Published in final edited form as:
Addict Biol. 2013 July ; 18(4): 709–716. doi:10.1111/j.1369-1600.2011.00349.x.

CYP2B6 SNPs are associated with methadone dose required for effective treatment of opioid addiction

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Abstract

Adequate methadone dosing in methadone maintenance treatment (MMT) for opioid addiction is critical for therapeutic success. One of the challenges in dose determination is the inter-individual variability in dose response. Methadone metabolism is attributed primarily to cytochrome P450 enzymes CYP3A4, CYP2B6, and CYP2D6. The *CYP2B6*6* allele [SNPs 785A>G (rs2279343) and 516G>T (rs3745274)] was associated with slow methadone metabolism. To explore the effects of *CYP2B6*6* allele on methadone dose requirement, it was genotyped in a well-characterized sample of 74 Israeli former heroin addicts in MMT. The sample is primarily of Middle Eastern/European ancestry, based on ancestry informative markers (AIMs). Only patients with no major co-medication that may affect methadone metabolism were included. The stabilizing daily methadone dose in this sample ranges between 13-260 mg (mean 140±52 mg). The mean methadone doses required by subjects homozygous for the variant alleles of the *CYP2B6* SNPs 785A>G and 516G>T (88, 96 mg, respectively) were significantly lower than those of the heterozygotes (133, 129 mg, respectively) and the non-carriers (150, 151 mg, respectively) (nominal $P = 0.012$, 0.048, respectively). The results remain significant after controlling for age, sex and the *ABCB1* SNP 1236C>T (rs1128503), that was previously shown to be associated with high methadone dose requirement in this population ($P = 0.006$, 0.030, respectively). An additional 77 *CYP2B6, CYP3A4* and *CYP2D6* SNPs were genotyped. Of these, 24 SNPs were polymorphic and none showed significant association with methadone dose. Further studies are necessary to replicate these preliminary findings in additional subjects and other populations.

Keywords

CYP2B6; Israel; methadone; opioid addiction; pharmacogenomics

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Conflict of interest None declared.

Authorship Contribution:
OL and MJK were responsible for the study concept and design. EP and MA are responsible for the clinical data. MR performed the DNA preparation and sequencing analysis. SH performed the statistical analysis. OL was responsible for data analysis and interpretation of findings. OL wrote the manuscript. All authors critically reviewed content and approved final version for publication.

INTRODUCTION

Methadone is the main pharmacotherapy of opiate addiction and a second-line opioid therapy for pain after morphine (Kreek et al., 2002). It is estimated that about a million people are currently in methadone maintenance treatment (MMT) worldwide. Successful treatment that prevents opiate use, withdrawal and craving relies in part on individual dose optimization and optimal dosage policies. Methadone is a synthetic opioid that is administered as a racemic mixture of (*R*)- and (*S*)-methadone enantiomers; the (*R*)-methadone is an active enantiomer at the mu-opioid receptor (Kreek, 2007). Methadone is a mu-opioid receptor full agonist and a modest noncompetitive N-methyl-D-aspartic acid (NMDA) receptor antagonist. Oral methadone is rapidly absorbed with peak plasma concentrations in two to four hours with the half-life of the racemic mixture in humans ranging from 16-28 hours (Kreek, 1973). It is metabolized primarily in the liver and also in the intestine and is slowly released back into plasma or bile, most of it bound to plasma proteins. Biotransformation of methadone is primarily by *N*-demethylation to an inactive major metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrolidine (EDDP), that can be converted by a second *N*-demethylation to inactive metabolite 2-ethyl-5-methyl-3,3-diphenylpyrroline (EMDP) (Inturrisi and Verebely, 1972a; Inturrisi and Verebely, 1972b).

Methadone metabolism in humans is attributed primarily to the cytochrome P450 (CYP) enzymes CYP3A4, CYP2B6, and CYP2D6 (e.g. (Foster et al., 1999; Wang and DeVane, 2003; Gerber et al., 2004; Kharasch et al., 2004; Crettol et al., 2005)). There is large inter-individual variability in expression and enzymatic activities of the CYP enzymes that may affect methadone clearance (Ingelman-Sundberg et al., 2007). The *CYP* genes are highly polymorphic with inter-ethnic differences in allele frequencies (Zhou et al., 2009). In addition to the metabolizing enzymes, other factors may be involved in methadone response including binding to plasma proteins, and drug transporters at the pharmacokinetic level, and genetic variation in opioid receptors at the pharmacodynamic level (Li et al., 2008). The variable activity of these factors may be caused by genetic, health, hormonal, and environmental factors.

The *CYP2B6*6* allele was associated with higher (*S*)-methadone plasma levels and prolonged QTc interval but showed no major influence on methadone dose requirement (Crettol et al., 2006; Eap et al., 2007). It was also associated with higher postmortem methadone concentration in blood suggesting a slow metabolism (Bunten et al., 2010). No associations with *CYP3A4* and/or *CYP2D6* variants and methadone dose requirement have been reported (Li et al., 2008).

