C>T; Arg144Cys] and 3* [1075A>C; Ile359Leu]), that have been shown to affect the activity of the enzyme. In two studies by Crettol et al., an association between *CYP2C9* genotypes and methadone response was not found (Crettol et al., 2005; Crettol et al., 2006).

CYP2C19

Several studies showed an involvement of CYP2C19 with a stereoselectivity towards the *(R)*-enantiomer (Gerber et al., 2004; Totah et al., 2007; Totah et al., 2008). As for the previous CYP, in vivo studies didn't show association with methadone plasma concentrations, dose requirements nor methadone treatment outcome (Crettol et al., 2005; Crettol et al., 2006; Crettol et al., 2007).

P-glycoprotein

Methadone is a substrate of P-glycoprotein which shows a weak stereoselectivity towards the *(S)*-enantiomer (Crettol et al., 2007). It is a trans-membrane protein of 1280 amino acids. It is expressed in tissues with barrier function, as liver, intestines, kidneys, lymphocytes, placenta and the endothelial

cells lining in the brain capillaries (Fojo et al., 1987; Li et al., 2008). The activity of P-glycoprotein in intestines and the brain blood barrier has been shown of some relevance in defining methadone concentrations (Li et al., 2008).

The P-glycoprotein is encoded by the multidrug resistance 1 (*ABCB1*) gene on chromosome 7p21. This gene is highly polymorphic and numerous variants have been associated with drug response (Hoffmeyer et al., 2000). The majority of studies focused on a nonsynonymous exon 26 SNP, 3435C>T; the homozygosity to the allele T showed lower in vivo duodenal P-glycoprotein expression (Hoffmeyer et al., 2000); also, the *ABCB1* 3435T allele may alter the stability of ABCB1 mRNA and is associated with lower mRNA concentrations (Wang et al., 2005; Wang & Sadee, 2006).

Genetic variability of *ABCB1* and effects on MMT has been studied. One study (Coller et al., 2006) with 60 opioid dependent subjects in MMT showed that *ABCB1* genetic variability influenced daily methadone requirements.

In another study (Crettol et al., 2006), on 245 MMT patients, the *ABCB1* 3435TT carriers presented lower though *(R,S)*-methadone plasma concentrations, but they didn't found any influence in therapeutic response.

Levran et al. (2008), studied the association between *ABCB1* polymorphisms and methadone dose requirements in 98 maintained patients, and they found that individuals bearing 3- locus genotype pattern TT-TT-TT (rs1045642,

rs2032582 and rs1128503) have more chance to require higher methadone doses.

Several studies have been conducted to assess the influence of haplotypes on the clinical response to methadone, with contradictory findings, probably related to methodological differences (methadone formulations, treatment duration and previous exposure to methadone) (Li et al., 2008).

In conclusion, genetic variability at *ABCB1* level may influence in the methadone dose requirements in opioid dependent subjects.

Taking all this data into account it seamed justified to perform a pharmacogenetic study on MMT efficacy and dose requirements, involving both, pharmacodynamic and pharmacokinetic factors.

2. OBJECTIVES

2. OBJECTIVES

2.1. Main objective

To study the variability in genes potentially associated to therapeutic response in patients enrolled in a methadone maintenance treatment for opioid dependence.

2.2. Specific objectives

- To study the genetic variability in coding genes implicated in pharmacodynamics of methadone.
- To study the genetic variability in the coding genes implicated in pharmacokinetics of methadone.

3. SUBJECTS AND METHODS

3. SUBJECTS AND METHODS

3. 1. Design

Association, cross-sectional study.

3.2. Patients

Participants in the study were subjects, both genders, older than 18 that fulfilled the following inclusion criteria: patients who met criteria for Diagnostic and Statistical Manual of Mental Disorders (4th Edition) [DSM-IV] opioid dependence disorder, and being in the same MMT program during at least 2 months.

Exclusion criteria were language-related barriers, severe cognitive impairment, or any medical disorder that would interfere with the research assessments.

3.3. Characteristics of MMT

All patients were recruited from CAS Barceloneta. CAS Barceloneta is an out-patient center for drug addicts located in the old part of the city of Barcelona (Spain). The area of influence is problematic, with small houses, high unemployment, many immigrants, a high percentage of people with legal problems, drug trafficking and prostitution. The staff is constituted by three psychiatrists, one psychologist, one specialist in infectious diseases, one social worker and three nurses.

The characteristics of the MMT provided were: no upper limit on methadone dosages prescribed; no restriction

on duration of treatment; forced discharge occurred only as a result of patients' violent behavior or drug trafficking; and that clinical management included individual counseling and encouraged drug abstinence.

3.4. Measures

3.4.1.Protocol "ad hoc"

A close-ended questionnaire (**Appendix 2**) was used to register the patient's demographic characteristics (age, sex, family situation); employment status (currently employed, number of months employed); criminal status (criminal history, imprisonment,), serological status (antibodies positive for HIV, HCV), substance use history (age of onset of regular use, days of use during the last 30 days, initial and current route of use and mean of amount use/day), any previous psychiatric treatment, and concomitant pharmacological treatments.

3.4.2. Psychiatric diagnoses

Substance use and non-substance use psychiatric disorders were diagnosed according to DSM-IV criteria using the Spanish version of the Psychiatric Research Interview for Substance and Mental Disorders (PRISM-IV) (Torrens et al., 2004). This structured interview is specially suited to provide diagnoses of primary and substance-induced disorders and to distinguish between the expected effects of intoxication or withdrawal of drugs, from psychiatric symptoms. The PRISM

has shown good to excellent test-retest reliability and validity across a variety of psychiatric diagnoses in substance abuse patients (Hasin et al., 2006; Torrens et al., 2004).

3.4.3. Personality Profile: Cloninger's Temperament and Character Inventory (TCI)

Personality profile was assessed by the Spanish version of the Temperament and Character Inventory -TCI- (Gutierrez et al., 2001), a self-administered dimensional questionnaire constructed by Cloninger et al. (Cloninger et al., 1994) to assess the seven basic dimensions of personality defined in his psychobiological model of personality. Cloninger et al. (1993) described four dimensions of temperament that have been related to different neurotransmitter system: "novelty seeking" (dopaminergic system), "harm avoidance" (serotonergic system), "reward dependence" (oxytocine), and "persistence" (noradrenergic system), which are independently heritable and manifested early in life. There are also three dimensions of character: "self-directedness", "cooperativeness", and "self-transcendence" that contain goals, values and coping strategies learned in the sociocultural milieu that evolve through the lifetime. The TCI has shown a moderate to high internal consistency for all personality dimensions in its validation in a Spanish sample (Gutierrez et al., 2001). Additionally, character dimensions have demonstrated to be a reliable, valid and low-cost tool for

detecting personality disorders in drug abusers (Gutierrez, et al., 2002).

3.4.4. Addiction Severity Index (ASI)

To measure the degree of impairment related to addiction, patients were assessed with the Spanish version of the Addiction Severity Index -ASI- (Gonzalez et al., 2002). The ASI instrument is a structured clinical interview developed to evaluate in a reliable, valid, and standardized way, the clinical status of substance abuse patients and to plan a specific treatment, as well as its use for research purposes (McLellan et al., 1980). The ASI gives 10-point problem severity ratings in each of six areas commonly affected by addiction (general health status, economic and working problems, alcohol use, other substances use, legal problems, family and social relationships and psychological status). The ASI instrument is highly reliable and valid (McLellan et al., 1980).

3.4.5. Urine controls and outcomes

The use of illegal heroin was assessed by reviewing the results of the last four urine testing performed over last 1-2 months months before blood sampling, once methadone dose has been stabilized; that means, i) patients don't experience opioid withdrawal symptoms, ii) patients don't refer frequent craving to use opioids, iii) they don't experience opioid reinforcement if they use heroin, and iv) patients don't present severe side effects.

Urinalyses were carried out at the centre, 1 day at random every 1 or 2 weeks, under supervision of the nursing staff to detect the presence of heroin consumption.

According to urinalyses, patients were classified as responders or nonresponders to MMT. Responders to MMT were those patients whose last four urinalysis tests were negative for illicit opioids, and nonresponders were those with two or more of their last four urinalysis tests positive for illicit opioids. Those patients with one positive urinalysis test were not included in the study.

3.4.6. Methadone analysis

An enantioselective determination of methadone and its metabolite EDDP, analyzed in 5 ml of peripheral blood 24 hours after last supervised methadone oral administration was done. A capillary electrophoretic system (CE, ^{3D}Hewlett-Packard) equipped with a diode-array detector (UV) was used for the enantioselective determination of methadone. (*S*)-dextrorphan and (*R*)-levorphanol were used as internal standards for (*S*)- and (*R*)-methadone, respectively. After a liquid-liquid extraction of 1 mL of plasma with *tert*-butilmethyleter (Torrens et al., 1998), resolution of the enantiomers was performed in an untreated fused-silica capillary of 48.5-cm total length (40-cm effective length) and a standard 50-µm optical path length cell. A constant voltage of 25 kV was applied and the cartridge temperature was maintained at 16ºC, The diode-array detector was set to

monitor the signal at 204 nm. Resolution was performed by using 1mM heptakis-(2,6-di-O-methyl)-β-cyclodextrin in 100 mM H_3PO_4, pH=2.5 as a running buffer (Kelly et al., 2003). Calibration curves were prepared for each analytical batch with appropriate volumes of the corresponding racemic mixture working solutions added to test tubes containing 1 mL of drug-free plasma, and were linear over 100-500 ng/mL calibration range of the corresponding enantiomers. Control plasma samples containing 150 (low control) and 350 ng/mL (high control) of methadone enantiomers were prepared in drug-free plasma and were kept frozen at –20°C in 1 mL aliquots until their use.

Peak-area ratios between compounds and I.S. were used for calculations. A weighted (1/concentration) least-square regression analysis was used (SPSS for Windows, version 12.0). Extraction efficiencies for (*R*)-methadone and (*S)*-methadone were calculated by comparing the peak areas of equal concentrations of drug extracted and non-extracted, being 99.8 and 86.7%, respectively. Four replicate analyses were performed with plasma samples corresponding to the first level of concentrations of the calibration curves, and 3 and 10 standard deviations (SD) of the calculated concentrations at this calibration level were used for estimating the limits of detection (LOD) and quantization (LOQ), respectively, being LOD 25.9 and 23.2 ng/mL and LOQ 78.5 and 70.2 ng/mL for (*R)* and (*S*)-enantiomer, respectively. Precision was calculated as the relative

standard deviation (RSD) of the quality control samples concentrations and there were 8.9% and 10.2% for (*R*) and (*S*)-methadone, respectively. Accuracy is expressed as the relative error of the calculated concentrations, being 8.2 and 10.9% for (*R*) and (*S*)-methadone, respectively.

