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ORIGINAL ARTICLE

β-Arrestin2 influences the response to methadone in opioid-dependent patients

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β-Arrestin2 (*ARRB2*) is a component of the G-protein-coupled receptor complex and is involved in μ-opioid and dopamine D_2 receptor signaling, two central processes in methadone signal transduction. We analyzed 238 patients in methadone maintenance treatment (MMT) and identified a haplotype block (rs34230287, rs3786047, rs1045280 and rs2036657) spanning almost the entire *ARRB2* locus. Although none of these single nucleotide polymorphisms (SNPs) leads to a change in amino-acid sequence, we found that for all the SNPs analyzed, with exception of rs34230287, homozygosity for the variant allele confers a nonresponding phenotype ($n = 73$; rs1045280C and rs2036657G: OR = 3.1, 95% CI = 1.5–6.3, $P = 0.004$; rs3786047A: OR = 2.5, 95% CI = 1.2–5.1, $P = 0.02$) also illustrated by a 12-fold shorter period of negative urine screening ($P = 0.01$). The *ARRB2* genotype may thus contribute to the interindividual variability in the response to MMT and help to predict response to treatment. *The Pharmacogenomics Journal* (2011) **11,** 258–266; doi:10.1038/tpj.2010.37; published online 1 June 2010

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Introduction

Opioid dependence (OD) is associated with serious medical, legal, social and psychiatric problems. It is treated worldwide generally with methadone, a synthetic μ-opioid receptor agonist, in the form of methadone maintenance treatment (MMT).[1,2] Successful treatment blocks the effects of opioids, reduces drug craving, and prevents relapses and adverse reactions.[3] However, a large interindividual variability in drug response has been observed and those who remain in treatment show only 60–70% success rates.[4,5] The response to a drug may depend on environmental and/or genetic factors. Thus, genetic variations in genes controlling the pharmacokinetics (for example, drug absorption, distribution, metabolism and elimination) and/or the pharmacodynamics (for example, interactions with receptors and signal transduction) may potentially influence the response to MMT. Most of the studies that have examined the genetic influence on response to methadone analyzed pharmacokinetic factors, such as isoforms of the cytochrome P450 family (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5). Although CYP isoforms are important in methadone metabolism (mainly CYP2B6 and CYP3A4), no or only weak influences were found on the therapeutic response and/or the dose requirement.[6–8] Conversely, the CYP2B6 slow metabolizer genotype, which is associated with a reduced ability to metabolize the more cardiotoxic (*S*)-methadone,[6,7] is associated with an increased risk of prolonged QTc interval.[9] Methadone is also a substrate of the permeability glycoprotein (P-gp), encoded by the *ABCB1* gene.[10]

Genetic polymorphisms of *ABCB1* were found to influence methadone trough plasma levels weakly, whereas contradictory results were reported on its influence on the dose requirement.[7,11–13]

An association of the ankyrin repeat and kinase domain containing 1 (*ANKK1*) variant (TaqI A_1) with nonresponse to MMT was also suggested in one study,[14] but not confirmed in two others.[15,16] Till now, few studies have been published examining pharmacodynamic factors. The μ-opioid receptor (encoded by the *OPRM1* gene), is a G-protein-coupled receptor and is the primary site of action of methadone.[17,18] A previous study has reported an association of the *OPRM1* rs1799971 (118A>G) single nucleotide polymorphism (SNP) with a decreased potency of methadone.[19] Consequently, the rs1799971G allele would have been expected to characterize the MMT nonresponder group, a hypothesis that was not confirmed in our previous report where the *OPRM1* rs1799971 polymorphism was analyzed, and no influence on response to methadone was observed.[16] However, in that study we observed a trend toward a shorter self-estimation of methadone holding time for patients with the rs1799971GG genotype,[16] which is in line with the hypothesis that the rs1799971G allele leads to a decreased potency of methadone. It can be tentatively hypothesized that, although the *OPRM1* rs1799971A>G SNP might lead to some changes of methadone potency, the effect is of small amplitude and does not, or only marginally, affect the overall response to treatment.[16]

