Pharmacogenetics of Methadone Response

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Abstract

The efficacy of methadone maintenance treatment (MMT) in opioid use disorder is well established but responses vary. The influence of methadone pharmacodynamics and pharmacokinetics on dose requirements and program outcomes remains controversial despite the increasing number of studies evaluating genetic influences on response to methadone treatment. Furthermore, patients require different doses (usually between 60 and 100 mg/day), and there are no clear data on a plasma concentration associated with treatment success. We review the evidence regarding the influence of genetics on pharmacokinetic and pharmacodynamic factors in terms of MMT outcome. We also analyse the influence of genetics on the occurrence of severe adverse events such as respiratory depression and ventricular arrhythmia in methadone treatment. The outcomes of MMT may be influenced by a combination of environmental, drug-induced, and genetic factors. The influence of pharmacokinetic genetic variability can be clinically managed by modifying the posology. A better understanding of pharmacodynamic factors could help in selecting the best opioid for

substitution treatment, but patient phenotype must still be considered when establishing a maintenance treatment. Pharmacogenetic studies represent a promising field that aims to individualize treatments according to genetic backgrounds, adapting medication and doses according to possible outcomes and the risk of adverse events.

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Electronic supplementary material

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Key Points

The results of methadone maintenance treatment (MMT) are influenced, both positively and negatively, by genetic, environmental, and drug-induced factors.
Treatment outcomes could be directly related to pharmacodynamic and/or pharmacokinetic factors.
The influence of genetic variability in the coding genes involved in the pharmacokinetics of methadone metabolism and transport can be clinically managed, usually by increasing or decreasing methadone doses or splitting the daily dose.

1. Opioid Addiction: A Relapsing Complex Disease

Opioid use disorder is a chronic, relapsing condition that involves elevated costs for individuals, families, and society. Opiates, opioids, and prescription opioids continue to be one of the most problematic drug groups worldwide. The 2016 *World Drug Report* by the United Nations Office on Drug and Crime estimated that 0.4% of the global population aged 15–64 years, or the equivalent of some 17 million people, had consumed opiates and opioids in 2014 [1]. Opioid addiction is a complex disease with interacting issues, including environmental factors (cues, conditioning, stress, etc.), drug-induced neurobiological changes (the reward circuits in the basal ganglia and extended amygdala, involving dopamine, corticotropin-releasing factor, and dynorphin; and impairments in executive functions mediated by glutamate in the prefrontal cortex, insula, and basal ganglia), and individual characteristics (genetics, medical and psychiatric comorbidity, personality, and stress responsiveness), that result in the

compulsive behaviour of drug seeking [2, 3, 4].

The two main treatment strategies in opioid dependence are abstinence oriented and medication assisted [5]. The goal of the former is to remove the abused opioid during a detoxification process and undergo a complete lifestyle change, whereas the objective of the latter is to stabilize brain neurochemistry by replacing a short-acting opioid with a long-term acting opioid that has relatively steady-state pharmacokinetics. Opioid agonist maintenance treatment is designed to minimize the effect of the euphoria associated with the administration of illicit opioids and eliminate opioid withdrawal syndrome [6].

Methadone maintenance treatment (MMT) is the most widely used therapy for heroin and prescription opioid use disorder and has been shown to be effective in opioid-dependent subjects who stay in treatment [7, 8, 9]. Criteria for successful MMT may vary between countries and/or centres but usually include improvement of retention in treatment [10, 11]; decreased illicit opioid use, risk behaviour related to HIV/sexually transmitted diseases [12], criminal behaviour related to drug use [13], and overdose mortality risk [14]; and augmented health-related quality of life [15]. No specific dose to achieve these “response criteria” has been established, and patients can require different doses [16]; this variability has been related in part to the pharmacologic properties of the molecule.

1.1. Methadone Pharmacokinetics

Methadone is a chiral molecule. This means the chemical structure includes an asymmetrical carbon atom that leads to two enantiomeric forms with the same chemical structure but different spatial dispositions so that one is the mirror image of the other. The methadone usually employed in the treatment of addiction and pain contains both enantiomers in the same proportion (racemate): (*R*)- or levo- or l-methadone and (*S*)- or dextro- or d-methadone. The pharmacodynamics of each enantiomer differs: (*R*)-methadone has a higher affinity for opioid receptors than *(S)*-methadone, and greater analgesic potency has also been reported [16]. The *(R)*-methadone enantiomer produces the main therapeutic effects of methadone, whereas (*S*)-methadone is usually associated with adverse events [16, 17, 18]. The pharmacokinetic profiles of the enantiomers also differ: the (*R*)-/(*S*)-methadone plasma concentration ratio varies during the 24 h after administration [19, 20]. Plasma concentrations also differ between subjects [18], which may explain range of responses to

treatment.

The marked inter-individual differences in methadone pharmacokinetics is considered the main explanation for the range of doses required to achieve treatment success [16]. These differences have been previously described in relation to bioavailability, plasma protein binding, volume of distribution, total body clearance, and elimination half-life. Oral bioavailability has been reported to range from 36 to 100% [16], with such a broad range partly explained by the action of intestinal cytochrome P450 (CYP)-3A4 [21]. A number of studies have also reported inter-individual variability in the free fraction of plasma methadone [22], in addition to a distribution volume with a mean of 4 l/kg and a range of 2–13 l/kg [23]. Although distribution volume has no direct effect on steady-state plasma concentrations, changes in the unbound fraction of methadone could modify the volume of distribution [20].

Results from studies focused on finding optimal methadone plasma concentrations for effective MMT have been contradictory [24, 25, 26]. Some were unable to find such a threshold concentration [26, 27, 28], whereas others described a relationship between methadone plasma concentration and outcomes such as presence of withdrawal symptoms [24, 29] and illegal drug use, detected with random urine analysis [25]. The proposed values usually range from 50 to 600 μg/l of (*R*,*S*)-methadone [30]. The measurement of methadone plasma concentrations has generally been recommended to confirm inadequate rather than optimum doses [31]. As the opioid effect of (*R*,*S*)-methadone is mainly due to the (*R*)-enantiomer, and some side effects (such as QTc prolongation) have been associated with (*S*)-methadone, differences in the (*R*)/(*S*)-methadone ratio could also explain the variability in treatment responses and tolerance. Furthermore, given the wide inter-individual variability of the (*R*)/(*S*)-methadone ratio, other studies have tried to correlate the concentration of (*R*)-methadone with therapeutic outcome [30, 32, 33].

Although the efficacy of MMT has been demonstrated, a significant number of patients still respond poorly [34]. An adequate dosage of methadone—usually ranging from 60 to 100 mg/day—is the main factor associated with successful MMT [10, 35] and is usually correlated with higher retention in treatment. The role of variables such as sex, ethnicity, education level, employment status, years of consumption, and previous treatments in response to MMT has not been confirmed [36, 37].

Nevertheless, the relationship between a patient's genetic background and their response to MMT has become a topic of interest. Most reports have focused on genetic polymorphisms in genes coding for methadone-metabolizing enzymes and transporter proteins (e.g., P-glycoprotein). Differences in response to or tolerance of MMT related not only to pharmacokinetic genetic variability but also to polymorphisms associated with pharmacodynamic factors—such as the μ opioid receptor, dopamine receptors, β-arrestin, and neurotrophins—have also been evaluated [38, 39]. In the next section, we describe the main approaches taken in pharmacogenetic studies.

1.2. Different Approaches to Study Pharmacogenetics

Approaches to studying the pharmacogenetic basis of response to drugs and vulnerability to drug addiction vary. Linkage studies use families to provide evidence of how close a genetic marker is to an allele causing the phenotype in question but are complicated to perform because it can be difficult to find families with more than one affected member.

Association studies may be performed with unrelated individuals and parent–offspring trios, and associations between specific genetic variants and outcomes/traits of interest are evaluated. Variants are selected based on previous hypotheses.

In genome-wide association studies (GWAS), the entire genome of different individuals is examined to establish whether any variant is associated with a trait or disease. Such an approach can be used to detect new loci in the genome and formulate hypotheses. More than 100,000 markers across the genome are generally employed.

Epigenetic studies analyse genetic information not encoded in the DNA sequence. DNA methylation and covalent histone modifications are the primary sources of epigenetic inheritances. Changes in methylation in genomic DNA are a mechanism of control of DNA expression. MicroRNA (miRNA) is another mechanism by which gene expression is regulated post-transcriptionally; miRNA consists of short-length (18–25 nucleotides) non-coding RNA. Epigenetics and miRNAs control each other, regulating the normal expression of genes.

It is important to be aware of some limitations that could affect the validity of

pharmacogenetic analysis results. Subjects should be correctly selected and a "good response" phenotype clearly defined. Samples from different ethnic or racial origins (population stratification) can be a source of inaccurate results because of varying therapeutic responses and allele frequencies. Another reason for errors in genetic studies is the multiple comparison issue. Studies that analyse multiple genes and/or single nucleotide polymorphisms (SNPs) must include a method to control for multiple comparison. Without this, a risk of false-positive (or type I error) is high. In GWAS studies, where more than 500,000 SNPs could be tested simultaneously, this is paramount, and p values around 5×10^{-8} are generally employed. Finally, a replication study increases the validity of positive results; lack of validation in many pharmacogenetic studies forces cautious interpretation [40].