3.4.7. Genetic analysis

A collection of 20 ml of blood was done to extract DNA from leukocytes to evaluate existing variants of coding genes of the following proteins:

- Brain-derived neurotrophic factor (*BDNF)*
- Mu-Opioid Receptor (*OPRM1*)
- Myocardin (*MYOCD*)
- Metabotropic glutamate receptors (*mGluR6* and *mGluR8)*
- Cryptochrome 1 (*CRY1)*
- Nuclear receptor subfamily 4, group A, member 2 (*NR4A2*)
- 1q31.2 (rs965972)
- 2q21.2 (rs1867898)
- Cytochrome P450: *CYP3A5*; *CYP2D6; CYP2B6; CYP2C9; CYP2C19*
- Multidrug Resistance 1 Gene (*ABCB1*)

BDNF SNPs were genotyped using the SNPlex™ platform (Applied Biosystems, Foster City, CA) according to the manufacturer instructions and analyzed on an Applied Biosystems 3730xl DNA Analyzer. Allele-calling was done by

clustering analysis using Genemapper software (Genemapper v.4.0).

Genotyping of *OPRM1*, *MYOCD*, *mGluR6*, *mGluR8*, *CRY1*, *NR4A2*, *1q31.2* and *2q21.2* was performed in the CEGEN (Spanish National Genotyping Centre), Universidad de Santiago Node, using the hME MassARRAY SNP genotyping system (Sequenom Inc., San Diego, CA).

For the study of *CYP3A5*, *CYP2D6*, *CYP2B6*, *CYP2C9*, *CYP2C19*, and *ABCB1*, the genotyping was performed using a DNA microarray (Progenika Biopharma, Derio, Spain). Details on the allelic variants monitored per gene as well as performance of the microarray have been previously described (Cuyas et al., 2010). The genotype distribution and corresponding variant allele frequency were calculated. According to the genotype, the phenotype was inferred and subjects classified in four potential categories: extensive metabolizer (EM; 'normal' activity), intermediate metabolizer (IM; decreased activity), poor metabolizer (PM; no enzyme activity), and ultrarapid metabolizer (UM; increased activity). The various phenotypes were predicted in the following manner: EM: combination of two functional alleles, or the presence of one functional allele in combination with a decreased-activity or non-functional allele; IM: presence of two decreased-activity alleles, or the combination of decreased-activity and non-functional alleles; PM: two no-

functional alleles; UM: presence of more than two functional, alleles (CYP2D6).

3.5. Statistical analysis

To minimize the effects of population stratification, we only studied individuals who were of Caucasian ethnicity. Differences in socio-demographic and clinical characteristics between groups were examined using Chi-square and one-way ANOVA tests, with Bonferroni and Tukey *post hoc* analysis, using the SPSS software analysis package version 12.0. All genotypes were checked for deviation from Hardy-Weinberg equilibrium. We used multivariate logistic regression analyses to examine associations between the SNPs in the responder and non-responder groups.

3.6. Ethical aspects

Written informed consent was obtained from each subject after they had received a complete description of the study and been given the chance to discuss any questions or issues. The study was approved by the Ethical and Clinical Research Committee of the institution (CEIC nº: 2001/1219/1).

4. RESULTS

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Title Page

Original Contributions

Influence of Cytochrome P450 and ABCB1 Genetic Variability on Methadone Pharmacokinetics, Dose Requirements, and Response

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Running title: Pharmacogenetics and methadone treatment

Original Contribution

Influence of Cytochrome P450 and ABCB1 Genetic Variability on Methadone Pharmacokinetics, Dose Requirements, and Response

Abstract

Although the efficacy of methadone maintenance treatment (MMT) in opioid dependence disorder is well established, the influence of methadone pharmacokinetics in dose requirement and clinical outcome remains controversial. The aim of this study is to analyze methadone dosage in responder and nonresponder patients considering pharmacogenetic and pharmacokinetic factors that may contribute to dosage adequacy. Opioid dependence patients (Diagnostic and Statistical Manual of Mental Disorders, [4th Edition] criteria) from a MMT community program were recruited. Patients were clinically assessed and blood samples were obtained to determine plasma concentrations of (R,S)-, (R) and (S)- methadone and to study allelic variants of genes encoding CYP3A5, CYP2D6, CYP2B6, CYP2C9, CYP2C19, and P-glycoprotein. Responders and nonresponders were defined by illicit opioid consumption detected in random urinalysis. The final sample consisted in 105 opioid dependent patients of Caucasian origin. Responder patients received higher doses of methadone and have been included into treatment for a longer period. No

differences were found in terms of genotype frequencies between groups. Only CYP2D6 metabolizing phenotype differences were found in outcome status, methadone dose requirements, and plasma concentrations, being higher in the ultrarapid metabolizers. No other differences were found between phenotype and responder status, methadone dose requirements, neither in methadone plasma concentrations. Pharmacokinetic factors could explain some but not all differences in MMT outcome and methadone dose requirements.

Key words: Methadone maintenance treatment, pharmacogenetics, pharmacokinetics, Cytochrome P450, CYP2D6

Introduction

Maintenance treatment of opioid dependence with methadone is a well known pharmacotherapy approach. However, there is a large interindividual variability in outcomes among subjects in methadone maintenance treatment (MMT).[1] In fact, between the 30% and 80% of patients receiving methadone are poor responders when retention in the MMT and/or illicit opioid use are considered as the main outcome variables.[2,3] Several factors like, poor coping self-efficacy,[4] mood states,[5] genetic polymorphisms in drug metabolizing enzymes,[6] and methadone pharmacokinetics[7] have been suggested as contributing factors. One of the main factors related to MMT success is the dose of methadone provided.[8-10] Although a strong correlation between methadone dose and concentrations in plasma has been reported,[11] this relationship may not be linear, and it has been shown that the determination of methadone plasmatic concentrations and their enantiomers is not useful to predict illicit opioid use, nor opioid withdrawal symptoms.[12,13]

Methadone is metabolized by the cytochrome P450 system. The major metabolizing enzymes are CYP3A4, CYP2B6 and CYP2D6 to a lesser extent.[14] Other isoenzymes, such as CYP1A2, CYP2C8, CYP2C9 and CYP2C19 could also be involved in methadone metabolism but there are contradictory data.15 Furthermore, methadone is a substrate for the P-glycoprotein transporter, a

transmembrane protein encoded by the multidrug resistance gene 1 (ABCB1),[16] that has been related to methadone dose requirements in previous studies.[14,17] The study of patients' genetic polymorphisms in genes encoding for methadone-metabolizing enzymes and transporters[14,18-20] has been an active area of research[15,21] but the clinical relevance in MMT outcome is unclear.

This cross-sectional study was designed to assess the influence of ABCB1 and cytochrome P450 genetic variability on methadone pharmacokinetics, dose requirements, and clinical response in opioid dependent patients included in a MMT program.

Material and Methods

Design and Patients

The study recruited opioid dependence patients who met criteria for Diagnostic and Statistical Manual of Mental Disorders (4th Edition) [DSM-IV] opioid dependence from a MMT program (MMT community program, CAS Barceloneta, Barcelona, Spain). The main characteristics of the MMT provided included: clinical management with individual counseling to encourage drug abstinence, methadone dosages as required (no restrictions for upper limit) and no restriction on treatment duration. Forced discharge occurred only as a result of patients' violent behavior or drug trafficking. To be eligible for the study, patients had to be Caucasian, enrolled in MMT for at least four months, and receiving a

stable methadone dose for the last two months. Exclusion criteria were as follows: language-related barriers, severe cognitive impairment, and any medical condition that would interfere with research assessments and refusal to take part in the study.

Clinical Assessment

A close-ended questionnaire was used to record patients' socio-demographic characteristics, serological status (Human Immunodeficiency Virus [HIV], Hepatitis C Virus [HCV]), history of substance use, and previous psychiatric pharmacological treatment as well as other concomitant treatments. Substance use disorders and other psychiatric disorders were diagnosed according to DSM-IV criteria, using the Spanish version of the Psychiatric Research Interview for Substance and Mental Disorders (PRISM-IV) for axis I and II (borderline and antisocial personality disorders).[22,23] The degree of addiction-related impairment was assessed using the Spanish version of the Addiction Severity Index (ASI).[24,25]

The use of illegal opiates was evaluated by reviewing the results of the last 4 urine tests performed over 2 months before study inclusion. Urinalyses for the detection of heroin consumption were carried out at the centre, 1 day at random every 1 or 2 weeks, under supervision of the nursing staff. It was considered that illegal opiates had been used when 2 or more urinalyses tested positive for heroin (in the last 4 drug tests in urine). These results were used to group patients as

responders (all drug tests were negative) and nonresponders (2 or more positive drug tests).

Written informed consent was obtained from each subject after they had received a complete description of the study and been given the chance to discuss any questions or issues. The study was approved by the Ethical and Clinical Research Committee of our institution (CEIC-IMAS).

Plasma Samples Analysis

A blood sample (5 mL) was taken 24 hours after the last supervised methadone oral administration. The (R)-, (S)- and (R,S)- methadone plasma concentrations were determined by capillary electrophoresis technique (CE-UV) after a liquid-liquid extraction of samples with tert-butylmethylether. A capillary electrophoretic system (CE, 3DHewlett-Packard) equipped with a diode-array detector (UV) was used for the enantioselective determination of methadone. (S)-dextrorphan and (R)-levorphanol were used as internal standards (I.S.) for (S)- and (R)-methadone, respectively. After a liquid-liquid extraction of 1 mL of plasma with tert-butylmethylether,[13] resolution of the enantiomers was performed in an untreated fused-silica capillary of 48.5-cm total length (40-cm effective length) and a standard 50-µm optical path length cell. A constant voltage of 25 kV was applied and the cartridge temperature was maintained at 16ºC, The diode-array detector was set to monitor the signal at 204 nm. Resolution was performed by using 1mM

heptakis-(2,6-di-O-methyl)-β-cyclodextrin in 100 mM H3PO4, pH=2.5 as a running buffer.[26]

Calibration curves were prepared for each analytical batch with appropriate volumes of the corresponding racemic mixture working solutions added to test tubes containing 1 mL of drug-free plasma, and were linear over 100-500 ng/mL concentration range of the corresponding enantiomers. Control plasma samples containing 150 (low control) and 350 ng/mL (high control) of methadone enantiomers were prepared in drug-free plasma and were kept frozen at –20°C in 1 mL aliquots until their use.