A potential role for the *DRD1* gene in addictive behavior was previously reported,[20–22] but the nonassociation between the *DRD1* DdeI genotype and response to MMT or heroin dependence did not support this role.[16] The dopamine D2 receptor (*DRD2*), another member of the G-protein-coupled receptor protein family, is significant in the rewarding effects of drug abuse.[23] In a previous report, we showed that the *DRD2* rs6277 (957C>T) SNP, which was found to be in linkage disequilibrium (LD) with the TaqI A variant,[24] is significantly associated with MMT outcome, with rs6277CC carriers being more frequently nonresponders (odds ratio (OR) = 2.4, $P = 0.02$).[16] In addition, the association of the newly identified *DRD2* rs6275 (939C>T) variant and the dose requirement has been recently reported.[25] A recent *in vitro* study suggested that the *DRD2* rs6277T allele alters the predicted mRNA folding, leading to a decrease in mRNA stability and a dramatic change in the dopamine-induced upregulation of *DRD2* expression.[24] However, there is presently a discrepancy between the *in vitro* and *in vivo* data as a recent study suggested that, *in vivo*, the rs6277TT carriers have the highest DRD2 binding potential.[26] *DRD2* variants are, however, unlikely to be the sole genetic modulator in the course of methadone therapy.

The number and morphology of receptors, together with variations in multiple downstream targets, can also potentially influence the response to treatment. The β-arrestin2 gene (*ARRB2*) spans approximately 11 kb of genomic DNA and consists of 14 exons. It is expressed in multiple tissues and organs, with especially high expression level in the brain. It regulates opioid signal transduction through promotion of receptor desensitization and internalization.[27–29] An association of the rs3786047, rs1045280, rs2271167 and rs2036657 SNPs in *ARRB2* with clinical response to morphine, another μ-opioid receptor agonist, was recently reported in cancer patients.[30] Apart from its classical function in receptor desensitization, *ARRB2* also functions as a signaling intermediate through a kinase/phosphatase scaffold, in response to dopamine receptor activation. This implicates *ARRB2* as a positive mediator of dopaminergic synaptic transmission.[31] In previous studies, *ARRB2* was found to be significantly associated with nicotine dependence, methamphetamine use disorder in a Japanese population and ethanol consumption in rats.[32–34] In the latter study, a novel variant associated with higher *ARRB2* transcript levels that confers the high-ethanol-preferring phenotype was observed.[32]

The aim of this study was to analyze *ARRB2* as a new candidate gene influencing methadone pharmacodynamics during MMT. We compared the association of alleles and genotypes of four SNPs (one synonymous and three in the noncoding region) spanning the *ARRB2* locus between MMT responders and nonresponders, and between patients requiring high and low methadone maintenance doses. Finally, as a secondary aim, the allele and genotype frequencies of the *ARRB2* gene were compared between the group of MMT patients and a control group of subjects screened for psychiatric disorders and substance dependence, to assess its role as a vulnerability gene to OD.

Materials and methods

Study design and patients

This is a retrospective cross-sectional study on a cohort of patients (278 included in total) described previously, for which the effects of dopamine D1, D2, μ-opioid and δ-opioid receptor on MMT response were analyzed.[16] In addition, the same cohort or part of it was previously used and described in other reports that showed the effect of *CYP2B6*, of other CYP isoforms, and of *ABCB1* genotypes on QT interval, methadone plasma concentrations and response to treatment.[6,7,9] For this study on the response to treatment (in contrast to the pharmacokinetics part), 40 patients had to be excluded for the following reasons: 10 patients were not Caucasian, 6 had a methadone dose out of the inclusion criteria, 23 had not had weekly urine checking and 1 for whom we could not obtain blood for genotyping. Thus, 238 heroin-dependent Caucasians (diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) criteria 304.02) on MMT were selected for this study. They were recruited in five methadone-dispensing centers in Geneva (Fondation Phenix, $n = 121$; Division d'Abus de Substances HUG, $n = 33$), Lausanne (Centre Saint-Martin, $n = 46$), Montreux (Unité Ambulatoire Spécialisée, $n = 23$) and Bern (Universitäre Psychiatrische Dienste, Integrierter Drogendienst, $n = 15$), Switzerland. The patients had been on MMT for a median duration of 43 months