We review the current evidence on genetic influences on pharmacodynamic and pharmacokinetic factors involved in MMT outcomes, methadone doses required, and methadone-related adverse events.

2. Pharmacodynamic Genetic Variability

Pharmacodynamics is the study of the biochemical and physiological effects of drugs and their mechanism of action, including correlations between their chemical structure and their actions and effects. Table 1 and the table in the Electronic Supplementary Material (ESM) summarizes the pharmacodynamic targets that have been studied, including the μ opioid receptor, dopamine receptor genes, β-arrestin 2, neurotrophins, and genes previously involved in the risk for opioid dependence disorder.

Table 1

The main studies assessing pharmacodynamic genetic variability and its influence on methadone maintenance treatment outcomes

Gene	Study	Sample characteristics	Main results
	Barratt et al. [54]	119 Caucasian MMT patients	Wild-type *OPRM1* subjects, an *ABCB1* variant haplotype group had significantly lower doses than *ABCB1* wild-type (35 ± 5 vs. 180 ± 65 mg/day; $p < 0.01$) and C trough (78 ± 22 vs. 177 ± 97 ng/ml; $p < 0.05$). In subjects with the most common *ABCB1* haplotype combination, the *OPRM1* 118 A/G genotype

				was associated with a significantly higher C trough than 118 A/A (250 ± 126 vs. 108 ± 36 ng/ml; $p = 0.016$)
	OPRM1	Hung et al. [55]	321 Han Chinese MMT patients: 92 low dose (< 55 mg/day); 150 medium dose (55–99 mg/day); 79 high dose (100–150 mg/day)	*OPRM1*, *ABCB1*, *CYP2B6*, *ANKK1*, and *DRD2* genetic variants were jointly correlated with optimal methadone dose (adjusted $r^2 = 53\%$)
		Crettol et al. [56]	238 Caucasian MMT patients: 165 R (40–120 mg/day), 73 NR (> 120 mg/day)	No association between genotype and MMT outcome: AA carriers: AA 118 (72%) R vs. 59 (81%) NR AG carriers: 44 (27%) R vs. 13 (18%) NR GG carriers: 3 (2%) R vs. 1 (1%) NR ($p = 0.3$)
		Fonseca et al. [58]	116 Caucasian MMT patients: 83 R (100 ± 68 mg/day); 33 NR (78 ± 43 mg/day)	No association between genotype and MMT outcome: R: 75% A 25% G vs. NR: 75% A 25% G; $p = 0.96$
		Wang et al. [59]	366 Han Chinese MMT patients (54.7 ± 28.1 mg/day). Methadone plasma concentrations 193.07 ± 121.76 ng/ml	No association with response, methadone dose, nor plasma concentrations with any of the SNPs
		Levran et al. [60]	227 Israeli patients undergoing stable MMT (141 ± 51 mg/day)	Carriers of the G allele required lower doses of methadone (132.4 mg ± 50.7 vs. 147.4 ± 49.7 mg; $p = 0.028$ in a dominant model)
		Smith et al. [51]	383 African American subjects in MMT	SNP associated with: mean methadone dose (68.0 ± 30.1 mg) and rs73568641 ($p = 2.8 \times 10^{-8}$); each minor (C) allele corresponded to an additional ~ 20 mg day^{-1} of oral methadone. Children being treated for surgical pain, rs73568641-C was associated with a higher required dose of morphine ($n = 241$, $p = 3.9 \times 10^{-2}$). Not replicated in Caucasian sample (77.8 ± 33.9 mg)
		Crist et al. [61]	283 European Americans in MMT. Mean maximum dose 99.6 ± 46.0 mg/day.	Methadone patients with the A/A genotype at rs10485058 were less likely to have opioid-positive urine drug screens than

		Opioid-positive urine drug screens 36.0%	those in the combined A/G and G/G genotypes group. RR 0.76, 95% CI 0.73–0.80; $p = 0.0064$)
OPRD1	Crist et al. [66]	77 African Americans (~~MTD~~ methadone mean dose= 99.7 ± 46.4) and 566 European Americans (~~MTD~~ methadone mean dose= 79.2 ± 26.5) undergoing treatment for opioid dependence (MMT or buprenorphine)	Methadone patients with the CC genotype were less likely to have opioid-positive urine tests than those in the combined CT and TT genotypes group (RR 0.52, 95% CI 0.44–0.60; $p = 0.001$)
DRD2	Crettol et al. [56]	238 Caucasian MMT patients: 165 R (40–120 mg/day); 73 NR (> 120 mg/day)	Carriers of 957CC genotype were more frequently NR: 12 vs. 25% ($p = 0.05$)
	Doehring et al. [68]	85 Caucasian MMT patients	*DRD2* rs6275 was associated with methadone dose and time to reach maximum dose; $p = 0.016$ and 0.005 for average and maximum dose, respectively (exact figures not shown in the original)
	Hung et al. [55]	321 Han Chinese MMT patients: 92 low dose (< 55 mg/day); 150 medium dose (55–99 mg/day); 79 high dose (100–150 mg/day)	*DRD2* variability was associated with methadone dose requirements, *DRD2* -214A>G or 939C>T allele had a twofold chance of requiring a lower methadone dose than non-carriers ($p = 0.001$)
	Bawor et al. [69]	240 MMT patients (85% of European origin). Methadone dose 89.5 ± 60.8 mg/day. R 167 (97.5 ± 67.5), NR 73 (71.2 ± 35.9)	The genetic variants were not significantly associated with continued opioid use: OR 1.27, 95% CI 0.511–3.182; $p = 0.603$
	Huang et al. [70]	138 male Taiwanese heroin addicts in MMT (56.8 ± 24.8 mg/day; range 3–140)	*DRD2* Taq1 B genotype was not associated with methadone use requirements
	Levran et al. [60]	227 Israeli patients undergoing stable MMT (141 ± 51 mg/day)	The two SNPs were associated with lower methadone dose. *ANKK1* rs7118900 A allele required lower methadone doses than non-carriers (119 ± 57.1, 128.5 ± 51.7 vs. 146.2 ± 50.1; $p = 0.028$). *DRD2* rs2283265 T allele required

			lower methadone doses than non-carriers (121.2 ± 51.4, 128.2 ± 53.2 vs. 144.4 ± 50.4; $p = 0.0496$, non-adjusted)
β-Arrestin2	Oneda et al. [76]	238 Caucasian MMT patients. Median methadone daily dose 125 mg (range 3–430 mg). Low-dose R 97 (37%); high-dose R 78 (33%); NR 73 (31%)	Homozygous for rs3786047, rs1045280 and rs2036657 were more probably NR (48 vs. 29 and 23%; $p = 0.01$; 47 vs. 29 and 25%; $p = 0.002$; 47 vs. 29 and 25%; $p = 0.002$ respectively)
BDNF	de Cid et al. [80]	91 Caucasian MMT patients: 68R (106.27 ± 70.96); 23 NR (90.00 ± 49.86)	Carriers of the CCGCCG haplotype of the subset formed by rs7127507, rs1967554, rs11030118, rs988748, rs2030324, and rs11030119 were at higher risk of being NR 4/42 vs. 1/135 (corrected $p = 0.0234$)
	Levran et al. [60]	227 Israeli patients undergoing stable MMT (141 ± 51 mg/day)	Of 13 *BDNF* SNPs analysed, three non-coding *BDNF* SNPs were associated with methadone dose. Lower methadone dose was 125.1 ± 44.9 (CC carriers of rs1491850) vs. highest methadone dose 158.7 ± 47.8 (CC carriers of rs10835210)
	Bawor et al. [69]	240 MMT patients (85% of European origin). Methadone dose 89.5 ± 60.8 mg/day. R 167 (97.5 ± 67.5); NR 73 (71.2 ± 35.9)	The genetic variants were not significantly associated with continued opioid use (OR 1.37, 95% CI 0.792–2.371; $p = 0.264$)
NTRK2	Levran et al. [60]	227 Israeli patients undergoing stable MMT (141 ± 51 mg/day)	Carriers of one or two of the variant C alleles of the rs2289658 SNP require relatively higher methadone doses (168 mg) than non-carriers (143 mg). Carriers of two A alleles of rs2120266 require relatively higher methadone doses (191 mg) than non-carriers
	Levran et al. [82]	72 MMT patients (Israeli population with Caucasian and Middle Eastern ancestry)	The mean daily methadone doses in subjects homozygous for the variant T allele (81.7 mg) were lower than those of the heterozygotes and the non-carriers (140 and 153 mg, respectively) ($p = 0.0002$ with recessive