Peak-area ratios between compounds and I.S. were used for calculations. A weighted (1/concentration) least-square regression analysis was used (SPSS for Windows, version 14.0). Extraction efficiencies for (R)-methadone and (S)-methadone were calculated by comparing the peak areas of equal concentrations of drug extracted and non-extracted, being 99.8 and 86.7%, respectively. Four replicate analyses were performed with plasma samples corresponding to the first level of concentrations of the calibration curves, and 3 and 10 standard deviations (SD) of the calculated concentrations at this calibration level were used for estimating the limits of detection (LOD) and quantization (LOQ), respectively, being LOD 25.9 and 23.2 ng/mL and LOQ 78.5 and 70.2 ng/mL for (R) and (S)-enantiomer, respectively. Precision was calculated as the relative standard deviation (RSD) of the quality control samples

concentrations and there were 8.9% and 10.2% for (R) and (S)-methadone, respectively. Accuracy is expressed as the relative error of the calculated concentrations, being 8.2 and 10.9% for (R) and (S)-methadone, respectively.

Genetic Analysis

A collection of 20 mL of blood was done to extract DNA from leukocytes to evaluate allelic variants of genes encoding the following proteins: cytochrome P450 3A5 (CYP3A5); cytochrome P450 2D6 (CYP2D6); cytochrome P450 2B6 (CYP2B6), cytochrome P450 CYP2C9 (CYP2C9), cytochrome P450 CYP2C19 (CYP2C19), and the Multidrug Resistance 1 transporter (ABCB1).

The genotyping was performed using a DNA microarray (Progenika Biopharma, Derio, Spain). Details on the allelic variants monitored per gene as well as performance of the microarray have been previously described.[27] The genotype distribution and corresponding variant allele frequency were calculated. According to the genotype, the phenotype was inferred and patients classified in four potential categories: extensive metabolizer (EM; 'normal' activity), intermediate metabolizer (IM; decreased activity), poor metabolizer (PM; no enzyme activity), and ultrarapid metabolizer (UM; increased activity). The various phenotypes were predicted in the following manner: EM: combination of two functional alleles, or the presence of one functional allele in combination with a decreased-activity or non-functional allele; IM:

presence of two decreased-activity alleles, or the combination of decreased-activity and non-functional alleles; PM: two no-functional alleles; UM: presence of more than two functional, alleles (CYP2D6).

Statistical Analysis

Descriptive statistics of all variables of interest are presented as means and standard deviation (SD) in case of quantitative variables, and by absolute and relative frequencies in case of categorical variables. Differences in sociodemographic and clinical characteristics between groups were examined using Chi-square, One-Way ANOVA and T student (when appropriate) tests. The phenotypes were compared with respect to methadone dose and plasmatic concentrations using one-way ANOVA together with Tukey post hoc analysis for pairwise comparisons. All analyses were performed with the statistical software package SPSS (SPSS Inc., Chicago, IL), version 14.0.

Results

Clinical Characteristics of Patients

From 169 eligible patients, 12 were non Caucasian and were excluded. Reliable information on patients' medical history and on the use of concurrent medication was obtained from 105 patients (71% male; mean age 38 years [SD=8]) by personal interview and by review of the clinical records. The characteristics of patients (already split in responders and

nonresponders) are represented in Table 1. The mean methadone dose of patients included in the study was 98 mg/day (SD=64). All but 2 patients were smokers and the 65% were taking concomitant treatments. Responders and nonresponders showed similar characteristics except of, days of heroin use in the last 30 day (responders 0 days [SD=1] vs. nonresponders 16 days [SD=10]), methadone dosage (responders 109 mg/day [SD=68] vs. nonresponders 72 mg/day [SD=43], p=0.007), months in methadone (Responders 52 months [SD=49] vs. nonresponders 21 months [SD=32], p=0.001), and in the Drug Use ASI scale and Legal Problems ASI scale.

Plasma samples were obtained from 79 patients. There were no differences between these 79 patients from which we obtained plasma and the rest (n=26, 11 responders and 14 nonresponders) of patients included in the study samples in terms of sociodemographic, neither medical nor psychopathological characteristics. Blood samples for genotyping were usually obtained at the inclusion of patients in the MMT. Blood samples for methadone determination were obtained once the patient was enrolled for 4 months at the MMT and dose was stabilized (according to inclusion criteria) in the MMT for 2 months. The main reason for not obtaining blood samples from all patients once dose was stabilized is the lack of cooperation for sample withdrawal.

Genotypes and Phenotypes

The frequencies of genotypes are represented in **Table 2** and those of the different phenotypes are represented in **Table 3**. No differences were observed in the distribution of genotypes and phenotypes for genes evaluated among responders and nonresponders patients except for an overrepresentation of UM subjects of CYP2D6 in responder patients.

Methadone Dose Requirements, Plasma Concentrations, and Phenotype

We studied the mean methadone dose, *(R)-, (S)-* and *(R,S)-* methadone plasma concentrations by phenotype for all genes evaluated. Results for all genes studied can be found in Supplementary materials (**Table 1 Supplemental Digital Contents**). Results were essentially negative except for *CYP2D6* (see **Table 4**). We found significant differences in methadone dose requirements and plasmatic concentrations depending on the phenotype status in CYP2D6, taking patients all together: The 5 UM received higher doses of methadone and also, presented higher *(R,S)-, (R*) and *(S)* methadone concentrations, compared to EM (Tukey post-hoc analysis). PM required marginal lower doses of methadone compared to other phenotypes. A similar trend of results was observed when grouping patients as a function of clinical outcome (see **Table 4**).

Discussion

Methadone patients categorized as responders and nonresponders on the basis of drug misuse while enrolled in the MMT differ on the daily dose of methadone they receive (109±68 mg/day vs. 72±43 mg/day). The lower dose of nonresponder patients cannot be explained by restrictions for upper limit in methadone dosage in the framework of the MMT.

A potential explanation of such dosage differences and clinical outcome may come from alterations in methadone pharmacokinetics due to genetic polymorphisms regulating it. The genetic polymorphisms of *CYP3A5, CYP2B6, CYP2C9, CYP2C19,* and *ABCB1* did not influence methadone doses. A small influence of *CYP2D6* in methadone doses and plasma concentrations was found. Mean plasma concentrations of *(R,S)*-methadone and of each enantiomer are not significantly different between responders and nonresponders, although concentrations in nonresponders were 30% lower than in responders in agreement with differences in dose requirements between both groups. Therefore, differences in clinical outcome cannot be justified on the basis of some kind of genetic differences in polymorphic drug metabolizing enzymes.

Concerning *CYP2D6* genetic polymorphism, its contribution on methadone metabolic disposition and dosage is controversial. Several reports suggest that its contribution is negligible[28,29], while others have shown that specific

inhibitors of CYP2D6 as paroxetine, markedly influence methadone disposition.[30,31] Five CYP2D6 UM subjects were identified among responder patients while none among nonresponders. The UM phenotype has been associated to lack of satisfaction of methadone treatment6 and with lower trough *(R,S)*-methadone plasma levels compared to other CYP2D6 phenotypes,[14] suggesting an increased methadone metabolic disposition. In this study, UM patients required high doses of methadone (about 180 mg), about twice to those provided to EM patients. Nevertheless this increased request of methadone is not related with an increased metabolic disposition as plasma concentrations of methadone and its enantiomers are the highest among all CYP2D6 phenotypes. The five PM patients included in this study (3 in the nonresponders and 2 in the responders groups) required marginally lower methadone doses and display twice methadone plasma concentrations of EM subjects, being methadone dosage quite similar. These results would imply an involvement of CYP2D6 in methadone metabolism. Discrepancies between CYP2D6 genotype and phenotype in terms of methadone metabolism have been already described.[32] The observed discrepancies could be related to interactions with other drugs as CYP2D6 has been implicated in the metabolism of other medications.[33] The effect of drug interactions could not be discarded in our results as the high proportion of patients taking concomitant medications (65%). Nevertheless present observations should apply to EM

patients, but not to PM patients (homozygotes for non-functional allelic variants) with a non-functional enzyme or to UM (homozygotes for more than two functional allelic variants) the most susceptible to drug interactions but also, those requiring the larger doses. A recent report suggests that CYP2D6 a non-inducible hepatic enzyme, may be induced at the brain level by nicotine.[34] As almost all participants were smokers, there may be a dissociation between plasma concentrations, hepatic metabolism and genotype, and brain drug requirements. However, the contribution of CYP2D6 to methadone metabolism as well the interaction with smoking deserves further studies.

It is apparent from the present study that interindividual pharmacokinetic differences among patients can be compensated by clinical management of the doses of methadone (e.g. dose requirements of UM patients). Although the absence of restrictions in dose increases in our MMT program, some patients with poor response to MMT do not accept increases in their methadone dose. It could be hypothesized that those patients show significant adverse events associated to methadone. *(S)*-methadone has been previously associated to adverse responses of *(R,S)*-methadone as negative mood effects –tension, fatigue, confusion…-.[5,35] No significant differences in the *(S)*-methadone plasmatic concentrations have been detected, nor in the *(R)/(S)* ratio in this sample. Other possible explanations could be a pharmacodynamic influence in the reluctance of a

considerable group of patients to increase methadone dose. An influence of a polymorphism on the DRD2 gene promoter (rs1800497 C>T) has been associated with both, the risk of opiate addiction, leading to the necessity of methadone substitution therapy, and the course of this therapy in terms of dosage requirements.[36] Other pharmacodynamic influences in those patients could be a difference related to the activation of kappa opioid receptors. Kappa opioid receptors have been involved in the response to drugs (cocaine, alcohol and opiates)[37] in opiate withdrawal and stress responsivity;[38] kappa agonists lower the levels of dopamine in the nucleus accumbens and act in a counter-modulatory manner to attenuate the increase in dopamine levels39 and induce a negative mood state.[38] Although lower than for the mu type, methadone also has some affinity for kappa opioid receptor, without differences in enantiomer affinity.40 The study of pharmacogenomics at this level could explain the implication of this receptor in MMT outcome.

The negative results in terms of the genotypes and phenotypes of drug metabolizing enzymes examined in this study are consistent with previous data.[14,18,41] Classically, the main strategies used to optimize the MMT programs have been focused in the methadone dose provided: high vs. low methadone doses [8-10] and treatment response has been evaluated in terms of retention in treatment and opioid consumption measured by positive urine tests. In recent years, aspects as patients' satisfaction with the MMT program

are considered important in the outcome[42,43] also, personal attitudes as coping self-efficacy have been recently received attention.[4]

Other factor to take into account in response to methadone maintenance treatment is the duration of treatment. As seen in our results, responder patients stayed in treatment more than twice than nonresponders (52 vs. 21 months). Some studies have shown that the results obtained after treatment over a period of less than 3 months were comparable to those obtained after no treatment at all[44] and others described reduction in drug use when patients remained in treatment for at least one year.[45]

The present findings should be interpreted taking into account some limitations of the study. Firstly, the sample size was small; complex study procedures in the framework of a longitudinal design (urine testing, blood analyses for genotyping, time consuming interviews) can result in a non-negligible number of patients with incomplete follow-up data. It is also remarkable that patients with poorer outcomes (for example, more illicit drug use) were more reluctant to accept to participate in clinical studies; this could imply a bias in the study results, but, on the other hand, to offer a payment for the participation it is not acceptable in the ethical committee. Lastly, we cannot exclude a risk of stratification effect, although all subjects were Caucasian.