(range: 2–294 months). Included patients were classified into three groups, that is, low-dose responders, high-dose responders and high-dose nonresponders, according to the following inclusion criteria. The first group of patients were responders to treatment, with methadone daily doses between 40 and 80 mg (low-dose responders, $n=87$). The second group of patients were responders to treatment requiring doses equal or superior to 120 mg per day (high-dose responders, $n=78$). The third group of patients were nonresponders (daily doses superior to 120 mg, $n=73$). Response to treatment was defined by a nonconsumption of heroin or cocaine, an absence of complaints of withdrawal symptoms, and a steady and regular attendance of the therapeutic program. Nonconsumption was based on the self-report of the patient and confirmed by the absence of positive urines for opiate or cocaine over at least 3 months before inclusion with random weekly urine checking. Nonresponse to treatment was defined by a regular consumption (at least once every 2 days) of opiate and/or cocaine based on the self-report of the patient, and confirmed by the presence of at least one positive urine for opiate or cocaine over the 3 months before inclusion. It must be mentioned that the proportion of nonresponders in this study does not reflect the proportion of nonresponders in the five centers where this study was conducted.

Among the 165 responders, 9 patients had one positive urine screening over the last 3 months before inclusion, whereas none of them had had a positive urine screening over the last month. All patients had been on MMT for more than 3 months except for four patients (three low-dose responders and one high-dose nonresponder) who had only been on MMT for 2 months at the time of inclusion (the three responders did not have a positive urine screening during the 2-month period before inclusion in the study). Among the included patients, 15 received doses of methadone outside the above-mentioned range at the time of inclusion (three of them, all responders, had methadone doses below 20 mg per day, that is, 3, 10 and 18 mg per day), mainly because they were in the process of reducing their methadone dose to stop the MMT. Such patients were included because they had fulfilled the inclusion criteria previously. Importantly, methadone plasma levels might be influenced by environmental factors including concomitant medications known to influence the main CYP isozymes involved in methadone metabolism, such as CYP3A4, CYP2B6, and to a lower extent, CYP2D6. The co-medications inducing CYP3A4 were considered as an exclusion criteria, whereas the co-medications inhibiting CYP3A4, CYP2B6 and CYP2D6 were not considered as exclusion criteria but were taken into account in statistical analyses.[35–40] Drugs which could inhibit methadone metabolism taken by patients in our cohort are: sertraline ($n=4$), paroxetine ($n=9$), levothyroxine ($n=3$), nefazodone ($n=2$) fluoxetine ($n=9$), levomepromazine ($n=4$), fluvoxamine ($n=4$), valproate ($n=4$), ethinylestradiol ($n=2$), venlafaxine ($n=5$), haloperidol ($n=1$) and thioridazine ($n=4$). Patient's characteristics, such as co-medications, and the genotype and phenotype influencing methadone kinetics and dynamics extracted from previous reports, possibly relevant for the present one, are reported in Table 1.[7,9]

Table 1 Detailed description of the 238 included patients

	Number	*Percentage*
Self-declaration		
Opiate consumption	34	14
Cocaine consumption	64	27
Cannabis consumption	146	61
Alcohol consumption[a]	80	34
Benzodiazepine consumption[b]	154	65
Amphetamine consumption	7	3
Patients taking concomitant medications[c]	187	79
Benzodiazepines	118	50
Antiepileptics	44	18
Antipsychotics	51	21
Nonselective serotonin reuptake inhibitor antidepressants	42	18
Selective serotonin reuptake inhibitors	47	20
Sedative hypnotics	40	17
CYP2B6 genotype[d]		
SM	78	33
EM	160	67
CYP3A4 genotype[d]		
CYP3A4*1/*1	222	94
CYP3A4*1/*1B	16	6
DRD2 rs6277 genotype[e]		
TT	83	35
CT	117	49
CC	38	16

Abbreviations: SM, slow metabolizer, presence of *6 allele; EM, extensive metabolizer, absence of *6 allele.
[a]Superior to 40 g per day for men and 20 g per day for women.
[b]Regardless of whether or not prescribed.
[c]Mean number of concomitant medications: 2 ± 1.7 per patients, range: 0–7.
[d]Genotyping data from a previous analysis.[7]
[e]Genotyping data from a previous analysis.[16]

The 217 subjects of the control group were Caucasians with no lifetime history of psychotic or mood disorders, alcohol or heroin dependence according to DSM-IV. These controls were recruited among orthopedic inpatients and outpatients at the University hospitals in Lausanne and Geneva. The ethics committees of the corresponding centers (Lausanne, Geneva and Bern) approved the study and written informed consent was obtained from all participants.