NGFB			mode)
	Levran et al. [60]	227 Israeli patients undergoing stable MMT (141 ± 51 mg/day)	Homozygosity (TT) for SNP rs2239622 resulted in a methadone mean dose (122 mg) that was lower than the mean dose of heterozygotes and non-carriers (140 and 146 mg, respectively), but the association only reached borderline significance
ALDH5A1	Fonseca et al. [83]	169 Caucasian subjects in MMT (89 R and 43 NR). R 83 (109 ± 71 mg/day); NR 33 (71 ± 45 mg/day)	Subjects carrying the T variant allele have a higher risk of being NR to methadone treatment (62.8 vs. 37.2%; $p = 0.0024$)
GRM6; *GRM8*; *NR4A2*; *CRY1*; *MYOCD*	Fonseca et al. [58]	116 Caucasian MMT patients: R 83 (100 ± 68 mg/day); NR 33 (78 ± 43 mg/day)	Patients carrying the A allele at rs1714984 (*MYOCD*) had an increased risk of being NR only when they were also carriers of the AG genotype at rs953741 (*GRM6*) (OR 10.83; 95% CI 2.52–46.66; $p = 0.006$)
KCNJ6	Lötsch et al. [84]	85 Caucasian subjects in MMT	The average daily dose of methadone during the first year was higher in the AA genotype (119.7 ± 49.6 vs. 77.5 ± 26.2 mg/day; $p = 0.003$)

CI confidence interval, *LD* linkage disequilibrium, *MMT* methadone maintenance treatment, ~~*MTD* maximum tolerated dose~~, *NR* non-responders, *OR* odds ratio, *R* responders, *RR* relative risk, *SNP* single nucleotide polymorphism

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2.1. μ Opioid Receptor (*OPRM1*)

Methadone, heroin, and morphine have high affinity for the μ receptor. The gene encoding the receptor, *OPRM1*, is located in chromosome 6 (6q24-q25) [41]. The most widely studied polymorphism in *OPRM1* is an SNP in exon 1 (rs1799971), involving a substitution A → G (A118G), which encodes an Asn40Asp amino acid substitution. The Asp40 residue results in a threefold increase in β-endorphin binding compared with the Asn40-containing protein (probably related to suppression of a potential glycosylation site in the N terminal region [42]) and differences in messenger RNA (mRNA) expression, with a decrease of 1.5 in the mRNA and tenfold lower receptor levels in the

G118 allele compared with the A118 allele. The authors hypothesized this could indicate a defect in transcription or mRNA maturation [43]. Carriers of this variant have reduced agonist-induced receptor signalling efficacy as shown in the post-mortem brain [44], differences in signal response to agonists [45], and stronger cortisol response to receptor blockade by naloxone, with a reduced agonist effect of morphine-6-glucuronide [46]. Several authors have studied the influence of this gene on opioid addiction disorder, with controversial results. A recent meta-analysis indicated that the G allele has a protective effect in substance dependence, but no significant results have been detected for opioid use disorder [47]. This SNP has also been studied in terms of naltrexone response in alcohol use disorder and the doses required to achieve analgesia in pain treatment [48, 49, 50, 51, 52]. However, the influence of this SNP on MMT outcome remains unclear. An experimental study reported an association between the 118G variant and decreased miotic potency of (*R*)-methadone in healthy volunteers [53]. When this gene was evaluated in MMT subjects, one study [54] reported an epistatic interaction of the polymorphism with the transporter gene ATP-binding cassette subfamily B member 1 (*ABCB1*) and methadone dose. *OPRM1* wild-type subjects plus *ABCB1* carriers of a haplotype variant required lower methadone doses than *ABCB1* wild-type carriers (35 ± 5 vs. 180 ± 65 mg/day; $p < 0.01$). Similarly, when the SNP was assessed in combination with other genes involved in methadone pharmacokinetics, the A118G SNP was associated with the maximum methadone dose [55]. However, both studies had high-risk or type I errors as they did not control for multiple testing. In contrast, other authors have found no association between polymorphisms at *OPRM1* alone and treatment response (based on heroin use) or with methadone doses [54, 55, 56], indicating that the influence of this SNP may be modulated by other pharmacodynamic and pharmacokinetic factors.

AQ5

Evaluations of other SNPs in *OPRM1* have produced some controversial results. One study evaluating the SNP rs10744287, previously associated with opioid addiction [57], found no association with MMT outcome (based on urine controls) [58]. Other authors evaluating the influence of other *OPRM1* SNPs on methadone adverse events (see Sect. 4.1.1) found no association with treatment response, methadone dose, or plasma concentrations [59].

Two studies recently described promising results regarding the influence of *OPRM1* SNPs on methadone requirements and treatment outcome. Levran et al. [60] investigated 11 genes related to the opioid, dopamine, glutamate, and

neurotrophin systems: 23 SNPs in the *OPRM1* gene, and one SNP (rs558025), were associated with methadone dose, and carriers of the G allele required lower doses of methadone (132.4 ± 50.7 vs. 147.4 ± 49.7 mg/day; $p = 0.028$ in a dominant model). The authors controlled for SNP multiple testing, and the p values were obtained by permutation analysis based on 40,000 replicates to allow for the fact that, for a given SNP, three modes of inheritance were tested (co-dominant, dominant, and recessive). Although the differences were statistically significant, they have little relevance in clinical practice. In a sample of opioid-dependent African American subjects, the SNP rs73568641 (of which the nearest gene—306 kilobases—is *OPRM1*) was associated with methadone dose. This association was replicated in the amount of morphine required to treat pain after surgery in a set of opioid-naïve African American children but was not observed in a Caucasian cohort [51]. Finally, 582 patients receiving methadone or buprenorphine/naloxone treatment were evaluated in terms of treatment response, based on the number of opioid-positive urine controls. A single haplotype, tagged by rs10485058, was significantly associated with patient urinalysis data in the methadone treatment group. The same SNP also predicted self-reported relapse rates in an independent population of Australian patients of European descent receiving opioid substitution therapy [61].

In light of the reviewed studies, it is not possible to draw clear conclusions about the influence of the *OPRM1* gene on treatment response. Most studies had not been replicated, and several cases were not controlled by multiple testing [54, 55], so the risk of a type I error was high. Moreover, the samples were heterogeneous (e.g., Caucasian, Chinese, Israeli, and African American), and G allele frequencies vary according to ethnicity, ranging from 0.12 to 0.20 in Whites (non-Hispanic, European descent), 0.19 to 0.24 in non-White Hispanics, and 0.01 to 0.04 in African Americans [62]. ~~Furthermore~~, ~~the~~ ~~frequency~~ ~~of~~ ~~the~~ This allele ~~has~~ ~~been~~ is found in 40–50% of Asian populations [43]. Although evidence seems to suggest that polymorphisms at *OPRM1* are crucial for opioid addiction, pain therapy, and methadone-related adverse events, there are no conclusive results with implications for MMT response or the dose of methadone required to achieve good outcomes.

2.2. Delta Opioid Receptor (*OPRD1*)

The *OPRD1* gene, located in chromosome 1p36, encodes the delta opioid receptor (DOR), a G-protein-coupled receptor. This receptor has been

implicated in reward, pain, addiction, and affective changes [63, 64]. Some authors have associated variants in *OPRD1* with the risk of opioid addiction [65]. One study evaluating the involvement of six SNPS in the *OPRD1* gene for an association with positive urine tests in patients receiving methadone and buprenorphine treatment reported that, in patients receiving methadone, carriers of CC genotype at rs678849 SNP were less likely to have opioid-positive urine tests than were heterozygous and non-carriers [66]. As the study included only a reduced sample of African Americans and was not replicated, it was not possible to establish a relationship between this gene and methadone response.

2.3. Dopamine D2 Receptor (*DRD2*) and Ankyrin Repeat and Kinase Domain Containing 1 (*ANKK1*)

The dopaminergic system is involved in the rewarding effects of opioids and drugs of abuse. Heroin increases dopamine concentrations in the reward areas of the brain (nucleus accumbens and caudate putamen). The D_2 receptor gene (*DRD2*) is located in chromosome 11 (11q22-23). Polymorphisms in this gene have been associated with addiction [67].

Crettol et al. [56] analysed the synonymous polymorphism rs6277 (C957T) in a group of 238 heroin-dependent subjects on an MMT programme. The carriers of the 957CC genotype were more frequently non-responders with a shorter period of negative results in urine tests [56]. In a study [68] in 85 MMT patients, average and maximum daily methadone doses were significantly associated with rs6275 (C939T) SNP. The carriers of the T allele needed higher methadone doses and required a longer time to reach the maximum methadone dose than non-carriers, but the exact figures were not included in the original article. Hung et al. [55] described an association between methadone doses and two SNPS of the *DRD2* gene, rs1799978 (A214G) and rs6275. In contrast, another study could not replicate the influence of rs1799978 on MMT outcome (measured by the continued use of illicit opioids) [69]. However, the sample size of the non-responder group was small, and significant differences in mean methadone doses between groups interfered with the results, as non-responders were treated with lower doses (difference of 26 mg). Another study [70] investigated whether *DRD2* *TaqIB* polymorphisms were related to methadone dose and personality traits and reported that differences in genotypes were not linked to daily methadone dose. One further study [60], which assessed SNPs from the *DRD2* and *ANKK1* genes in a sample of stable MMT patients, found two SNPs to be associated with differences in methadone dose: the rs7118900 at

ANKK1 (non-synonymous) and rs2283265 at *DRD2* (intronic). Carriers of the variant A allele of *ANKK1* rs7118900 and the variant T allele of *DRD2* rs2283265 required lower methadone doses than non-carriers [60].