In summary, the present results suggest a small influence of enzymes involved in methadone metabolism and transport on MMT outcome. This influence is usually managed by clinicians increasing methadone doses and dividing them twice a day (b.i.d.). The interest should to be driven towards the genetics of pharmacodynamics in methadone treatment response.

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Author disclosure information

The authors declare no conflicts of interest.

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Table 1. Main sociodemographical and clinical characteristics of responder and nonresponder patients groups.

	Responders N= 76	**Nonresponders N= 29**	**P[a]**
Male (%)	53 (70)	21 (72)	1.000
Age, mean ± SD	39 ± 7	36 ± 9	0.076
Years at school ± SD	9 ± 3	8 ± 3	0.060
Single (%)	30 (41)	13 (45)	0.629
Criminal background (%)	40 (54)	18 (62)	0.248
Live with family (%)	58 (78)	19 (66)	0.764
Employed (%)	22 (30)	10 (42)	0.205
HIV + (%)	31 (41)	9 (31)	0.380
HCV + (%)	59 (78)	18 (62)	0.139
Lifetime psychiatric comorbidity (%)	45 (74)	14 (48)	0.416
Months of heroin use ± SD	144 ± 80	121 ± 67	0.192
Days of heroin 30 days ± SD	0 ± 1	16 ± 10	**< 0.001**
Days of cocaine 30 days ± SD	2 ± 6	7 ± 12	0.123
Nicotine cigarettes/day ± SD	22 ± 11	26 ± 13	0.172
Concomitant medication (%)			
benzodiazepines	39 (51)	9 (31)	0.080
antiretrovirals	13 (17)	5 (17)	1.000
anticonvulsants	9 (12)	0 (0)	0.060
SSRI	13 (17)	1 (3)	0.106
other antidepressants (non-SSRI)	9 (12)	4 (14)	0.750
antypsychotics	14 (18)	3 (10)	0.388

antibiotics	6 (8)	1 (3)	0.670
any concomitant medication	53 (70)	15 (52)	0.110
Months in methadone ± SD	52 ± 49	21 ± 32	**0.001**
Methadone dosage (mg/day) ± SD	109 ± 68	72 ± 43	**0.007**
Methadone plasma concentrations (ng/ml) ± SD[b]			
Total	587 ± 501	443 ± 246	0.121
(R)-	311 ± 259	238 ± 131	0.136
(S)-	276 ± 288	205 ± 121	0.370
ASI scores ± SD			
General Health	3 ± 2	4 ± 2	0.184
Work	4 ± 3	3 ± 3	0.670
Alcohol Use	1 ± 2	1 ± 1	0.127
Drug Use	4 ± 2	6 ± 2	**0.001**
Legal	1 ± 2	3 ± 3	**0.001**
Social	3 ± 3	3 ± 2	0.789
Psychological	3 ± 3	3 ± 3	0.855

[a] Bold numbers indicate statistically significant differences between patients

[b] Plasma concentrations of methadone were obtained from 79 subjects (65 responders and 14 non-responders)

SD=standard deviation; HIV= human immunodeficiency virus; HCV= hepatitis C virus; SSRI= Selective Serotonin Reuptake Inhibitors; ASI= Addiction Severity Index.

Table 2. Genotype frequencies of *CYP3A5*, *CYP2D6, CYP2B6, CYP2C9, CYP2C19 and ABCB1* between responder and nonresponder groups.

	Responders N= 76 (%)	**Nonresponders N=29 (%)**	**P**
CYP3A5 Genotype			
*1/*1	1 (1)	1 (3)	
*1/*3	11 (15)	2 (7)	0.446
*3/*3	64 (84)	26 (90)	
CYP2D6 Genotype			
*1/*1	4 (5)	2 (7)	
*1/*2	12 (16)	4 (14)	
*1/*3	2 (3)	0 (-)	
*1/*4	16 (21)	3 (10)	
*1/*5	1 (1)	0 (-)	
*1/*6	1 (1)	0 (-)	
*1/*9	2 (3)	0 (-)	
*1/*10	2 (3)	0 (-)	
*1/*41	1 (1)	3 (10)	
*2/*2	6 (8)	2 (7)	
*2/*3	1 (1)	0 (-)	
*2/*4	9 (12)	6 (21)	0.211
*2/*5	1 (1)	1 (3)	
*2/*6	1 (1)	1 (3)	
*2/*9	2 (3)	0 (-)	
*2/*35	1 (1)	0 (-)	
*2/*41	1 (1)	3 (10)	
*3/*17	1 (1)	0 (-)	
*4/*4	2 (3)	3 (10)	
*5/*41	2 (3)	0 (-)	
*10/*41	1 (1)	0 (-)	
*35/*35	1 (1)	0 (-)	
*35/*41	0 (-)	1 (3)	
**1/*2* x3[a]	3 (4)	0 (-)	
**2/*2* x 3[a]	2 (3)	0 (-)	
CYP2B6 Genotype			
*1/*1	31 (41)	7(24)	
*1/*4	18 (24)	6 (21)	0.225
*1/*5	5 (7)	5 (17)	
*4/*4	5 (7)	1 (3)	
*4/*5	2 (3)	3 (10)	
CYP2B6 Genotype			
G/G	27 (36)	14 (48)	0.566
G/T	18 (24)	4 (14)	
T/T	2 (3)	1 (3)	

CYP2C9 Genotype			
*1/*1	53 (70)	19 (66)	0.425
*1/*2	14 (18)	7 (24)	
*1/*3	6 (8)	2 (7)	
*2/*2	0 (-)	1 (3)	
*2/*3	2 (3)	0 (-)	
*3/*3	1 (1)	0 (-)	
CYP2C19 Genotype			
*1/*1	54 (71)	19 (66)	0.260
*1/*2	71 (29)	9 (31)	
*2/*2	0 (-)	1 (3)	
ABCB1 genotype			
C/C	24 (32)	14 (48)	0.266
C/T	39 (51)	12 (41)	
T/T	13 (17)	3 (10)	

[a] Patients with 3 functional alleles of *CYP2D6*

Table 3. Phenotype frequencies of *CYP3A5*, *CYP2D6, CYP2B6, CYP2C9, CYP2C19 and ABCB1* between responder and nonresponder groups.

	Responders N= 76 (%)	**Nonresponders N=29 (%)**	**P[a]**
CYP3A5 Phenotype			
Extensive	10 (13)	3 (10)	1.000
Poor	66 (87)	26 (90)	
CYP2D6 Phenotype			
Extensive	64 (84)	26 (90)	
Ultrarapid	5 (7)	0 (0)	**0.032**
Intermediate	5 (7)	0 (0)	
Poor	2 (3)	3 (10)	
CYP2B6 Phenotype			
Extensive	54 (86)	18 (82)	0.471
Intermediate	7 (14)	4 (18)	
CYP2B6 Phenotype			
Extensive	27 (57)	14 (74)	0.385
Intermediate	18 (38)	4 (21)	
Poor	2 (4)	1 (5)	
CYP2C9 Phenotype			
Extensive	67 (88)	26 (90)	0.779
Intermediate	4 (5)	2 (7)	
Poor	5 (7)	1 (3)	
CYP2C19 Phenotype			
Extensive	69 (91)	26 (90)	0.258
Intermediate	7 (9)	2 (7)	
Poor	0 (0)	1 (3)	
ABCB1 Phenotype			
Extensive	24 (32)	14 (48)	0.266
Intermediate	39 (51)	12 (41)	
Poor	13 (17)	3 (10)	

[a] Bold numbers indicate statistically significant differences between patients

Table 4. Mean methadone dose, *(R)*-, *(S)*- and *(R,S)*-methadone plasmatic concentrations by phenotype of *CYP2D6* in the responder and nonresponder patients

	Methadone dose (mg/day)[a] (N) mean ± SD [range]	**P[b]**	***(R,S)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**	***(R)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**	***(S)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**
All patients								
***CYP2D6* Phenotype**								
Extensive	(90) 95 ± 60 [15-400][d]	**0.043**	(68) 503 ± 416 [31-2461] [d]	**0.002**	(68) 263 ± 207 [16-978] [d]	**<0.001**	(68) 239 ± 256 [15-1889] [d]	**0.048**
Ultrarapid	(5) 177 ± 96 [105-340] [d]		(5) 1275 ± 484 [740-2050] [d]		(5) 707 ± 267 [413-1084] [d]		(5) 568 ± 262 [327-966] [d]	
Intermediate	(5) 92 ± 60 [15-160]		(2) 368 ± 35 [343-393]		(2) 215 ± 30 [194-237]		(2) 152 + 5 [149-156]	
Poor	(5) 87 ± 67 [30-200]		(4) 756 ± 716 [332-1825]		(4) 416 ± 382 [193-987]		(4) 341 ± 336 [107-838]	
Responder Patients								
***CYP2D6* Phenotype**								
Extensive	(64) 105 ± 64 [25-400]	0.120	(56) 512 ± 443 [31-2461] [d]	**0.002**	(56) 268 ± 219 [16-978] [d]	**<0.001**	(56) 245 ± 276 [15-1889] [d]	**0.044**
Ultrarapid	(5) 177 ± 96 [105-340]		(5) 1275 ± 484 [739-2050] [d]		(5) 707 ± 267 [413-1084] [d]		(5) 568 ± 262 [327-966] [d]	
Intermediate	(5) 92 ± 60 [15-160]		(2) 368 ± 35 [343-393]		(2) 216 ± 30 [194-237]		(2) 153 + 5 [149-156]	
Poor	(2) 102 ± 73 [55-200]		(2) 1164 ± 936 [502-1825]		(2) 622 ± 517 [256-987]		(2) 542 ± 419 [246-838]	
Nonresponder Patients								
***CYP2D6* Phenotype**								
Extensive	(26) 72 ± 43 [15-200]	0.654	(12) 459 ± 264 [163-1058]	0.580	(12) 243 ± 142 [101-577]	0.753	(12) 216 ± 127 [62-481]	0.429
Ultrarapid	(0) -		(0) -		(0) -		(0) -	
Intermediate	(0) -		(0) -		(0) -		(0) -	
Poor	(3) 60 ± 33 [30-95]		(2) 349 ± 23 [333-365]		(2) 210 ± 23 [193-226]		(2) 139 ± 46 [107-172]	

[a] Data from the 105 patients included
[b] Bold numbers indicate statistically significant differences between patients
[c] Plasmatic concentrations were obtained for 79 patients
[d] Statistical significant differences were found between Ultrarapid compared to Extensive metabolizers (Tukey *post hoc* analysis) $p<0.05$