DNA extraction, SNPs selection and genotyping

To select the SNPs in the *ARRB2* gene, we used the dbSNP databases of the NCBI and the International HapMap project. Four SNPs (rs34230287 in the promoter, rs3786047 in intron 1, rs1045280 in exon 11 and rs2036657 in the 3′

Table 2 Selected *ARRB2* SNPs, relative position and frequency

dbSNP ID	*SNP location*	*Alleles (NT_010718)*	*MAF (NCBI)*
rs34230287	Promoter	−159C>T	0.14
rs3786047	Intron 1	1082G>A	0.29
rs1045280	Exon 11	8622T>C	0.42
rs2036657	3′ UTR	111436A>G	0.30

Abbreviations: *db*, NCBI reference ID; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

UTR) were selected on the basis of high heterozygosity (minor allele frequency >0.14), uniform coverage of the gene and previous relevant reports (that is, functionality and disease-association) (Table 2).[30,33,34,41,42] All the SNPs were genotyped by real-time polymerase chain reaction (PCR) with 5′-nuclease allele discrimination assays (ABI PRISM 7000 Sequence Detection System; Applied Biosystems, Rotkreuz, Switzerland) according to the manufacturer's protocol (assay ID: C__60483877_10, C__27500850_10, C__8718195_20 and C__11954713_10 for the SNPs –159C>T, 1081G>A, 8622T>C and 111436A>G, respectively). To ensure the quality of the genotyping, consistent results were required for 9 control samples added to each 96-well reaction plate, previously genotyped by direct sequencing (Supplementary Materials).

Quantification of methadone enantiomers

The plasma concentration of (*R*)- and (*S*)-methadone were measured by liquid chromatography coupled with mass spectrometry, as described previously.[6]

Statistical analysis

Measured (*R*)- and (*S*)-methadone plasma levels were corrected by methadone daily dose (in mg per day). The χ^2-test with OR was used to compare allele and genotype frequencies across the three groups, between the responder and nonresponder groups and between the high- and low-dose groups. The Mann–Whitney *U*-test or the Kruskal–Wallis test were used to determine group differences in methadone plasma level, methadone daily dose, MMT duration, duration since the last positive urine screening and patients' estimation of methadone holding time. Logistic regression models were used to analyze the combined influence of *CYP2B6*, *CYP3A4*, *DRD2* and *ARRB2* on clinical response considered as a binary outcome. Pairwise LD between *ARRB2* SNPs was assessed using the Haploview (4.1; Cambridge, MA, USA) program with the option of determining LD blocks according to the criteria defined by Gabriel *et al*.[43] All tests were two sided and a *P*-value less than or equal to 0.05 was considered statistically significant. Correction for multiple testing was not applied as none of the analyzed *ARRB2* markers is independent ($D'>0.95$).[44,45] All analyses were performed with the STATA software (version 10.0; StataCorp, College Station, TX, USA).

Results

We included 238 Caucasian patients on MMT (184 men, 54 women). Of these, 56 patients were treated with drugs which could inhibit methadone metabolism. However, as no differences ($P>0.6$) were observed in (*R*)-, (*S*)-, or (*R*,*S*)-methadone plasma levels at trough or peak between patients with or without medications influencing methadone metabolism, we carried out the subsequent analysis on the whole cohort. The geometric means of (*R*)-, (*S*)- and (*R*,*S*)-methadone trough plasma levels in patients with and without medications influencing methadone metabolism were: 1.9 ng ml^{-1} mg (range: 0.6–3.9) versus 2.0 ng ml^{-1} mg (range: 0.4–12.8), $P=0.8$; 1.7 ng ml^{-1} mg (range 0.3–4.3) versus 1.8 ng ml^{-1} mg (range 0.1–15.0), $P=0.7$; 3.5 ng ml^{-1} mg (range: 0.9–8.3) versus 3.8 ng ml^{-1} mg (range: 0.5–27.7), $P=0.7$, respectively. At peak, the geometric means of (*R*)-, (*S*)- and (*R*,*S*)-methadone plasma levels in patients with and without medications influencing methadone metabolism were: 2.8 ng ml^{-1} mg (range: 1.0–6.0) versus 2.8 ng ml^{-1} mg (range: 1.1–5.9), $P=0.9$; 2.9 ng ml^{-1} mg (range: 0.7–6.5) versus 3.0 ng ml^{-1} mg (range: 0.7–7.7), $P=0.6$; 5.7 ng ml^{-1} mg (range: 1.6–10.9) versus 5.8 ng ml^{-1} mg (range: 1.9–12.7), $P=0.8$, respectively.