Finally, in the year 2000, an exploratory study found that rs1800497 (*Taql A1*), an allele located 10 kb from the 3′ region of the DRD2 in disequilibrium linkage (DL) with the SNP rs6277, appeared to be associated with MMT response [71]; this finding was not replicated in a smaller sample [72].

In conclusion, while multiple studies have analysed *DRD2* genetic variability, participants have come from diverse ethnicities (even within the same publication but with no specific information given) and the aspects evaluated have varied (dose, illicit opioid use, time to reach maximum dose), which precludes the collection of useful data and comparison of results.

2.4. Beta-Arrestin

β-Arrestin 2 is a component of the G-protein-coupled receptor complex. It is involved in μ opioid and D_2 receptor signalling. It also regulates the number of functional receptors expressed on the neuron surface by desensitization and internalization of these G protein-coupled receptors [73]. *β-Arrestin 2* knockout mice do not become morphine tolerant when compared with wild-type mice, and the impact of different opioid agonists varies depending on the activity of β-arrestin 2 protein [73]. Polymorphisms in the coding gene *β-arrestin 2* have been associated with differential risk for developing cocaine and nicotine addiction [74, 75]. Our literature search revealed only one study [76] that evaluated the influence on treatment outcome of genetic variability at *β-arrestin 2* (*ARRB2*); results were based on random urine controls (non-responders were maintained at methadone doses > 120 mg/day). The authors evaluated 238 subjects receiving MMT and described a haplotype block that was associated with risk for poor response. Homozygosity for the variant allele at rs3786047, rs1045280, and rs2036657 was associated with a non-responding phenotype. However, none of these SNPs induced changes in the final protein, and replication studies are lacking, which makes it difficult to explain the mechanism involved.

2.5. Neurotrophins

Neurotrophins are a protein family essential for central nervous system development; they are also involved in the survival and regeneration of

damaged neurons, learning, memory, and brain plasticity [77].

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 evidence indicates that it may be involved in the mechanisms underlying substance abuse [78, 79].

An exploratory study in 91 subjects receiving MMT assessed *BDNF* genetic variability, analysing 21 SNPs across the *BDNF* genomic region. Haplotype block analysis showed a low-frequency haplotype (2.7%) that was more common in the non-responder group [80]. Another study analysed 13 *BDNF* SNPs, of which three non-coding SNPs (rs10835210, rs7934165, and rs1491850) were associated with methadone doses and hypothesized to play a regulatory function [60]. The same study analysed 15 SNPs at the neurotrophic receptor tyrosine kinase 2 (*NTRK2*) gene; six intronic SNPs (rs2120266, rs2289658, rs4358872, rs1948308, rs2378676, and rs4877900) were found to be associated with methadone dose. A possible association between *BDNF* G196A (rs6265) and MMT outcome was evaluated using urine to assess treatment response in a sample of 240 patients but the variant was not associated with poor treatment outcomes [69]. Levran et al. [60] studied the same SNP but found no association even though they included a different population ancestry (Israeli).

Nerve growth factor β polypeptide (*NGFB*) could play a key role in opioid treatment as it has been associated with the expression of the DOR in the cell membrane [81]. Genotypes of 14 SNPs of *NGFB* were analysed for involvement in response to MMT, and a significant difference was observed in methadone doses required depending on the genotype at rs2239622: the mean daily methadone doses in subjects homozygous for the variant T allele (81.7 mg) were lower than those of the heterozygotes and the non-carriers (140 and 153 mg, respectively) [82]. A later study did not confirm this association, although homozygous subjects required lower doses than heterozygotes and non-carriers (122 vs. 140 vs. 146 mg); however, the differences were non-significant, and the clinical significance of the variation was minimal [60].

The evidence found for genes encoding for neurotrophins has limitations similar to those described in previous sections. We found few studies evaluating neurotrophic factors (three for *BDNF*, one for *NTRK2*, and two for *NGFB*), and those we did find were conducted in patients of different ethnicity, and none

replicated results in the same SNP. In cases describing significant differences, either the haplotype was low frequency [80] or the differences in the daily dose of methadone had no clinical relevance [82]. For these reasons, we cannot conclude that the genetic variability in genes coding for neurotrophins could clearly determine methadone response.

2.6. Succinic Semialdehyde Dehydrogenase (*ALDH5A1*)

The succinic semialdehyde dehydrogenase enzyme SSADH or ALDH5A1 is responsible for the transformation of succinic semialdehyde to succinic acid, which then enters the Krebs cycle. Succinic semialdehyde is the metabolite of gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter of the brain. In addition, it is the precursor and metabolite of gamma hydroxybutyric acid (GHB). Our group genotyped a genetic variant in *ALDH5A1* (rs2760118; cC538T) and found that subjects carrying the variant T allele had a higher risk of being non-responders to methadone treatment (62.8 vs. 37.2%). This variant involves an amino acid substitution and results in a considerable reduction in enzyme activity (83%). The possible explanation for such an association could be a reduction in enzyme activity, which would increase endogenous GHB and GABA levels and thus induce symptoms such as sedation and impaired psychomotor performance, neuropsychological adverse events that could be responsible for a greater propensity to relapse in genetically predisposed patients [83]. Although the results appear very promising, the small sample size and lack of replication studies mean that results should be interpreted with caution.

2.7. Other Genes

Based on the results of a GWAS by Nielsen et al. [57] aimed at detecting new loci associated with opioid dependence, our investigation group [58] performed an association study to evaluate a possible association between response to MMT and polymorphisms of genes involved in opioid dependence. We found an association between MMT response status and genetic variability in the cryptochrome 1 (*CRY 1*) gene, which is involved in circadian rhythms. In addition, we also described an epistatic effect between the genes coding for the transcription factor myocardin (*MYOCD*) and the metabotropic glutamate receptor 6 (*GRM6*) [58].

Another gene studied for its possible association with MMT is potassium voltage-gated channel subfamily J member 6 (*KCNJ6*), which encodes for

potassium inwardly rectifying channels (Kir3.2, GIRK2) involved in opioid receptor transmission. The authors genotyped 85 patients receiving MMT, 352 pain patients receiving opioid treatment, and 51 healthy controls. The mean dose of methadone during the first year was higher in patients with the rs2070995 AA genotype (119.7 ± 49.6 vs. 77.5 ± 26.2 mg/day; $p = 0.003$). Moreover, a trend, but not a significant result, was observed in patients treated with opioids for analgesia in the carriers of the AA genotype ($p = 0.093$) [84].

3. Pharmacokinetic Genetic Variability

It has been classically described that methadone is extensively metabolized in the liver, mainly by CYP3A4. Both in vitro and in vivo studies have shown that the isoenzymes of cytochrome P450, CYP3A4, CYP2D6, CYP2B6, and CYP2C19, are involved in methadone metabolism [25, 85, 86, 87, 88, 89]. The pharmacokinetics of methadone are also influenced by the activity of a membrane transporter, P-glycoprotein, a trans-membrane protein expressed in tissues with barrier function (intestine, brain). Its activity in intestines and the blood–brain barrier (BBB) has shown relevance in defining methadone concentrations [38], and a recent review of clinical interaction studies discussed the influence of P-glycoprotein in methadone brain access [89].

Genetic variability in genes coding for metabolizing enzymes and transporters has been extensively studied. Overall, variability at this level can influence enzyme activity, changing methadone plasma concentrations. It should be taken into account that some of these enzymes display stereoselectivity towards one of the enantiomers, and differences in their activity alter the (*R*)/(*S*)-methadone ratio and lead to variations in therapeutic response and adverse events. Table 2 summarizes the genes involved in the metabolism and transport of methadone.