Table 1 (Supplemental Digital Content). Mean methadone dose requirements of patients (n=105) and *(R)*-, *(S)*- and *(R,S)*-methadone plasma concentrations (n=79) following phenotypes of genes evaluated (*CYP3A5*, *CYP2D6, CYP2B6, CYP2C9, CYP2C19 and ABCB1)*

	Methadone dose (mg/day)[a] (N) mean ± SD [range]	**P[b]**	***(R,S)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**	***(R)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**	***(S)*-Methadone (ng/ml)[c] (N) mean ± SD [range]**	**P[b]**
***CYP3A5* Phenotype**								
Extensive	(2) 30 ± 7 [25-35]	0.312	(1) 40-	0.540	(1) 20-	0.516	(1) 20-	0.657
Poor	(13) 100 ± 56 [35-200]		(12) 568 ± 574 [40-1825]		(12) 310 ± 329 [20-987]		(12) 258± 251 [20-838]	
Very Poor	(90) 100 ± 65 [15-400]		(66) 568 ± 450 [31-2461]		(66) 300 ± 226 [16-1084]		(66) 268 ± 271 [15-1889]	
***CYP2D6* Phenotype**								
Extensive	(90) 95 ± 60 [15-400][d]	**0.043**	(68) 503 ± 416 [31-2461][d]	**0.002**	(68) 263 ± 207 [16-978][d]	**<0.001**	(68) 239 ± 256 [15-1889][d]	**0.048**
Ultrarapid	(5) 177 ± 96 [105-340][d]		(5) 1275 ± 484 [740-2050][d]		(5) 707 ± 267 [413-1084][d]		(5) 568 ± 262 [327-966][d]	
Intermediate	(5) 92 ± 60 [15-160]		(2) 368 ± 35 [343-393]		(2) 215 ± 30 [194-237]		(2) 152 + 5 [149-156]	
Poor	(5) 87 ± 67 [30-200]		(4) 756 ± 716 [332-1825]		(4) 416 ± 382 [193-987]		(4) 341 ± 336 [107-838]	
***CYP2B6* Phenotype**								
Extensive	(72) 105 ± 68 [15-400]	0.869	(58) 619 ± 492 [31-2461]	0.591	(58) 327 ± 256 [16-1084]	0.506	(58) 293 ± 287 [15-1889]	0.739
Intermediate	(11) 102 ± 48 [35-165]		(9) 527 ± 392 [54-1097]		(9) 267 ± 188 [33-577]		(9) 259 ± 210 [21-598]	
***CYP2B6* Phenotype**								
Extensive	(41) 100 ± 72 [15-400]	0.776	(27) 577 ± 527 [31-2461]	0.987	(27) 306 ± 250 [16-978]	0.920	(27) 271 ± 358 [15-1889]	0.952
Intermediate	(22) 109 ± 71 [25-340]		(17) 611 ± 596 [40-2050]		(17) 319 ± 316 [20-1084]		(17) 293 ± 286 [20-966]	
Poor	(3) 82 ± 13 [70-95]		(1) 724 -		(1) 327 -		(1) 397 -	
***CYP2C9* Phenotype**								
	(72) 103 ± 71 [15-400]	0.483	(54) 628 ± 510 [40-2461]	0.158	(54) 338 ± 260 [20-1084]	0.091	(54) 290 ± 301 [20-1889]	0.361

Extensive Intermediate Poor	(21) 90 ± 45 [20-190] (12) 83 ± 45 [25-190]		(17) 445 ± 340 [31-1178] (8) 356 ± 297 [40-929]		(17) 217 ± 175 [16-684] (8) 196 ± 187 [20-567]		(17) 228 ± 176 [15-603] (8) 159 ± 113 [20-362]	
CYP2C19 **Phenotype**								
Extensive Intermediate Poor	(73) 101 ± 69 [15-400] (31) 95 ± 50 [15-190] (1) 20 -	0.420	(54) 581 ± 497 [40-2461] (25) 518 ± 403 [31-1607] (0) -	0.578	(54) 298 ± 241 [20-1084] (25) 298 ± 251 [16-978] (0) -	0.999	(54) 283 ± 303 [20-1889] (25) 220 ± 161 [15-629] (0) -	0.328
ABCB1 **Phenotype**								
Extensive Intermediate Poor	(38) 97 ± 61 [15-270] (51) 102 ± 69 [25-400] (16) 91 ± 56 [15-190]	0.828	(25) 667 ± 621 [40-2461] (41) 494 ± 341 [31-1282] (13) 568 ± 474 [40-1607]	0.352	(25) 326 ± 273 [20-1084] (41) 282 ± 220 [16-929] (13) 293 ± 261 [20-978]	0.775	(25) 341 ± 396 [20-1889] (41) 212 ± 150 [15-598] (13) 275 ± 228 [20-697]	0.163

[a] Data on methadone dose was obtained in 105 patients

[b] Bold numbers indicate statistically significant differences between patients

[c] Plasma concentrations were obtained in 79 patients

[d] Statistical significant differences were found between Ultrarapid compared to Extensive metabolizers (Tukey *post hoc* analysis) $p < 0.05$

5. DISCUSSION

5. DISCUSSION

Main results have been presented as independent articles in the previous section of this dissertation. This section will discuss the most important aspects of the different papers divided in four subsections:

- Sample characteristics and MMT outcome
- Pharmacodynamics and MMT outcome
- Pharmacokinetics and MMT outcome
- Future research

5.1. Sample characteristics and MMT outcome

5.1.1. Responders and nonresponders

The study recruited a sample of patients in MMT, divided in responders and nonresponders. The inclusion process into the study has been slow in order to obtain a very specific phenotype profile. Rigorous phenotypic assessment is essential for all studies of addiction genetics because poor or inadequate phenotypic assessments lead to incorrect results. Such assessment entails the use of a number of instruments to evaluate personality traits, comorbid disorders, and a detailed history of initiation of drug use, and progression to addiction. The carefully phenotypic assessment is one of the strengths of the study.

As heroin use is the most frequent criteria used to assess the eficacy of opioid dependence disorder treatments, we defined responders and nonresponders according to the use, at least twice, of illicit opioids detected in random

urinalysis during the previous 2 months, once methadone dose has been stabilized. As we aimed to define a strict phenotype we decided to exclude those subjects with subthreshold urine controls, that is, patients with just one positive urine control.

Heroin use is the most frequent criteria used to assess the efficacy in opioid disorders treatments. Also, MMT outcome could be defined by other criteria: retention in treatment, concomitant use of other illicit substances, criminal activity, risk behaviors for HIV and other sexually transmitted diseases and quality of life, among others. In recent years, other aspects of methadone treatment have received interest, as patient's satisfaction with methadone treatment, and mood states (Elkader et al., 2009a; Elkader et al., 2009b; Perez de los Cobos et al., 2007). Those factors have also been associated with treatment retention (Villafranca et al., 2006) and substance use (Carlson & Gabriel, 2001).

5.1.2. Sociodemographic characteristics

We didn't found significant differences in terms of gender, age, criminal background, family situation, working status, or co-occurring infectious diseases (hepatitis C and HIV infections) in MMT response between both groups of patients. Other authors have found similar results (Kamal et al., 2007).

5.1.3. Psychiatric comorbidity

When comparing patients in terms of psychiatric comorbidity, we observed that there were no differences in terms of prevalence of any life-time non-substance use disorder among sub-groups of patients.

It has been reported that dually diagnosed patients in drug addiction presented poorer outcomes in both disorders (substance use related and non-substance use related): (i) comorbid patients presented more emergency admissions (Martín-Santos et al., 2006), (ii) more psychiatric admissions (Lambert et al., Schmitt, 2003), (iii) increased prevalence of suicide (Aharonovich et al., 2002) and (iv) more severe impairment (Conner et al., 2008).

However, studies focused in methadone maintenance treatment patients show different results. Astals (2009) described that co-occurring mental disorders were not related to retention in treatment in a sample of MMT patients. Furthermore, in a retrospective study of factors that affect outcome in MMT (measured with illegal opioid use detected in urine controls), dual diagnosed patients tended to have higher rates of abstinence (Kamal et al., 2007).

5.1.4. Personality profile

Personality profile was studied with Cloninger's Temperament and Character Inventory (TCI). The Spanish version of TCI has already demonstrated to be a reliable tool to assess personality characteristics of substance use disorders

patients. The instrument provided a dimensional model of personality and the character dimension Self-Directedness was strongly associated with the presence and severity of any personality disorder (Gutierrez et al., 2002).

In our study, the most relevant result was the lower scores in Cooperativeness subscale in the responder group (de Cid et al., 2008). Cooperativeness was formulated to account for individual differences in identification with and acceptance of other people and reveals an inclination toward social tolerance, empathy, helpfulness and compassion. This scale together with Self-Directedness were negatively correlated with the risk of personality disorder (Gutierrez et al., 2008; Pelissolo & Corruble, 2002). Nevertheless, in our results, there were no differences in the life-time prevalence of personality disorders between responders and nonresponders.

Other authors have described similar results in terms of Cooperativeness and outcome in pharmacological treatment studies, for example in eating disorders (Klump et al., 2004) where lower Cooperativeness scores were observed in the responder group. However, other studies on major depression and panic disorder, showed opposite results (Hirano et al., 2002; Kronstrom et al., 2010; Marchesi et al., 2006). Although a sample size bias can not be discarded, these differences could be diagnosis specific, or might be related to the TCI assessment time (Marchesi et al., 2006).

5.1.5. Addiction severity profile

In the ASI scores, nonresponders presented higher severity in the Drug Use Problems and Legal Problems compared to responders.

Differences on the Drug Use scale were expected, as by definition, nonresponders patients were using heroin in a regular basis. The ASI Drug Problems scale evaluated the use of all substances, except alcohol. The results were the reflect of heroin use because, we recorded the number of days of cocaine and heroin use during the last 30 days, and differences were observed only in the number of days on heroin (Fonseca et al., 2010).

Nonresponder patients also presented more severe scores in the Legal Problems scale. Due to the characteristics of the instrument was not possible to distinguish between legal problems related or non related to drug use in our sample. Inclusion in a MMT program has shown to reduce criminal activity (Dolan et al., 2005).

5.1.6. Methadone dosage

Dosage of methadone is a well known factor contributing to response to treatment (Amato et al., 2005b; Farre et al., 2002). Although the mean doses in nonresponders were lower, doses in both groups were within the therapeutic range (almost 80 mg/day) (Amato et al., 2005b) and higher than the minimum dose of 50 mg/day recommended in a meta-analysis (Farre et al., 2002).