The median age of the patients was 35 years (range: 21–54 years) and 222 patients were smokers (93%). The median methadone daily dose was 125 mg (range: 3–430 mg per day); 97 patients (37%) were low-dose responders, 78 (33%) high-dose responders and 73 (31%) were nonresponders. At trough and at peak, the methadone plasma levels were significantly lower in the nonresponder group, confirming the results of a previous study with a slightly smaller subset of patients.[6] The geometric means of methadone trough plasma levels in low-dose responders, high-dose responders and nonresponders were, respectively: (*R*)-methadone: 2.4 ng ml^{-1} mg (range: 1.0–12.8), 1.8 ng ml^{-1} mg (range: 0.4–3.9) and 1.6 ng ml^{-1} mg (range: 0.6–3.6), $P=0.0001$; (*S*)-methadone: 2.4 ng ml^{-1} mg (range: 0.7–15.0), 1.5 ng ml^{-1} mg (range: 0.1–4.3) and 1.3 ng ml^{-1} mg (range: 0.2–3.7), $P=0.0001$; (*R*,*S*)-methadone: 4.9 ng ml^{-1} mg (range: 1.7–27.7), 3.2 ng ml^{-1} mg (range: 0.5–8.3) and 2.9 ng ml^{-1} mg (range: 1.0–7.1), $P=0.0001$. At peak, they were, respectively: (*R*)-methadone: 3.1 ng ml^{-1} mg (range: 1.6–6.0), 2.6 ng ml^{-1} mg (range: 1.1–5.9) and 2.6 ng ml^{-1} mg (range: 1.1–5.0), $P=0.003$; (*S*)-methadone: 3.6 ng ml^{-1} mg (range: 1.2–7.7), 2.5 ng ml^{-1} mg (range: 0.8–6.3) and 2.8 ng ml^{-1} mg (range: 0.7–5.1), $P=0.0001$; (*R*,*S*)-methadone: 6.7 ng ml^{-1} mg (range: 2.9–12.7), 5.1 ng ml^{-1} mg (range: 2.0–12.2) and 5.5 ng ml^{-1} mg (range: 2.0–9.7), $P=0.0002$.

More information on these three groups, including demographics, MMT duration, methadone daily dose, consumption of other drugs and duration since the last positive urine screening for opiate or cocaine, has been reported elsewhere.[16] In addition, 217 subjects were included as a control group (110 men, 107 women; median age: 43 years, range: 17–76 years).

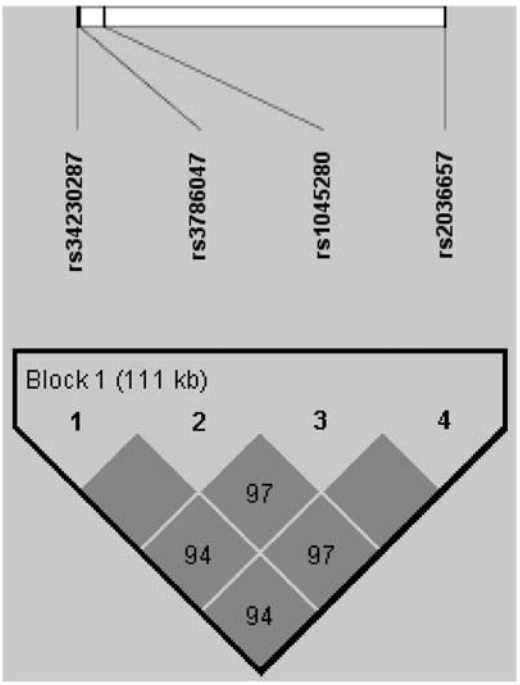

Figure 1 The haplotype block spanning four SNPs on the *ARRB2* gene from the promoter to the 3′ UTR. Values in the squares are *D*′ values and represent the linkage disequilibrium between the SNPs.