Table 2

Main studies assessing pharmacokinetic genetic variability and its influence in methadone maintenance treatment (MMT) outcome

Study	Sample characteristics	Gene(s)/SNP(s)	Results
Metabolizing genes (CYP)			
Eap et	235 MMT subjects: 18	*CYP2D6*	Different phenotypes associated with methadone plasma concentrations: for (*R*)- ($p = 0.024$), (*S*)-

al. [107]	PM, 228 EM, 10 UM		(p = 0.033), and (*R,S*)-methadone (p = 0.026) concentrations to dose-to-weight ratios
Crettol et al. [25]	209 MMT subjects (199 White). Dose 140 ± 82 mg	*CYP2B6*; *CYP2C9*; *CYP2C19*	*CYP2B6* genotype influences (*S*)-methadone and, to a lesser extent, (*R*)-methadone plasma levels (105 vs. 122 vs. 203 ng·kg/ml for the non-carriers of allele *6, heterozygous and homozygous, respectively (p = 0.0004). *CYP2C9* and *CYP2C19* genotypes do not influence methadone plasma levels
Crettol et al. [92]	245 Caucasian MMT patients (divided in low-dose R, high-dose R, high-dose NR). Mean methadone dose 134 ± 82 mg (range 3–430 mg/day)	*CYP1A2*; *CYP3A4*; *CYP3A5*; *CYP2B6*; *CYP2D6*; *CYP2C9*; *CYP2C19*	*CYP3A4*, *CYP2B6* and, to a minor extent, *CYP2D6* were involved in methadone metabolism
Coller et al. [108]	51 MMT subjects (45 White, 5 indigenous Australian, 1 Asian)	*CYP2D6*	No influence of any polymorphism in (*R*)-, (*S*), and (*R,S*)-methadone clearance
Fonseca et al. [93]	105 Caucasian MMT patients. R 76 dose: 109 ± 68; NR 29 dose: 72 ± 43	*CYP3A5*; *CYP2B6*; *CYP2D6*; *CYP2C9*; *CYP2C19*	*CYP2D6* metabolizing phenotype differences were found, higher probability of being NR in the group of UM (p = 0.032)
Hung et al. [55]	321 Han Chinese MMT patients. 92 low dose (< 55 mg/day); 150 medium dose (55–99 mg/day); 79 high dose (100–150 mg/day)	*CYP2B6*	rs3745274 (G516T) SNP was associated with differences in methadone doses: low-dose patients were more likely to carry the TA and TG haplotypes than were medium- and high-dose methadone patients (p < 0.001)
Wang et al. [98]	366 Han Chinese MMT patients. Methadone dose 54.7 ± 28.1 mg/day. Methadone plasma concentrations 193.07 ± 121.76 ng/ml	*CYP2B6*	rs10403955, rs3745274, rs2279345, and rs707265 were associated with a higher clearance, a lower plasma concentration, and a lower C/D ratio of (S)-methadone (p = 0.0017)
	178 Taiwanese MMT patients (R 62 and NR		Methadone maintenance dose, *CYP2B6* rs2279343

Lee et al. [102]	116). Methadone daily dose 50.8 ± 30.5 mg/day (range 5–250)	*CYP2B6*; *CYP2C19*	(785G) allele, and *ABCB1* rs2032582 (2677T) allele had positive effects on the methadone plasma concentration
Levran et al. [94]	74 Israeli MMT patients. Mean dose 140 ± 52 mg/day (range 3–260)	*CYP3A4*; *CYP2B6*; *CYP2D6*	*CYP2B6* genetic variability was involved in MMT dose requirements: rs3745274 GG carriers required higher doses of methadone (150.3 ± 8.1) than GT (128.6 ± 9.1) and TT (96.3 ± 15.3), (p = 0.048). rs2279343 AA carriers required higher doses of methadone (151.4 ± 8.4) than AG (132.6 ± 8.9) and GG (88.3 ± 11.9), (p = 0.012)
Dennis et al. [103]	Meta-analysis of seven articles	*CYP2B6*	Homozygous for the *CYP2B6*6* genotype have higher trough (*R*) and (*S*) methadone plasma concentrations, but no influence on MMT response
Wang et al. [96]	366 Han Chinese MMT patients. Methadone dose 54.7 ± 28.1 mg/day. Methadone plasma concentrations 193.07 ± 121.76 ng/ml	*CYP2B6*; *CYP3A4*; *CYP2C19*	SNPs on *CYP2B6* were associated with plasma (*S*)-methadone concentration; SNPs on *CYP3A4* were associated with withdrawal symptoms and side effects; and SNPs on *CYP2C19* were associated with methadone dose
Tsai et al. [101]	366 Han Chinese MMT patients. Methadone dose: 54.7 ± 28.1 mg/day. Methadone plasma concentrations 193.07 ± 121.76 ng/ml	*CYP2B6*; *CYP3A4*; *CYP2C19*	Methadone dose was influenced by *CYP2C19* gene dose in patients with negative urine controls but not in those with positive results
Dobrinas et al. [100]	From 276 Caucasian MMT patients, 12 and 35 selected individuals with high (*S*)-methadone plasma exposure and low (*S*)-methadone plasma exposure, respectively	*CYP2B6*	High concentrations of (*S*)-methadone—high levels of rs35303484 Low concentrations of (*S*)-methadone—low levels of rs3745274 and rs2279344; and high levels of rs3211371
			A modest correlation was

Mouly et al. [21]	81 stable patients (85.2% Caucasians; 9.9 Africans; 4.9% Asians) divided into quartiles with respect to the median daily dose	*CYP2B6*; *CYP3A5*; *CYP2C19*; *CYP2D6*	observed between liver/intestinal *CYP3A4* activity and methadone dose at steady state, but none of the genetic polymorphisms showed influence in the methadone dose
Transporter gene (*ABCB1*)			
Coller et al. [115]	60 opioid-dependent subjects in MMT and 60 healthy controls	rs9282564 (A61G); rs2229109 (G1199A); rs1128503 (C1236T); rs2032582 (G2677TorA); rs1045642 (C3435T)	No influence on disease status. *ABCB1* genotype influenced in the methadone dose required: AGCTT genotype associated with lower doses
Crettol et al. [119]	245 Caucasian MMT patients (low-dose R, high-dose R, high-dose NR). Mean methadone dose 134 ± 82 mg (range 3–430)	rs1045642 (C3435T)	Lower plasma concentrations were found in the TT carriers, but no influence on dose or response
Levran et al. [116]	98 MMT patients	rs1045642 (C3435T); rs6949448; rs2235067; rs2032583; rs2032582 (G2677TorA); rs1128503 (C1236T); rs1922242; rs2520464; rs3789243	Patients with TT-TT-TT genotype pattern of rs1045642, rs2032582 and rs1128503 have more chance to require higher doses of methadone
Fonseca et al. [93]	105 Caucasian MMT patients. R 76 dose 109 ± 68; NR 29 dose 72 ± 43	rs1045642 (C3435T)	No association with response, methadone dose, or plasma concentrations
Hung et al. [55]	321 Han Chinese MMT patients (92 low dose; 150 medium dose; 79 high dose)	rs1045642 (C3435T)	Associated with differences in methadone doses
Barratt et al.	119 Caucasian MMT patients	rs9282564 (A61G); rs2229109 (G1199A); rs1128503 (C1236T);	Wild-type *OPRM1* subjects, the carriers of an *ABCB1* variant haplotype required significantly lower doses of methadone than *ABCB1* wild-types Among subjects with the

[54]		rs2032582 (G2677TorA); rs1045642 (C3435T)	most common *ABCB1* haplotype, the 118 A/G genotype was associated with higher methadone plasma concentrations
Lee et al. [102]	178 Taiwanese MMT patients (R 62 and NR 116). Methadone daily dose 50.8 ± 30.5 mg/day (range 5–250)	rs2032582 (G2677TorA)	Methadone maintenance dose, *CYP2B6* 785G allele, and *ABCB1* 2677T allele had positive effects on the methadone plasma concentration
Bart et al. [118]	206 MMT patients (76 Hmong and 130 non-Hmong)	rs2032582 (G2677TorA)	Hmong ethnicity reduced CL/F by approximately 30% and the rs2032582 GG genotype was associated with a 20% reduction in CL/F
Dennis et al., 2014 [103]	Meta-analysis of seven studies		No significant association between the *ABCB1* polymorphism and the trough ~~*(R), (S)*~~ *(R,S)*- plasma concentrations, methadone dose, or methadone response
Zahari et al. [117]	148 MMT males. Methadone dose 72.70 ± 28.25 mg/day (range 20–160)	rs1128503 (C1236T); rs2032582 (G2677TorA); rs1045642 (C3435T)	Association of CGC/TTT diplotype (1236C>T, 2677G>T/A, and 3435C>T) with dose-adjusted serum methadone. Patients with CGC/TTT diplotype had 32.9% higher dose-adjusted serum methadone
Other genes/SNPs			
Yang et al. [120]	344 Han Chinese MMT patients	rs17180299	This SNP would influence the regulation of plasma (*R*)-methadone through epigenetic histone modification of *H3K9me3*. This SNP accounted for 9.541% of the variation in the plasma concentration of the (*R*)-methadone

C/D concentration-to-dosage, *CL/F* apparent total clearance, *CYP* cytochrome P450, *EM* extensive metabolizers, *MMT* methadone maintenance treatment, *NR* non-responders, *PM* poor metabolizers, *R* responders, SNPs single nucleotide polymorphisms, *UM* ultra-rapid metabolizers

3.1. *CYP3A4*

CYP3A4 has been shown to be involved in the formation of 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) [90] in a non-enantioselective way [91]. The activity of this enzyme varies widely between individuals and can be affected by health status and environmental aspects. Genetic variability does not affect the pharmacokinetics of methadone in any significant manner, and most SNPs occur with low allelic frequencies. Crettol et al. [92] conducted an in vivo study and reported that the carriers of the *CYP3A4*1B* (rs2740574) variant presented a 1.4-fold increase for (*S*)-methadone and a 1.1-fold increase for (*R*)-methadone concentrations, although this group was more likely to take lower doses of methadone. A French study assessed the influence of environmental and genetic factors (liver/intestinal CYP3A4 activity and polymorphisms of the *OPRM1*, *DRD2*, catechol-*O*-methyltransferase [*COMT*], *ABCB1*, *CYP2B6*, *CYP3A5*, *CYP2C19*, and *CYP2D6* genes) on methadone doses. Although they observed a modest correlation between liver/intestinal CYP3A4 activity and methadone dose at steady state, none of the genetic polymorphisms was shown to play a role with respect to dose [21]. In addition, a study by Fonseca et al. [93] and an additional analysis by Levran et al. [94] in 74 Israeli patients receiving MMT found no association between *CYP3A4* and methadone doses.