Is noticeable that, according to the philosophy of the center, there was no upper limits on methadone dosage, some patients with poor response to MMT did not accept increases in their methadone dose. The first reason could be related to patient beliefs': many patients referred that they did not want to increase methadone doses because they felt dependent on the drug, and they thought it would be more difficult to suppress in the future. It could also be hypothesized that those patients show significant were not satisfied with the treatment or presented side effects events associated to methadone.

5.1.7. Length of MMT

The length participation of patients in the MMT could also be an important issue. In our study, statistical differences between groups were observed, responder patients tend to stay for longer (more than twice than nonresponders) in the MMT (Fonseca et al., 2010; Fonseca et al., submitted). Some studies have shown that the results obtained after treatment over a period of less than 3 months were comparable to those obtained after no treatment at all (Simpson, 1981). According to other publications, a reduction in drug use was observed in patients remaining in treatment for at least one year (Kamal et al., 2007; Teesson et al., 2006).

5.2. Pharmacodynamics and MMT outcome

Previous studies in MMT pharmacodynamics have been focused mainly in the study of candidate genes related with methadone mechanism of action: the study of *OPRM1* receptor and dopamine D2 receptor genetic variability.

The candidate gene *OPRM1* has previously been related to opioid treatment response, mainly in analgesia and alcohol dependence (Haile et al., 2008; Reynolds et al., 2008; Wu et al., 2009). The more commonly studied SNP (A118G), confers a change in the resultant protein. So, the carriers of the homozygous variant (GG) require higher opioid doses to achieve pain relief when they are treated with morphine (Klepstad et al., 2004). In alcohol dependence disorder, carriers of an Asp40 allele in *OPRM1* showed a modest better response to naltrexone treatment (Kranzler & Edenberg, 2010). However, when genetic variability of this receptor has been studied in MMT results have been negative (Crettol et al., 2008b) as in our study. These negative findings may be due to these variants being specifically involved in the heroin dependence phenotype but not in the individual differences in the response to methadone treatment in heroin addiction.

The pharmacodynamic study of this work has been focused in genes encoding for proteins previously associated with heroin addiction. Firstly, the study of BDNF which has been previously classified as pro-addictive factor because acts to potentiate the rewarding effects of drugs (Yamada, 2008). On the other hand, *GRM6*, *MYOCD* and *CRY1* have

been related to heroin addiction following a different approach: a genome wide study (Nielsen et al., 2008); this kind of approach allow to confirm the involvement of previously identified genes as well as, to find evidence for the involvement of genes and genomic regions not previously associated with addiction nor treatment response.

We have identified one haplotype (*BDNF*) and genetic variants (*GRM6*, *MYOCD* and *CRY1*) with low frequency that modulates the response to MMT. Nevertheless, given this low frequency, it seems clear that other factors should be considered.

5.2.1. BDNF genetic variability and MMT outcome

The results of our work (De Cid et al., 2008) suggested that *BDNF* variability confers a differential susceptibility to MMT response in opioid dependent patients. The implication of BDNF variability to therapeutic response has been previously suggested with regards to the efficacy of other psychiatric treatments such as prophylaxis with lithium carbonate for bipolar mood disorders (Rybakowski et al., 2005) and antidepressants for unipolar depression (Gratacòs et al., 2008).

We identified one haplotype with a low frequency that confers a poorer response in patients undergoing MMT. Endogenous opioids (beta-Endorphin, endomorphin-1 and endomorphin-2) significantly increase BDNF mRNA levels in the frontal cortex, hippocampus and amygdala, with most of

these effects reversed by naltrexone (Zhang et al., 2006). Also, *BDNF* has been linked to both, the dopaminergic system (Seroogy et al., 1994) and the noradrenergic system (Akbarian et al., 2002), that play a relevant role in opioid dependence disorder. Therefore, it is not unlikely that *BDNF* could affect the response to MMT. Differential response to MMT could be a consequence of a reduction of brain plasticity arising from an altered expression and functionality of *BDNF*.

5.2.2. GRM6, MYOCD genetic variability and MMT outcome

As seen in the results section, our study (Fonseca et al., 2010) showed a positive association between response to MMT in heroin dependent patients and different variants in genes *GRM6* and *MYOCD* previously reported to confer susceptibility to heroin addiction (Nielsen et al., 2008). There is a pharmacogenetic epistatic effect between SNPs in *GRM6* and *MYOCD* that modulate, partially, the inter-individual variation to MMT response.

The metabotropic receptor gene GRM6 encodes a glutamate receptor subunit. Glutamate receptors have been related to the pathophysiology of mood disorders and addiction (Kalivas, 2009; Lavreysen & Dautzenberg, 2008). Also, impaired glutamate homeostasis is critical in the pathophysiology of addiction, and alterations in the function and expression of metabotropic glutamate receptors have been reported in the development of addiction to drugs of

abuse such as opiates (Kalivas, 2009; Kelley, 2004; Nestler, 2001).

The myocardin gene (*MYOCD*) pertains to a family of powerful myogenic serum response factor coactivators involved in cell proliferation, migration and control of smooth muscle gene expression (Imamura et al., 2010; Pipes et al., 2006); the variant rs1714984 is located in the second intron of the myocardin gene. In a recent study, MYOCD and serum response factor (SRF) overexpression in small cerebral arteries appears to initiate arterial hypercontractility and cerebral blood flow dysregulation, which are associated with Alzheimer's Disease (Chow et al., 2007).

The finding of an association between *MYOCD* and *GRM6* has not been previously described in the literature. Because *MYOCD* has been associated with changes in central nervous system blood flow (Chow et al., 2007) these changes might affect glutamatergic neurotransmission in those carriers of the heterozygous genotype of GRM6.

5.2.3. CRY1 genetic variability and MMT outcome

CRY1 encodes the cryptochrome 1 (Cry1); it is a circadian gene involved in the molecular regulation of the clock machinery that has been implicated in both addiction and mood disorders vulnerability (Kosobud et al., 2007). Although differences between groups disappeared with correction for multiple testing, we detected a difference in genotype distributions between responders and nonresponders.

It can be hypothesized that a genetic change in *CRY1* could affect cytokine tumor necrosis factor-alpha (TNF-α) activity. The TNF-α is a cytokine involved in inflammation, and neuroinflammation of the central nervous system and seems to participate in the sensitizing and addictive effects of opiates (Petrzilka et al., 2009).

5.3. Pharmacokinetics and MMT outcome

5.3.1. Methadone plasma concentrations and MMT outcome

In our work (Fonseca et al., submitted), mean plasma concentrations of (*R,S*)-methadone and of each enantiomer were not significantly different between responders and nonresponders, although concentrations in nonresponders were 30% lower than in responders in agreement with differences in dose requirements between both groups. Therefore, differences in clinical outcome cannot be justified on the basis of some kind of genetic bias in drug metabolizing and transport enzymes. The effect of drug interactions could not be discarded in our results as the high proportion of patients taking concomitant medications (65%). Also, interactions with other substances as cannabis and cocaine have to be taken into account (Hallinan et al., 2009; McCance-Katz et al., 2010).

Due to great differences in interindividual methadone dosage adequacy, many researchers have previously tried to find a minimum methadone plasma concentration to assure the treatment efficacy (as described in the introduction

section), but results on this subject have been inconclusive. As methadone enantiomers present differences in pharmacokinetic and pharmacodynamic parameters, authors have studied the association of *(R)*-methadone plasmatic concentrations and methadone outcome. Similarly to racemic methadone, the determination of plasmatic concentrations of methadone enantiomers does not contribute significantly to evaluate MMT outcome. Moreover, in clinical management, medical evaluation of objective signs (heroin detection in urinalyses) and subjective symptoms (withdrawal symptoms, self-reported opioid craving) is sufficient for dosage titration in most patients.

5.3.2. Cytochrome P450 and ABCB1 *genetic variability and methadone doses, plasmatic concentrations and MMT outcome*

According to our results, the genetic polymorphisms of *CYP3A5, CYP2B6, CYP2C9, CYP2C19*, and *ABCB1* did not influence methadone doses; the negative results in terms of the genotypes and phenotypes of drug metabolizing enzymes examined in this study are consistent with previous data (Crettol et al., 2005).

A small influence of *CYP2D6* in methadone doses and plasma concentrations was found. The contribution of *CYP2D6* on methadone metabolic disposition and dosage is controversial. Several reports suggest that its contribution is negligible (Shiran et al., 2009), while others have shown that

specific inhibitors of *CYP2D6* as paroxetine, markedly influence methadone disposition (Begre et al., 2002; Wang & DeVane, 2003).

On the other hand, *ABCB1* genetic variability has been previously associated with methadone dose requirements by some authors (Coller et al., 2006; Levran et al., 2008). Other authors (Buchard et al., 2010) did not found any relationship between *ABCB1* genotype and the amounts of methadone or EDDP detected in postmortem blood samples; the authors didn't find a correlation between the *ABCB1* genotype nor with the R/S ratio; although it has been described that P-glycoprotein exhibits weak stereoselectivity toward the *(S)*-enantiomer (Crettol et al., 2007).

5.4. Future research

MMT outcome should be understood as the result of the combination of multiple factors: genetic, environmental and drug induced. It is apparent from the present study that interindividual pharmacokinetic differences among patients can be compensated by clinical management of the doses of methadone. The study of other pharmacodynamic influences in the reluctance of a considerable group of patients to increase methadone dose should be studied (i.e. differences related to the activation of kappa opioid receptors).

The future research also has to take into account the interaction between genetic factors, environmental factors

and drug-induced factors (as epigenetic modifications induced by substances) influencing methadone response.

6. CONCLUSIONS

6. CONCLUSIONS

- The result of a good or a poor outcome in MMT would be in part, the result of different positive and negative influences of genetic, environmental and drug induced factors.

- Effects of methadone could be directly related to pharmacodynamic or/and pharmacokinetic factors.

- Among *pharmacodynamic factors* *BDNF*, *MYOCD* and *GRM6* variability contribute to the variability in MMT outcome.

- *BDNF* variability confers a differential susceptibility to MMT response in opioid dependent patients, independently of personality traits, environmental cues, methadone dosage, and the presence of medical and psychiatric comorbidity.

- There is also a pharmacogenetic epistatic effect between SNPs in *MYOCD* and *GRM6* that may modulate the inter-individual variation to MMT response.

- In reference to the influence of the genetic variability in the coding genes implicated in *pharmacokinetics* of methadone metabolism and transport (*CYP3A5, CYP2B6, CYP2D6, CYP2C9, CYP2C19, ABCB1*), the clinical

relevance is small, due to the influence of genetic polymorphisms in metabolizing enzymes is usually managed by clinicians increasing and splitting methadone doses.

– The study of methadone pharmacogenetics and future development of genetic tests predicting its effectiveness in the treatment of individual patients is an issue of great clinical interest.