Table 3 Response to MMT according to the *ARRB2* genotypes in 238 MMT patients (percentage in brackets)

Genotype	*Response to MMT*		P-*value additive model*	P-*value recessive model*
	Responder	*Nonresponder*		
rs34230287				
CC	93 (56)	40 (55)		
CT	63 (38)	27 (37)		
TT	9 (5)	6 (8)	0.7	0.4
rs37866047				
GG	77 (47)	35 (48)		
GA	70 (42)	21 (29)		
AA	18 (11)	17 (23)	0.02	0.01
rs1045280				
TT	78 (47)	34 (47)		
TC	71 (43)	21 (29)		
CC	16 (10)	18 (25)	0.005	0.002
rs2036657				
AA	78 (47)	34 (47)		
AG	71 (43)	21 (29)		
GG	16 (10)	18 (25)	0.005	0.002

Additive model (grouped by three genotypes) and recessive model (grouped by the presence of the dominant allele vs recessive allele).

LD among SNPs

We analyzed four polymorphisms across the *ARRB2* gene in all patients according to their response to treatment and methadone dosing. Importantly, LD analysis revealed an obvious consistency of the genotypes between the four SNPs, by detecting almost complete LD ($D' > 0.94$, $r^2 > 0.59$) (Figure 1).

ARRB2 *and MMT response*

In the MMT population cohort, the observed allele frequencies were 34% for rs3786047A, rs1045280C and rs2036657G, and 26% for rs34230287T, respectively. Deviation from Hardy–Weinberg equilibrium was observed for the SNPs rs3786047 ($P = 0.03$), rs1045280 ($P = 0.04$) and rs2036657 ($P = 0.04$) in the MMT population cohort, but was not observed in 217 control subjects ($P > 0.5$). The deviation from Hardy–Weinberg in patients but not in controls suggests that these SNPs are associated with undergoing MMT. No deviation from Hardy–Weinberg equilibrium was observed for the SNP rs34230287 ($P > 0.5$).

The SNPs rs3786047, rs1045280 and rs2036657 were significantly associated with response to treatment ($P < 0.02$; Table 3), contributing to 3% of MMT response variability. The frequency of the rs1045280 and rs2036657 carriers homozygous for the variant allele was 25% in nonresponders ($n = 73$) and 10% in responders ($n = 165$, $\chi^2 = 9.3$, $P = 0.002$; Table 3), whereas it was 23 and 11% for the rs3786047 SNP in nonresponders and responders, respectively ($\chi^2 = 6.2$, $P = 0.01$; Table 3). The increased risk of nonresponse to MMT for the variants rs1045280C and rs2036657G can be expressed by an OR of 3.1 (95% CI = 1.5–6.3, $P = 0.004$), and by an OR of 2.5 (95% CI = 1.2–5.1, $P = 0.02$) for the variant rs3786047A. In line with those results, the rs3786047AA, rs1045280CC and rs2036657GG carriers had a significantly shorter duration since the last positive urine screening for opiate or cocaine, as a measurement of nonresponse (median duration for SNPs rs1045280 and rs2036657: homozygous recessive 0.5, heterozygous 7, wild type 5 months, $P = 0.01$; median duration for SNP rs3786047: homozygous recessive 2, heterozygous 7, wild type 5 months; $P = 0.04$). However, these variants do not seem to modulate the course of the therapy in terms of dose requirement ($P > 0.3$; data not shown), duration of MMT ($P > 0.3$; data not shown) and patients' estimation of methadone holding time ($P > 0.5$; data not shown).

No significant difference was observed in rs34230287 genotype frequencies when comparing the three inclusion groups ($P = 0.8$), the responder and nonresponder groups ($P = 0.7$; Table 3) and the high- and low-dose groups ($P = 0.7$). Moreover, the results remained nonsignificant when a recessive model of inheritance was considered ($P = 0.4$; Table 3). The duration since the last positive urine screening for opiate or cocaine was not different according to rs34230287 genotype or allele ($P = 0.4$; data not shown), as well as the median methadone daily dose ($P = 0.5$; data not shown), the duration of MMT ($P = 0.3$; data not shown) and the patients' estimation of methadone holding time ($P = 0.3$; data not shown).