These results indicate that the genetic variability of the *CYP3A4* gene has little influence on MMT, but its activity could be changed by environmental factors such as concomitant medication.

3.2. *CYP2B6*

In vitro and in vivo studies have reported that CYP2B6 is a contributor to methadone metabolism in different ethnic populations, with stereoselectivity towards (*S*)-methadone [95, 96]. Multiple SNPs within the *CYP2B6* gene (19q13.2), with varying prevalence in allele frequency depending on the ethnicity of the population studied, have been described [97]. Individuals carrying the rs3745274 and rs2279343 SNPs (*CYP2B6*6*/**6* homozygous individuals) showed low CYP2B6 protein levels, resulting in changes in (*S*)-methadone plasma concentrations and only weak (*R*)-methadone plasma concentrations [25, 92, 98]. One study including healthy volunteers with different *CYP2B6* genotypes evaluated differences in methadone enantiomers after single doses of oral and intravenous methadone. The metabolism of both enantiomers was reduced in *CYP2B6*6* carriers and increased in *CYP2B6*4*

(rs3745274 and rs2279343) carriers [99].

In terms of the influence of *CYP2B6* gene on the concentrations of (*S*)-methadone, some polymorphisms have been associated with plasma levels of this enantiomer. A subsample of patients receiving methadone treatment underwent a resequencing of the *CYP2B6* gene (12 had a high plasma concentration and 35 low) and were then compared with the rest of the subjects. The authors found a marked prevalence of the rs35303484 (*11; Ac136G; M46V) polymorphism in the high (*S*)-methadone concentration group; the low (*S*)-methadone group exhibited a lower frequency of the rs3745274 (*9; Gc516T; Q172H), rs2279344 (c822 +G183A), and rs8192719 (c1294+ C53T) polymorphism and a high frequency of the rs3211371 (*5; Cc1459T; R487C) polymorphism [100]. Another study [101] evaluated the influence of *CYP2B6* and its interaction with the nuclear receptor subfamily 1 group I member 2 (*PXR*) gene (which regulates the expression of the CYP450s) and showed that the combination could contribute to significant differences in (*S*)-methadone concentrations. When comparing different combinations of rs1464603, rs1464603, and rs2472681 at *PXR* and rs2279345, rs707265, and rs2279345 at *CYP2B6*, a genotype combination was produced that related to lower concentrations of (*S*)-methadone: 146.57 ± 102.47/141.83 ± 98.38/71.06 ± 44.08 ($p = 0.0003$), 139.76 ± 99.01/143.95 ± 99.50/71.06 ± 44.08 ($p = 0.0007$), and 153.72 ± 101.85/138.93 ± 98.19/73.60 ± 53.62 ($p = 0.001$), respectively.

Clinical studies with opioid-dependent subjects receiving MMT had mixed results. Three reported no influence of the *CYP2B6* gene in terms of MMT response [25, 92, 93], whereas another three showed differences in the methadone dose requirements depending on the genotype [55, 94, 102].

One meta-analysis of seven articles reporting data on possible associations between a *CYP2B6*6* polymorphism and methadone dose, plasma concentrations, and MMT outcomes observed that patients homozygous for the *CYP2B6*6* genotype had higher trough (*R*)- and (*S*)-methadone plasma concentrations but found no differences in terms of methadone dose or treatment response [103].

The data currently available indicates that the influence of the *CYP2B6* gene on methadone pharmacology may be related not only to a single polymorphism (*CYP2B6*6*) but also to different interacting effects of multiple polymorphisms and interactions with other genes related to both pharmacokinetics and

pharmacodynamics.

3.3. *CYP2D6*

In vitro studies have also demonstrated a role of the CYP2D6 enzyme in the formation of EDDP from methadone, with a stereoselectivity towards (*R*)-methadone [86, 104]. On the other hand, methadone has been described as an inhibitor of *CYP2D6*, and this should be taken into account in polypharmacy [105]. *CYP2D6* displays strong genetic polymorphism; some are non-functional or have reduced function and others are duplicated, increasing the expression of the protein. Different allele combinations cause a range of phenotypes in terms of metabolism activity: two non-functional alleles lead to a poor metabolizer (PM) phenotype, at least one functional allele leads to an extensive metabolizer (EM) phenotype, and multiple copies of a functional allele lead to an ultra-rapid metabolizer (UM) phenotype [106]. A number of phenotypes have been associated with variations in methadone plasma concentrations: PM subjects had higher methadone plasma concentrations than did EM and UM subjects [92, 107]. These results have not been replicated [108].

Two studies have found some influence of *CYP2D6* on methadone dose requirements. Fonseca et al. [93] described an effect of *CYP2D6* phenotype status on response outcome, with UM subjects requiring higher doses of methadone. Levran et al. [94] observed that a non-coding SNP (rs3892097) was associated with daily methadone dose. Both studies were based on small sample sizes, evaluated more genes, and, although they controlled for multiple testing, could lead to a risk for false-positive results.

3.4. *CYP2C19*

Several studies have evaluated the involvement of the CYP2C19 enzyme in methadone metabolism and its possible stereoselectivity. Although results have generally been controversial, a number of in vitro experiments indicated a minor involvement of CYP2C19 with stereoselectivity towards the (*R*)-enantiomer [85, 87, 91]. Crettol et al. [25, 92] did not demonstrate an association with methadone plasma concentrations, dose requirements, or methadone treatment outcome. Another study evaluated allelic combinations of *CYP450* genetic variants (two from *CYP2C19*, four from *CYP2B6*, and five from *CYP3A4*) in 366 heroin-dependent patients receiving MMT for a possible influence on daily dose or illicit opioid use. The interaction analysis reported some influence of *CYP2C19* on methadone dose, but data were not presented in the paper [96].

The same authors published a second paper evaluating an association between *CYP2C19* metabolising phenotype (based on genotype at rs4244285 and rs4986893) and methadone doses and found that EM subjects consumed higher doses of methadone than intermediate metabolizers and PM (58.78 ± 32.69, 57.64 ± 28.69, and 40.45 ± 22.17 mg/day; $p = 0.004$ and 0.001, respectively) [109]. As with the previous genetic studies, the sample size was quite small and multiple tests were performed. Two different papers were published [96, 109], but the sample characteristics were the same for both and it is not clear whether one was a replication study.

3.5. P-glycoprotein

Methadone is a substrate of P-glycoprotein, which shows weak stereoselectivity towards the (*S*)-enantiomer [110]. The P-glycoprotein is encoded by the multidrug resistance 1 (*ABCB1*) gene (chromosome 7p21). This gene is highly polymorphic, and numerous variants have been associated with drug response [111]. In vitro studies evaluating interactions between P-glycoprotein and methadone have shown that methadone is an inhibitor of the wild-type human protein [112] in a non-competitive way [113]. The same authors observed that different variants of P-glycoprotein presented less inhibition potency, explaining in part the disparities in the amount of methadone required for successful treatment when other substrates of P-glycoprotein are administered [113].

At a clinical level, the majority of studies focused on a non-synonymous SNP: rs1045642 (C3435T). T allele homozygotes showed lower in vivo duodenal protein expression [114]. There is a considerable degree of agreement regarding the influence of *ABCB1* genotypes on methadone plasma concentrations [55, 92, 103, 113, 115, 116, 117], which is not usually related to a single SNP but to specific haplotypes.

One study described differences in (*R*)- and (*S*)-methadone clearance depending on the polymorphisms at rs2032582 and on ethnicity [118]. However, there was no clear association with response to treatment [94, 119]. One pilot study evaluating environmental and genetic factors involved in response to MMT found that, in a sample of 178 patients, the *ABCB1* 2677T allele was associated with both methadone plasma concentrations and MMT outcome (based on self-report and urine controls) [102].