7. REFERENCES

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8. GLOSSARY

8. GLOSSARY

Addiction: is a chronic, neurobiologic disease, with genetic, psychosocial, and environmental factors influencing its development and manifestations. It is characterized by behaviors that include one or more of the following: impaired control over drug use, compulsive use, continued use despite harm, and craving.

Affinity: The strength with which a drug binds to its receptor is termed its affinity.

Agonist: Drugs that activate receptors in the brain are termed agonists. Agonists bind to receptors and turn them on.

Allele: one of two or more versions of a gene.

Amino Acid: Amino acids are a set of 20 different molecules used to build proteins. The amino acid sequences of proteins are encoded in the genes.

Amygdala: It is part of the limbic system and plays an important role in motivation and emotional behavior.

Antagonist: A substance that tends to nullify the effect of another. A drug that binds to a receptor without eliciting a response.

Bioavailability: The ability of a drug to enter the body.

Brain Derived Neurotrophic factor (BDNF): A protein member of the nerve growth factor family. It is induced by cortical neurons, and is necessary for survival of striatal neurons in the brain. This protein may play a role in the

regulation of stress response and in the biology of mood disorders.

Buprenorphine: A semi-synthetic opioid partial agonist that is a derivative of thebaine. Used for the treatment of opioid addiction.

Candidate gene: a gene whose chromosomal location is associated with a particular disease or other phenotype. Because of its location, the gene is suspected of causing the disease or other phenotype.

Carrier: an individual who carries and is capable of passing on a genetic mutation associated with a disease and may or may not display disease symptoms.

Chromosome: an organized package of DNA found in the nucleus of the cell.

Complex Disease: or multifactorial; is caused by the interaction of multiple genes and environmental factors.

Craving: Unnaturally strong desire/urge for a substance.

Cryptochrome 1 gene (CRY1): regulator of the circadian feedback loop.

Cytochrome P450: a superfamily of hundreds of closely related hemeproteins found throughout the phylogenetic spectrum, from animals, plants, fungi, to bacteria. They include numerous complex monooxygenases. In animals, serve two major functions: biosynthesis of steroids, fatty acids, and bile acids; and metabolism of endogenous and a wide variety of exogenous substrates.

Cytochrome P4501A2: cytochrome P450, family 1, subfamily A, polypeptide 2.

Cytochrome P4502B6: cytochrome P450, family 2, subfamily B, polypeptide 6.

Cytochrome P4502C19: cytochrome P450, family 2, subfamily C, polypeptide 19.

Cytochrome P4502C9: cytochrome P450, family 2, subfamily C, polypeptide 9.

Cytochrome P4502D6: cytochrome P450, family 2, subfamily D, polypeptide 6.

Cytochrome P4503A4: cytochrome P450, family 3, subfamily A, polypeptide 4.

Cytochrome P4503A5: cytochrome P450, family 3, subfamily A, polypeptide 5.

Deletion: a type of mutation involving the loss of genetic material.

Delta opioid receptor: A receptor on the surface membrane of nerve cells activated by opioid agonists.

Detoxification: The metabolic process by which the toxic qualities of a poison or toxin are reduced by the body.

Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR): a disease classification manual for mental disorders, published by the American Psychiatric Association.

Dopamine: One of the catecholamine neurotransmitters in the brain; it is derived from tyrosine and is the precursor to norepinephrine and epinephrine; dopamine is a major transmitter in the extrapyramidal system of the brain, and

important in regulating movement; dopamine receptors mediate its action.

Duplication: a type of mutation that involves the production of one or more copies of a gene or region of a chromosome.

EDDP, 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine: a metha**done inactive metabolite.**

Electrophoresis: is a laboratory technique used to separate DNA, RNA, or protein molecules based on their size and electrical charge.

EMDP, 2-ethyl-5-methyl-3,3-diphenyl-1-pyrrolidine: a methadone inactive metabolite.

Enantiomer: compounds that have the same relationship as an object and its mirror image.

Endogenous opioid: Opioids that are produced naturally in the body which have analgesic properties They are endorphins, enkephalins, and dynorphins.

Enzyme: a biological catalyst (almost always a protein). It speeds up the rate of a specific chemical reaction in the cell.

Epigenetics: an emerging field of science that studies heritable changes caused by the activation and deactivation of genes without any change in the underlying DNA sequence of the organism.

Epistasis: is a circumstance where the expression of one gene is affected by the expression of one or more independently inherited genes.

Exon: is the portion of a gene that codes for amino acids.

First degree relative: is a family member who shares about 50 percent of their genes with a particular individual in a family. First degree relatives include parents, offspring, and siblings.

Gene: The basic physical unit of inheritance. Genes are passed from parents to offspring and contain the information needed to specify traits. Genes are arranged, one after another, on structures called chromosomes. A chromosome contains a single, long DNA molecule, only a portion of which corresponds to a single gene.

Gene environment interaction: is an influence on the expression of a trait that results from the interplay between genes and the environment.

Gene expression: is the process by which the information encoded in a gene is used to direct the assembly of a protein molecule.

Genome-wide association study (GWAS): is an approach used in genetics research to associate specific genetic variations with particular diseases. The method involves scanning the genomes from many different people and looking for genetic markers that can be used to predict the presence of a disease.

Genotype: an individual's collection of genes. The term also can refer to the two alleles inherited for a particular gene.

Haplotype: a set of DNA variations, or polymorphisms, that tends to be inherited together. A haplotype can refer to a

combination of alleles or to a set of single nucleotide polymorphisms (SNPs) found on the same chromosome.

Heroin (diacetylmorphine): Heroin is a full opioid agonist at the mu receptor.

Heterozygous: refers to having inherited different forms of a particular gene from each parent.

Homozygous: is a genetic condition where an individual inherits the same alleles for a particular gene from both parents.

Insertion: a type of mutation involving the addition of genetic material.

Intron: a portion of a gene that does not code for amino acids.

Isomers: compounds with the same molecular formula and bonds, the arrangement of atoms is different.

Kappa opioid receptor: A receptor on the surface membrane of nerve cells activated by opioids

Levo-alpha acetyl methadol (LAAM): An opioid agonist medication derived from methadone that is effective for up to 72 hours.

Linkage: the close association of genes or other DNA sequences on the same chromosome. The closer two genes are to each other on the chromosome, the greater the probability that they will be inherited together.

Locus: the specific physical location of a gene or other DNA sequence on a chromosome.

Maintenance treatment: treatment based on the administration of an agonist during an indefinite amount of time. This allows the patient to prepare and make the necessary changes that would otherwise make long term abstinence unlikely.

Metabotropic glutamate receptors: are a family of G protein-coupled receptors. mGlu6 is involved in stimulation of photoreceptors.

Methadone: a semisynthetic full opioid agonist prescribed for the management of moderate to severe pain and for the treatment of opiate dependence.

Mu opioid receptor: A receptor on the surface membrane of nerve cells that mediates opioid analgesia, tolerance, and addiction through drug-induced activation.

Multidrug resistant gene 1 (*ABCB1*): a member of the family of ATP binding cassette (ABC) transporters.

Myocardin gene (*MYOCD*): A gene that encodes a nuclear protein, which is expressed in heart, aorta, and in smooth muscle cell-containing tissues. It functions as a transcriptional co-activator of serum response factor (SRF) and modulates expression of cardiac and smooth muscle-specific SRF-target genes, and thus may play a crucial role in cardiogenesis and differentiation of the smooth muscle cell lineage.

Naltrexone: An opioid antagonist, works by blocking opioid receptors in the brain, without activating them, therefore, blocking the effects of opioids.

Neurotransmitter: Chemical substances, synthesized and released by nerve cells, or glandular hormones that excite or inhibit other nerve, muscle, or gland cells by producing a brief alteration in the postsynaptic membrane of the receiving cell.

Neurotrophins: proteins with closely related structures that are known to support the survival of different classes of embryonic neurons. Are implicated in enhancing neuronal differentiation, inducing proliferation, influencing synaptic functions, and promoting the survival of neurons that are normally destined to die during different phases of the development of the central and peripheral nervous system.

Opiate: A drug derived directly from the opium poppy plant.

Opioid: Drugs that are derived naturally from the opium poppy plant (i.e., morphine and opium) and that are synthetically produced in the lab (i.e., methadone and oxycodone).

Opioid dependence: A chronic brain disease that involves a physical, psychological, and behavioral need for an opioid drug.

Pharmacodynamics: Study of the biochemical and physiological effects of drugs and the mechanisms of their actions, including correlation of these actions and effects with the chemical structure of the drug.

Pharmacogenomics: a branch of pharmacology concerned with using DNA and amino acid sequence data to inform drug development and testing. An important application of

pharmacogenomics is correlating individual genetic variation with drug responses.

Pharmacokinetics: Study of the action of drugs in the body over a period of time, including the processes of absorption, distribution, localization in tissues, biotransformation, and excretion.

Phenotype: is an individual's observable traits. Some traits are largely determined by the genotype, while other traits are largely determined by environmental factors.

Polymorphism: one of two or more variants of a particular DNA sequence. The most common type of polymorphism involves variation at a single base pair. Polymorphisms can also be much larger in size and involve long stretches of DNA.

Psychiatric comorbidity: a mental disorder, according to DSM-IV diagnosis, that is present in an individual diagnosed of a substance use disorder.

R,S: stereochemical descriptors by the Cahn-Ingold-Prelog system.

Racemate: mixture of 1:1 enantiomers.

Relapse: A recurrence of symptoms after a period of remission.

Remission: A period of time in which the signs and symptoms of the addiction have disappeared.

Replacement therapy: is the medical procedure of replacing an addictive full agonist (such as heroin) with a longer acting but less euphoric full agonist opiate (i.e. methadone).

Reward: The process that reinforces behavior. It is mediated at least in part by the release of dopamine into the nucleus accumbens. Human subjects report that reward is associated with feelings of pleasure.

Single nucleotide polymorphism (SNP): a type of polymorphism involving variation of a single base pair.

Stereoselectivity: the preference selection of a stereoisomer compared to the other.

Substance Abuse: a maladaptive pattern of substance use leading to clinically significant impairment or distress.

Ventral Tegmenal Area (VTA): group of neurons located close to the midline on the floor of the midbrain. The VTA is widely implicated in the drug and natural reward circuitry of the brain, cognition, motivation, drug addiction, and several psychiatric disorders.

Vulnerability: a condition of the body that increases the likelihood that the individual will develop a particular disease. Vulnerability is influenced by a combination of genetic and environmental factors.

Withdrawal: a predictable group of signs and symptoms resulting from abrupt removal of, or a rapid decrease in, the regular dosage of a psychoactive substance.

9. APPENDIX

Fonseca F, Marti-Almor J, Pastor A, Cladellas M, Farré M, de la Torre R, et al. Prevalence of long QTc interval in methadone maintenance patients. Drug Alcohol Depend. 2009; 99(1-3): 327-32.