Multivariate analysis combining previous results with ARRB2 *genotype*

In vivo, CYP3A4 and CYP2B6 are the major CYP isoforms involved in methadone metabolism.[7,9] Thus, a multivariate analysis considering the two isoforms was conducted along with the *ARRB2* genotypes. Being CYP2B6 extensive (CYP2B6*6 noncarrier) or slow metabolizer (CYP2B6*6 carrier) and/or CYP3A*1B carrier or noncarrier did not improve significantly the logistic regression model for clinical response ($P>0.2$; data not shown). In a previous study we found that *DRD2* rs6277CC is significantly associated with nonresponse to MMT (OR = 2.4, $r^2=2\%$, $P=0.02$),[16] we therefore analyzed the combined effect of this SNP along with the *ARRB2* SNPs using logistic regression. *ARRB2* rs3786047, rs1045280 and rs2036657, but not rs34230287 SNPs significantly improved logistic regression models ($r^2=6\%$, $P=0.001$, 0.0005, 0.0005, 0.08, respectively) for the clinical response, pointing toward a combined effect on the MMT modulation by gene variants in *DRD2* and *ARRB2*.

Opioid addiction

In the control group, the observed allele frequencies were 30% for rs3786047A, rs1045280C and rs2036657G, and 22% for rs34230287T. For all the SNPs analyzed, the allele and genotype frequencies were not significantly different between the MMT patients and the control group ($P\geqslant0.08$) (Table 4).

Table 4 Opioid addiction according to the *ARRB2* genotypes in 238 MMT patients (yes) versus 217 controls (no)

Genotype	*Opioid addiction*		P-*value additive model*	P-*value recessive model*
	No (%)	*Yes (%)*		
rs34230287				
CC	135 (62)	133 (56)		
CT	70 (32)	90 (38)		
TT	12 (6)	15 (6)	0.4	0.8
rs37866047				
GG	105 (48)	112 (47)		
GA	92 (42)	91 (38)		
AA	20 (9)	35 (15)	0.2	0.08
rs1045280				
TT	105 (48)	112 (47)		
TC	92 (42)	92 (39)		
CC	20 (9)	34 (14)	0.2	0.1
rs2036657				
AA	105 (48)	112 (47)		
AG	93 (42)	92 (39)		
GG	19 (9)	34 (14)	0.2	0.08

Additive model (grouped by three genotypes) and recessive model (grouped by the presence of the dominant allele vs recessive allele).

Discussion

ARRB2 is an intracellular protein, which can regulate the number of functional receptors expressed on the cell surface at a given time. The μ-opioid and the dopamine D2 receptors, both of which mediate rewarding properties of common drugs of abuse, are regulated by *ARRB2*.[31,46–48] In addition, *ARRB2*-mediated kinase/phosphatase scaffolding of serine/threonine kinase (Akt) and protein phosphatase 2A is responsible for the regulation of Akt by dopamine receptors. As such, *ARRB2* provides an alternative pathway by which D2 class receptor activation leads to the expression of dopamine-associated behaviors.[31] Recently, it has been reported that *ARRB2*$^{-/-}$ and *ARRB2*$^{+/-}$ mutant rats consumed significantly less and showed less preference for ethanol than their wild-type littermate control counterparts.[32]

We hypothesized that genetic variations in the *ARRB2* gene might be implicated in the rewarding pathway during MMT and thus influence response to treatment and/or methadone dose requirements. By analyzing four SNPs in *ARRB2*, we identified a haplotype block spanning almost the entire *ARRB2* locus, a feature conserved in rats, for which a haplotype spanning the entire *ARRB2* locus was also described and reported to confer an ethanol-preferring phenotype.[32] In a previous study on *ARRB2* and nicotine dependence, the haplotype block spanning the SNPs rs3786047 and rs1045280 was found in the European American population, but not in African Americans, indicating that haplotype specificity exists across ethnicity.[33] For all SNPs analyzed, with the exception of rs34230287, the risk of being nonresponders during MMT for homozygous carriers of the variant allele was increased up to threefold. Furthermore, an up to 12-fold shorter duration since the last positive urine screening was observed for homozygous carriers of the variant allele. In addition, for the same SNPs, deviation from the Hardy–Weinberg equilibrium in the MMT population was found, but not in the control population, which suggests an association of those SNPs with undergoing MMT.[49] This is due to the fact that in genomic regions associated with the trait of interest, Hardy–Weinberg equilibrium is expected to be distorted in cases.[50,51] Thus, given the careful quality control procedure for genotyping, we are confident that deviation from Hardy–Weinberg equilibrium in our patient cohort was not due to genotyping errors. However, we cannot exclude that such deviation was due to failure of one of the assumptions underlying Hardy–Weinberg expectations (for example, random mating, no selection, no migration and so on).