An interesting study by Barratt et al. [54] showed that interactions between polymorphisms in *ABCB1* and *OPRM1* genes could result in differences in

methadone doses required. The authors observed that, among wild-type subjects at A118G SNP, an *ABCB1* variant haplotype group required significantly lower doses of methadone than *ABCB1* wild-type subjects. On the other hand, among those with the most common *ABCB1* haplotype combination, the A118G genotype was associated with higher methadone plasma concentrations [54].

A meta-analysis [103] evaluated studies investigating *ABCB1* and its influence on methadone dose, plasma concentrations, and treatment response and found no association between the *ABCB1* polymorphism and the trough (*R*), (*S*) concentrations, methadone dose, or response.

As P-glycoprotein is an efflux transporter acting at the BBB, genetic variability at this level would affect concentrations at the central nervous system rather than plasma concentrations. In conclusion, genetic variability at the *ABCB1* level may have a minor influence on methadone dose requirements in opioid-dependent subjects, interacting with other factors (pharmacodynamics and pharmacokinetics). However, such variability can be managed at the clinical level by modifying the methadone dose.

3.6. rs 17180299

One study used GWAS to identify the pharmacokinetic determinants of methadone (*R*)- and (*S*)-enantiomer concentrations. A significant SNP, rs17180299, was identified as accounting for 9.5% of the variation in plasma concentrations of the methadone (*R*)-enantiomer [120]. This SNP is located in an intergenic region, but there is a relationship between rs17180299 and heterochromatic histone H3 lysine 9 trimethylation (*H3K9me3*) in the primary T-regulatory cells from peripheral blood [121]. The authors suggested that rs17180299 could influence the regulation of plasma (*R*)-methadone through the epigenetic histone modification of *H3K9me3*. The same study identified 17 haplotypes in spondin 1 (*SPON1*), GSG1-like (*GSG1L*), and *CYP450* genes associated with the plasma concentration of methadone (*S*)-enantiomer.

4. Genetic Variability and Methadone-Related Adverse Events

4.1. Minor Adverse Events

Methadone-related adverse events are similar to those of other opioid agonists and are generally mild and tolerable, the most common being constipation,

sweating, and insomnia [122]. Both pharmacodynamic and pharmacokinetic genes have been studied with respect to the presence of adverse events in MMT (Table 3).

Table 3

Main studies assessing genetic variability and its influence in adverse events in methadone maintenance treatment

Study	Sample characteristics	Gene (SNPs)	Results
Minor adverse events			
Wang et al. [59]	366 Han Chinese MMT patients	*OPRM1*; rs1074287; rs6912029; rs1799971; rs12209447; rs510769; rs3798676; rs553202; rs499796; rs7748401; rs495491; rs10457090; rs589046; rs3778152; rs563649; rs2075572	Changes in libido scores (dominant model) and insomnia scores (recessive model) were associated with rs1074287; rs6912029; rs12209447; rs510769; rs3798676; rs7748401; rs495491; rs10457090; rs589046; rs3778152; rs563649; rs2075572, after adjusting for age, sex, and BMI
Wachman et al. [123]	86 newborns and their mothers (98% White, non-Hispanic)	80 SNPs in 14 candidate genes: *OPRM1*; *OPRD1*; *OPRK1*; *PENK*; *POMC*; *PDYN*; *PNOC*; *OPRL1*; *COMT*; *GAL*; *BDNF*; *SLC6A2*; *SLC6A3*; *SLC6A4*	Carriers of the G allele at *OPRM1* (rs1799971) were at lower risk of experiencing neonatal withdrawal syndrome due to MMT in their mothers
Zahari et al. [124]	165 Malay males in MMT	*OPRM1*; rs1799971; rs2075572	The AC/AG diplotype for the A118G and IVS2 + G691C polymorphisms is associated with better sleep quality
			Six SNPs from

Wang et al. [125]	366 Taiwanese MMT patients	*OPRK1* 17 SNPs	rs7843965 to rs1051660 (intron 2 to exon 2) were significantly associated with body weight. A haplotype of four SNPs, rs7832417-rs16918853-rs702764-rs7817710 (exon 4 to intron 3), was associated with bone or joint aches and with the amount of alcohol use. The haplotype rs10958350-rs7016778-rs12675595 was associated with gooseflesh skin, yawning, and restlessness withdrawal symptoms
Chen et al. [126]	366 Han Chinese MMT patients	*CYP3A4* (rs4646440 and rs224248)	The SNPs were associated with the severity of withdrawal symptoms and methadone adverse effects
Zahari et al. [128]	148 Malay male MMT patients	*CYP2B6* (*6 allele) rs3745274	The *CYP2B6*6* allele was associated with a lower pain threshold and lower pain tolerance
Tian et al. [127]	366 MMT patients in Taiwan	*UGT2B7*; rs6600879; rs6600880; rs4554144; rs11940316; rs7438135; rs7662029; rs7668258; rs7439366; rs4292394; rs6600893	Significant associations with severity of withdrawal symptoms, pupil size and tremor
Serious adverse events			
Wong et al. [130]	21 methadone-related deaths	*CYP 2D6 alleles*; rs35742686 (*CYP2D6 *3*), rs3892097 (*CYP2D6 *4*), whole gene deletion (*CYP2D6 *5*)	The prevalence of poor metabolizers was higher but not significantly different from that of a control group
			CYP2B6: In the group of

Bunten et al. [131, 132]	40 individuals with deaths associated with MMT	*CYP2B6* *6 (rs3745274); *OPRM1* (rs1799971)	slow metabolizers, the post-mortem plasma concentrations of methadone were higher than other genotypes. *OPRM1*: Carriers of the 118GA genotype presented higher benzodiazepine plasma concentrations in the methadone-related deaths, but not in the morphine-related deaths
Richards-Waugh et al. [133]	136 methadone accidental overdoses: 133 involved methadone-only overdoses; 95 combined methadone/benzodiazepine overdoses	*CYP3A4*; rs2246709; rs3735451; rs4646437; rs2242480; rs4987161; rs4986910; rs2740574	Two SNPs: rs2242480 and rs2740574 demonstrated an apparent enrichment within the methadone-only overdose fatalities compared with the control group and the general population
Ahmad et al. [134]	125 Caucasian methadone fatalities	*CYP2B6*; rs2279344; rs3211371; rs3745274; rs4803419; rs8192709; rs8192719; rs12721655; rs35979566	The frequencies of SNPs rs3745274 (*9) and rs8192719 (C21563T) were enhanced in the methadone-only group. Higher blood methadone concentrations were observed in individuals who were genotyped homozygous for SNP rs3211371 (*5) (1.67 ± 0.85) as compared with either the heterozygote (0.52 ± 0.08) or homozygous ancestral genotype (0.59 ± 0.05), $p < 0.05$
Icick et al. [135]	108 Caucasian stable MMT patients	*OPRM1*; rs1799971	The A118G polymorphism was not associated with lifetime suicide attempts. Suicide risk was associated with major depression diagnosis
Hajj et al. [143]	82 stable methadone patients. Methadone dose 57 (range 10–320)	*KCNH2*: rs1805123; *KCNE1*: rs1805127; *KCNE1*: rs2236609	Each copy of a Lys allele at codon 897 of *KCNH2*, the gene that encodes the cardiac potassium voltage-gated channel hERG, was associated with a15.4 ms longer Qtc

Eap et al. [144]	179 Caucasian MMT patients. Methadone dose 145 ± 83 mg/day	*CYP2B6* (*6) rs3745274	The mean QTc was higher in *CYP2B6* slow metabolizers (439 ± 25 ms) than in extensive metabolizers (421 ± 25 ms; $p = 0.017$)
Wang et al. [109]	366 MMT patients in Taiwan	*CYP2C19*; rs4986893; rs4244285	Methadone daily doses of both the extensive metabolizers (58.78 ± 32.69 mg/day; $p = 0.004$) and intermediate metabolizers (57.64 ± 28.69 mg/day; $p = 0.001$) were significantly higher than that of the poor metabolizers (40.45 ± 22.17 mg/day). Poor metabolizers had higher plasma concentrations of both dose-corrected plasma concentrations of (*R*)-methadone ($p = 0.002$) and (*R*)-EDDP ($p = 0.03$) than extensive metabolizers
Carlquist et al. [146]	25 MMT patients: Caucasian (74%), Hispanic (19%), African American (3%), Native American (3%)	*CYP2C19*	Carriers of the *CYP2C19*2* variant presented higher concentrations of plasma EDDP, (*S*)-EDDP, and (*R*)-EDDP ($p = 0.004$). The methadone dose and the plasma EDDP concentration corrected for dose were both significantly associated with QTc

BMI body mass index, *EDDP* 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine, *MMT* methadone maintenance treatment, *SNP* single nucleotide polymorphism

4.1.1. *OPRM1*

Wang et al. [59] reported that 11 SNPs at *OPRM1* were associated with insomnia and changes in libido. In neonates, carriers of the G allele in the A118G SNP were at lower risk of experiencing neonatal withdrawal syndrome due to their mother's MMT treatment [123]. Zahari et al. [124] studied sleep quality and *OPRM1* genetic variability and found an association between a diplotype of two SNPs and sleep quality among males receiving methadone

treatment.