" Estudi farmacogenòmic de pacients amb trastorn mental greu: addictes a opiacis que no responen al tractament de manteniment amb metadona "

Grupo de Investigación aplicada en Trastorno por uso de substancias-IMAS-Hospital del Mar

Grupo de Psiquiatría Genética-IMIM

1. Datos sociodemográficos, historia toxicológica y datos de tratamiento

Iniciales del sujeto: ..... Núm. Protocolo: .....

Fecha recogida información: ...../...../..... Fecha analítica: ...../...../..... Hora: …../…….

Grupo: ☐ (A. Sujetos con metadona estable. B. Sujetos que inician PMM)

	Realizado	Fecha	Investigador/a
Consentimento Informado			
Datos sociodemográficos			
Historia toxicológica			
Datos de tratamiento			
Controles de orina			
Muestra de tóxicos			
Bioquímica			
Extracción genética			
Enviado CRG			
Extracción niveles Sangre			
Enviado IMIM			
PRISM			
TCI			
ASI			

DATOS SOCIODEMOGRÁFICOS

1.----------→	**Sexo**	1 HOMBRE 2 MUJER
2. ¿Qué edad tiene?	**Edad**	_____ Edad
3. ¿Cuál es su fecha de nacimiento?	**Fecha de nacimiento**	____/____/____ (día, mes ,año)
PREGUNTAR SI DESCONOCIDO: **4.** ¿De qué raza es?	**Raza**	1 BLANCO 2 NEGRO 3 GITANO 4 MAGREBI 5 OTROS (ESPECIFICAR) _____
5. ¿Cuál es su estado civil actual? ¿Convive en pareja? ¿Ha estado casado en alguna ocasión?	**Estado civil actual**	1 NUNCA CASADO - **PASAR A P.8** 2 CASADO 3 DIVORCIADO 4 SEPARADO 5 VIVE EN PAREJA 6 VIUDO
PREGUNTAR SI DESCONOCIDO: **6.** ¿Se ha divorciado en alguna ocasión? **SI AFIRMATIVO:** ¿Cuántas veces?	**Número de veces divorciado**	___ Nº DE VECES DIVORCIADO
PREGUNTAR SI DESCONOCIDO: **7.** ¿Ha enviudado en alguna ocasión? **SI AFIRMATIVO:** (¿Cuántas veces?)	**Número de veces enviudado**	___ Nº DE VECES ENVIUDADO
8. ¿Ha tenido algún hijo? ¿Tiene hijos adoptivos?	**Si ha tenido hijos** - hijastros que no han sido adoptados = "1" - hijos biológicos o adoptivos = "3"	1 NO – **PASAR A P.10** 3 SI
9. ¿Cuántos hijos tiene en <u>total</u>? (incluyendo hijos adoptivos) ¿Qué edad tienen?	**Número de hijos** - siga las indicaciones de P.8	___ NUMERO DE HIJOS
10. ¿Cuánto tiempo estuvo estudiando? (¿Qué estudios tiene?)	Nivel máximo de escolarización	Años escolarización: _____ 1 NO-ESCOLARIZACIÓN 2 CERTIFICADO ESCOLAR 3 GRADUADO ESCOLAR 4 CURSOS DE BUP 5 ACABO BUP 6 FORMACIÓN PROFESIONAL 7 COU 8 CURSOS DE UNIVERSIDAD 9 DIPLOMATURA 10 LICENCIATURA 11 ------- 12 DOCTORADO

11. ¿Ha realizado el servicio militar?

Si ha realizado el servicio militar

- sólo en reserva = "1"
- marina mercante = "1"
- prestación social = "1"

1 NO – **PASAR A P. 12**

3 SI

12. ¿ha estado en alguna ocasión en prisión o ha sido encarcelado durante una noche o más?

SI AFIRMATIVO:

¿Por qué motivo fue encarcelado?

Encarcelamiento

- Cárcel, en calabozo durante una noche o más = "3"
- Si es detención juvenil o ha estado en un reformatorio, codificar "3" y marcar casilla

ESPECIFICAR MOTIVO/S

_____
_____
_____
_____

1 NO – **PASAR A P.14**

3 SI

❑ DETENCION JUVENIL O REFORMATORIO

13. ¿Cuál fue el periodo más largo que pasó en prisión?

Duración del encarcelamiento más largo

____ DIAS ____ AÑOS

____ SEMANAS

____ MESES

14. ¿Con quien vive?

Todos los ocupantes del domicilio

ESPECIFICAR SI OTROS

_____
_____
_____
_____

SEÑALAR TODAS LAS PERTINENTES

1 VIVE SOLO
1 ESPOSO/A
2 HIJOS/AS
3 PADRES
4 HERMANOS/AS
5 PAREJA DEL SEXO CONTRARIO
6 PAREJA DEL MISMO SEXO
7 OTRO AMIGO/A
8 COMPAÑERO DE PISO, NO AMIGO
9 PISO PROTEGIDO
10 HUESPED
11 TIO/A
12 FAMILIARES POLITICOS
13 ABUELOS
14 OTROS FAMILIARES
15 MASCOTAS (ESPECIFICAR)
16 OTROS (ESPECIFICAR)

15. ¿Está usted trabajando? SI AFIRMATIVO: ¿Trabaja en jornada completa, es decir, 40 horas o más a la semana? ¿Qué tipo de trabajo realiza? SI NEGATIVO: **¿Tiene una invalidez?** ¿Está estudiando actualmente? (¿Qué está estudiando? ¿A tiempo completo o parcial?)	**Estado laboral actual** - señalar la situación en el día de la entrevista - si está hospitalizado, señalar la situación previa al ingreso hospitalario - "Invalidez temporal": desempleado por enfermedad/invalidez temporal - "invalidez permanente" incluye prestaciones de la seguridad social o pensión - sólo actividades ilegales = "5" ESPECIFICAR EMPLEO ACTUAL _____ _____ _____	SEÑALAR TODAS LAS PERTINENTES 1 EMPLEADO A TIEMPO COMPLETO - 40 HORAS O MÁS 2 EMPLEADO A TIEMPO PARCIAL- MENOS DE 40 HORAS 3 EMPLEADO DE BAJA POR ENFERMEDAD 4 EMPLEADO PERO TEMPORALMENTE SUSPENDIDO 5 DESEMPLEADO 6 DESEMPLEADO, INVALIDEZ TEMPORAL 7 DESEMPLEADO, INVALIDEZ PERMANENTE 8 JUBILADO 9 ESTUDIANTE A TIEMPO COMPLETO 10 ESTUDIANTE A TIEMPO PARCIAL 11 SUS LABORES
25. ¿Cuánto duró el periodo más largo de tiempo que trabajó en un mismo empleo?	**Periodo más largo que trabajó en un mismo empleo**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES

HISTORIA TOXICOLÓGICA: Droga principal

26. ¿Cuál es la droga por la que se trata en este centro?	**Droga principal**	1 HEROÍNA 2 COCAÍNA 3 ALCOHOL 4 CÁNNABIS 5 HIPNOSEDANTES 6 OTROS
27. ¿A qué edad inició el consumo de la droga principal?	**Edad de inicio de consumo de la droga principal**	_____ Edad
28. ¿Cuánto consume al día?	**Consumo diario droga principal**	_____ mg/día
29. ¿Cada cuanto consume?	**Frecuencia de consumo**	_____
30. ¿Cómo suele consumir?	**Vía de consumo principal**	1 ESNIFADA 2 VIA ORAL 3 FUMADA 4 ENDOVENOSA 5 OTROS (ESPECIFICAR) _____
31. ¿Cuánto tiempo ha durado la abstinencia máxima para este tóxico?	**Tiempo de abstinencia máxima**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES

32. ¿Cuánto tiempo ha durado la abstinencia total para este tóxico?	**Tiempo de abstinencia total**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES

HISTORIA TOXICOLÓGICA: Heroína

33. ¿A qué edad inició el consumo de **heroína**?	**Edad de inicio de consumo de heroína**	_____ Edad
34. ¿Cuánto consume al día?	**Consumo heroína**	_____ mg/día
35. ¿Cada cuanto consume?	**Frecuencia de consumo**	_____
36. ¿Cómo suele consumir?	**Vía de consumo principal**	1. ESNIFADA 2. VIA ORAL 3. FUMADA 4. ENDOVENOSA 5. OTROS (ESPECIFICAR) _____
37. ¿Cuánto tiempo ha durado la abstinencia máxima de heroína?	**Tiempo de abstinencia máxima heroína**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES
38. ¿Cuánto tiempo ha durado la abstinencia total de heroína?	**Tiempo de abstinencia total heroína**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES
39. ¿Cuánto tiempo ha durado el consumo total de heroína?	**Tiempo total de consum de heroína**	____ DIAS ____ AÑOS ____ SEMANAS ____ MESES

TRATAMIENTO ACTUAL

60. ¿Cuándo inició el programa actual de metadona?	Fecha inicio PMM actual	____/____/____ (día, mes ,año)
60.a. ¿Cuál es la dosis actual que toma?	**Dosis actual de metadona**	_____ mg/día
60.b. ¿Cuándo tomó la última dosis de metadona?	Fecha y hora de la última dosis de metadona	____/____/____ (día, mes ,año) _____/_____ (hora)
61. ¿Dónde se le dispensa en este momento la metadona?	Lugar de dispensación de PMM	1 CAS BCNTA 2 METABUS 3 FARMACIA 4 C.D. GENERALITAT 5 OTROS
62. ¿Tiene "take-home"? ¿Con qué frecuencia recoge la metadona en su lugar habitual de dispensación?	Régimen de "take-home"	1 NO TH 2 TH FINES DE SEMANA 3 2 DÍAS/SEM (no fines de semana) 4 1 DÍA/SEMANA 5 1 DÍA/2 SEMANAS 6 OTROS

63. ¿Qué tratamientos está tomando actualmente? Medicación concomitante

_____
_____
_____
_____
_____
_____
_____
_____
_____
_____
_____

OTROS DATOS

PESO: .....KG **TALLA**: ..... CM

CONSUMO DE TABACO

NÚM. DIAS DE CONSUMO ÚLTIMO MES.....

CIGARRILLOS/DÍA: .....

FUNCIÓN HEPÁTICA GOT: .....

GPT: .....

GGT: .....

SEROLOGÍA: HIV □ 0. Negativo □1.Positivo

VHC □ 0. Negativo □1. Positivo

ÚLTIMOS CONTROLES DE ORINA REALIZADOS PARA OPIÁCEOS

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1.Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

ÚLTIMOS CONTROLES DE ORINA REALIZADOS PARA COCAÍNA

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo

FECHA: ...../...../..... RESULTADO □ 0. Negativo □1. Positivo