To date, rs34230287 is the only polymorphism that has been functionally tested and shown to modulate *ARRB2* gene transcription.[41] No functional studies have been conducted for the other four SNPs. Moreover, none of them leads to a change in the amino-acid sequence. Hence, we can only speculate that they might affect expression level rather than function. However, we cannot exclude the possibility that the *ARRB2* locus contains causal SNPs linked

to the SNPs analyzed in this study. In any of those scenarios, either a loss- or a gain-of-function might be postulated to explain the effect of the causal variants. Because mice lacking *ARRB2* showed enhanced reward due to impaired desensitization of the μ-opioid receptors,[46,48] gain-of-function mutations might lead to enhanced desensitization, impaired reward and, thus, confer the nonresponding phenotype observed in our study. Conversely, a loss of function might also explain the nonresponding phenotype observed in *ARRB2*-mutated patients. Indeed, a previous report revealed that dopamine-mediated locomotor responses to drugs are reduced in *ARRB2* knockout mice.[28] This finding was substantiated by the observation of response reduction to amphetamine and apomorphine and elevated dopamine neurotransmission in the absence of *ARRB2*.[31] Such remarkable differences as a consequence of deficiency in molecules associated with G-protein-coupled receptor desensitization highlight the complex role of the *ARRB2* molecule in the regulation of G-protein-coupled receptor signaling.[31] One possible explanation is that some receptors are negatively regulated by *ARRB2* through receptor desensitization/internalization, whereas others use this same molecule as a mediator of positive signaling.[31] Further studies will undoubtedly be needed to delineate the role of these different pathways associated with dysregulation of dopaminergic neurotransmission.

Interestingly, an association of the rs3786047, rs1045280, rs2271167 and rs2036657 *ARRB2* SNPs with the clinical response to morphine was observed in cancer patients.[30] However, the nonresponders were more likely to carry the common allele.[30] In addition, the deletion of *ARRB2* in mice potentiates and extends morphine analgesia due to impaired receptor desensitization,[47,48] an effect partially balanced by local overexpression of *ARRB2* in brain regions involved in pain control.[52] Therefore, in contrast to what was observed in the reward pathway, *ARRB2* expression and function appears to be negatively correlated with analgesia. Thus, the opioid receptors in brain regions contributing to reward are not necessarily regulated in the same way as in the pain-processing center.

DRD2 has been found to modulate response to MMT,[16,25] whereas contradicting results have been reported for other genes. In this study, we show for the first time that not only the receptor but also the pathway upstream might mediate MMT. Finally, on the basis of the finding of an association of *ARRB2* with methamphetamine use disorder in a Japanese population,[34] we compared the genotype frequencies of specific SNPs in *ARRB2* between the MMT patients and a control group, screened for psychiatric disorders and substance dependence. None of the analyzed SNPs was found to be associated with OD.

A limitation of this study is that no patients with methadone doses between 81 and 119 mg per day were included, and the generalization of the present findings to such patients remains to be shown. However, due to the large interindividual variability in the metabolism of methadone, very variable plasma levels of methadone are measured for the same dose.[2] In this study, as expected, a broad and uniform range of methadone plasma levels were measured, with no apparent gap in particular values.[6,7,9] It appears therefore unlikely that the absence of inclusion of patients with methadone doses between 81 to 119 mg per day could have influenced the present results.

In summary, to the best of our knowledge, this is the first report that finds an association between *ARRB2* gene variants and differential response to opioid substitution therapy in addicted patients. On the basis of the present and on previous findings with other genes, it might be possible to better predict individual sensitivity to methadone, and thus to determine the most effective treatment for each individual patient. This is of utmost importance in personalized therapy approaches and might significantly help to avoid under-, as well as overtreatment. However, further studies are needed to replicate these results in independent cohorts, to determine the functional significance of the *ARRB2* genetic variants and to explore their significance in populations of different ethnicities.

Conflict of interest

The authors declare no conflict of interest.

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