4.1.2. Kappa Opioid Receptor 1 (*OPRK1*)

The kappa opioid receptor has also been associated with minor MMT-related adverse events. Methadone binds to the kappa opioid receptor but with low affinity. From a selection of 17 SNPs in the *OPRK1* gene, six were significantly associated with body weight, a low frequency haplotype (6.1%) of four SNPs—rs7832417-rs16918853-rs702764-rs7817710 (exon 4 to intron 3)—was associated with bone or joint aches, and the haplotype rs10958350-rs7016778-rs12675595 was associated with gooseflesh skin, yawning, and restlessness withdrawal symptoms [125]. This same sample of subjects receiving MMT has been used in other studies [59, 109, 126, 127] also analysing multiple SNPs and genes, which signifies that the results have a high risk of a type I error.

4.1.3. *CYP3A4*

In terms of pharmacokinetics, an association between *CYP3A4* genotype and withdrawal symptoms and methadone-related adverse events was observed in patients receiving MMT [126]. This study evaluated withdrawal syndrome in 366 Han Chinese with the Clinical Opioid Withdrawal Scale (COWS) and found that two SNPs (rs4646440 and rs2242480) showed an association with total scores of the scale. Four SNPs (rs3735451, rs4646440, rs2242480, rs4646437, and rs2246709) were also associated with methadone-related adverse events, mainly sedation.

4.1.4. *CYP2B6*

Hyperalgesia is frequently reported by formerly opioid-dependent patients. The *CYP2B6* gene has been studied to ascertain its possible influence on pain sensitivity in patients receiving MMT. In a set of 148 such patients who were assessed using the cold presser test, the *CYP2B6*6* allele carriers had a shorter cold pain threshold and pain tolerance time than non-carriers [128].

4.1.5. UDP Glucuronosyltransferase Family 2 Member B7 (*UGT2B7*)

Tian et al. [127] also evaluated the genetic basis of withdrawal syndrome severity in patients receiving MMT, focussing on polymorphisms at the *UGT2B7* gene. In urine-positive subjects, ten SNPs were significantly associated with withdrawal symptoms and tremor. Other studies have shown

that methadone is an inhibitor of this gene, presenting cross-tolerance with morphine, although methadone is not a substrate of this gene [129].

4.2. Severe Adverse Events

Two major adverse events are related to methadone: respiratory depression and ventricular arrhythmia [122].

4.2.1. Overdose Risk

Studying the pharmacokinetics of methadone could help describe the different risks for overdose. Wong et al. [130] evaluated a number of *CYP2D6* alleles—rs35742686 (*CYP2D6* *3), rs3892097 (*CYP2D6* *4), and whole gene deletion (*CYP2D6* *5 variant), associated with poor drug metabolism—in 21 deaths involving methadone. The prevalence of PMs was higher but not significantly different from that in the control group, but the small sample size could have decreased statistical power. *CYP2B6* encoding gene was also assessed in the risk of overdose deaths [131, 132]. The authors described an association with *CYP2B6*6*/**6* genotype (PM phenotype): carriers had higher post-mortem plasma concentrations of methadone than did other genotypes. In addition, the same study also detected an association between the A118G SNP at *OPRM1*, and heterozygotes presented higher benzodiazepine plasma concentrations than 118AA carriers (0.69 ± 0.363 vs. 1.66 ± 0.749; $p = 0.004$) in methadone-related but not in morphine-related deaths, indicating a pharmacodynamic–pharmacokinetic interaction.

Two SNPs, rs2242480 and rs2740574, of the main enzyme involved in methadone metabolism, *CYP3A4*, were associated with accidental methadone overdoses; they were found more frequently in those deaths compared with the control group, indicating a possible contribution to the methadone PM phenotype [133]. Finally, a recent study reported an association between the *CYP2B6* SNPs rs3745274 and rs35979566 and methadone overdoses [134].

In many cases of overdose, it is difficult to distinguish between an accidental death and a suicide attempt. In a study examining the risk of intentional overdose in patients stabilized on MMT, the authors found an association with suicide attempts and a diagnosis of major depression but not with differences in the A118G SNP [135].

4.2.2. QTc Prolongation

The prolongation of the QTc interval on electrocardiograms, and cardiac arrhythmias such as Torsades de Pointes, has been extensively studied [136, 137, 138]. Patients receiving methadone treatment with QTc prolongation usually present with other risk factors [139], such as cocaine and alcohol use, cardiotoxic drugs [140], and higher doses of methadone [141]. The mechanisms of QTc prolongation have been related to the inhibition of the cardiac potassium channel hERG induced by methadone, with (*S*)-methadone showing more potent inhibition than (*R*)-methadone [142]. Genetics at this level have also been studied to detect patients susceptible to developing this severe adverse event. One polymorphism at the potassium voltage-gated channel subfamily H member 2 (*KCNH2*, rs1805123 number of 897Lys) encoding for the potassium channel has been associated with longer QTc intervals in patients receiving methadone treatment [143].

Eap et al. [144] found differences in the QTc interval depending on the metabolizer status at *CYP2B6*: PM of *CYP2B6* presented longer QTc intervals than EM. As cardiac adverse events have been related to the (*S*)-enantiomer, the authors compared the administration of (*R,S*)- versus (*R*)-methadone and described a reduction in the QTc interval by substituting (*R,S*)-methadone for the active (*R*)-enantiomer [145]. Moreover, two studies reported that a polymorphism at the *CYP2C19* gene (rs4244285) was associated with QTc interval, methadone doses, (*R*)-methadone/methadone plasma concentration ratio, and EDDP (main methadone metabolite) concentrations [109, 146]. An interesting study [142] evaluated the different contributions of genetic and non-genetic factors to the effects of methadone on QTc changes. Polymorphisms at *CYP2B6*, *ABCB1* influenced the clearance of (*S*)-methadone, as well as levels of α-1 acid glycoprotein (AAG), a protein that also binds methadone in plasma [147]. Patients with doses of methadone > 240 mg/day and a low clearance profile had a higher risk of prolonged QTc (8 vs. 18%). It is also important to take into account that AAG binds plasma methadone, showing differences among the variants of the protein.

5. Conclusions

This review aimed to present a thorough summary of published studies regarding genetic influences on outcomes in MMT. The results presented in this paper show both considerable heterogeneity and contrasting results. The latter could be due to differences in study methods: small sample sizes, lack of control for multiple testing, and the risk of a stratification effect due to varying ethnic

origins. Moreover, factors associated with the characteristics of the subjects and methadone programmes (different definitions of "good response," the presence of and/or therapeutic approach regarding medical and psychiatric comorbidities, and services other than methadone) may have played a role. Accurate phenotypes are imperative in pharmacogenomic studies.

Despite the limitations, we can draw some conclusions from the studies reviewed. *OPRM1* SNP rs1799971 (A118G) alone is not associated with response to methadone treatment but may have an influence via interactions with other genes (mainly involved in pharmacokinetics). In the genes coding for enzymes involved in methadone metabolism, studies have found influences from *CYP2B6*, *CYP2D6*, and *CYP2C19* in methadone dose requirements. It is not possible to confirm a positive or negative association in the rest of the studied genes because of a lack of replication analysis. Methadone-related adverse events (such as sleep disturbance and sexual dysfunction) have been associated with different SNPs at the *OPRM1* gene. Finally, genotypes involving slow methadone metabolism have been involved with a higher risk of QTc enlargement.

Outcomes of MMT are the result of a combination of environmental, drug-induced, and genetic factors. They could be directly related to pharmacodynamic and/or pharmacokinetic factors. The influence of genetic variability in the coding genes involved in the pharmacokinetics of methadone metabolism and transport can be clinically managed, usually by increasing or decreasing methadone doses or splitting the daily dose. A better understanding of pharmacodynamic factors could help to select the best opioid for treatment; for example, the kappa antagonistic effect of buprenorphine would be beneficial in cases of comorbidity with depression [148].

The high variability in methadone metabolism and plasma concentrations and its relationship with serious adverse events, such as QTc prolongation, makes the implementation of regular electrocardiogram monitoring in patients receiving methadone treatment mandatory.

Although pharmacogenetic studies seemed promising years ago, they have not met expectations regarding their application to MMT. When considering therapeutic options for the treatment of opioid addiction, clinicians need information regarding genetic conditioning on methadone metabolism, enantiomer clearance, and the presence of genotypes associated with the risk of

adverse effects. They must also consider the other phenotypic characteristics of their patients.

Compliance with Ethical Standards

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Conflict of interest Dr. Francina Fonseca and Dr. Marta Torrens have no conflicts of interest.

6. Electronic supplementary material

Below is the link to the electronic supplementary material.

Supplementary material 1 (DOCX 25 kb)

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