To further extend previous studies indicating influence of the *CYP2B6*6* allele on methadone metabolism, we investigate the potential effects of *CYP2B6*6* allele on methadone dose requirement in a well characterized sample with rigorous clinical care and complete clinical and pharmacy records including only subjects with no co-medication that may affect methadone metabolism. In addition, we have explored the effect of additional *CYP2B6* polymorphisms as well as polymorphisms in *CYP3A4* and *CYP2D6*.

MATERIAL AND METHODS

Study Subjects

Our sample consisted of 74 (45% female) unrelated former severe heroin addicts in MMTP from Dr. Miriam and Sheldon G. Adelson Clinic for Drug Abuse, Treatment and Research, Tel Aviv, Israel. The ages range from 18-65 years (mean 38 years). The sample consists of the 64 Israeli Jewish subjects (86%) and 10 non-Jewish Israeli subjects. The Jewish subjects are divided into two main groups: Ashkenazi (24%) and non-Ashkenazi (57%). Five

subjects are Jewish of mixed or unknown origin. All subjects had one or more years of daily multiple uses of heroin and at least one withdrawal or failure in a detoxification center. Patients underwent repeated random and observed urine tests. Medical charts were reviewed for prescribed co-medications. Trough methadone plasma levels were obtained as described (Adelson et al., 2007). Patients drank the methadone under observation in the clinic for at least 4 days before blood was drawn for methadone plasma level. The inclusion criteria for this specific study were: a) At least 6 months in MMT; b) Negative urine for illicit opiates, cocaine and benzodiazepine for at least 4 weeks prior to obtaining blood specimen for methadone plasma level; c) Stable methadone dose for at least 2 weeks; and d) No co-medication except for medications that are not known to affect methadone metabolism (e.g. acetylsalicylic acid, metformin, statin) or prescribed benzodiazepine. The study was approved by the Helsinki Committee of the Tel-Aviv Sourasky Medical Center and The Rockefeller University Hospital Institutional Review Board and all subjects signed informed consent for genetic studies.

Gene polymorphisms analyses

Genomic DNA was extracted from whole-blood samples using standard techniques. Genotyping was performed using the drug-metabolizing enzyme and transporter DMET™ Plus Premier Pack (Affymetrix, Santa Clara, CA, USA) and analyzed using the DMET™ Console Software (Affymetrix). The DMET™ Plus Panel covers more than 90 percent of the current ADME Core markers (Dumaual et al., 2007). A total of 79 polymorphisms were genotyped in *CYP2B6, CYP3A4,* and *CYP2D6* (23, 25 and 31, respectively), including one copy number variation that can be detected by 9 probes (complete gene deletion, *CYP2D6*5*). Eight samples were run in duplicate with 99% concordance. Sequencing analyses were performed for SNPs that failed on the array or SNPs that were not called by the DMET software (*CYP2B6* rs45482602,rs28399500, rs3745274 and rs8192719, and *CYP3A4* SNP rs67666821). Three polymorphic SNPs: rs4803418 and rs36079186 (*CYP2B6*) and rs5030655 (*CYP2D6*) were excluded from analysis because of low call rate. Primers for PCR and sequencing were designed using software Primer3 (Supplementary Table S1). PCR amplifications were performed using AmpliTaq Gold® using a GeneAmp® PCR system 9700 (Applied Biosystems (ABI), Foster City, CA, USA). PCR amplification consisted of 10 min at 94°C, eight ‘touch-down’ cycles of 30 s at 94°C, 30 s at 63–56°C and 30 s at 72°C, followed by 35 cycles of 30 s at 94°C, 30 s at 56°C and 30 s at 72°C with a final step of 7 min at 72°C. Amplicons were purified with ExoSAP-IT® (Affymetrix) and run on an ABI 3730xl® DNA Analyzer (Applied Biosystems). Electropherograms were scored using the Sequencer 4.5 software (Gene Codes Corporation, Ann Arbor, MI, USA).

Ancestry informative markers (AIMs) analyses—AIMs genotyping was performed on a 1536-plex GoldenGate Custom Panel (GS0007064-OPA, Illumina, San Diego, CA, USA) that included 186 AIMs (Hodgkinson et al., 2008). Genotyping was performed at the Rockefeller University Genomics Resource Center according to the manufacturer’s protocol. Analysis was performed using BeadStudio genotyping software, v2.3.43 (Illumina). Genotype data were filtered based on SNP call rates (>99.5%), cluster separation score and deviation from HWE and 168 AIMs were used for analysis. Biographic Ancestry Scores (e.g. fractions of genetic affiliation of the individual in each of a predetermined number of clusters) were estimated by Structure 2.0 using 1051 CEPH subjects represented in the Human Genome Diversity Cell Line Panel (HGDP-CEPH) as reference, with K=7.

Statistical analyses—HWE and pair-wise linkage disequilibrium (D' and r^2) were estimated using R and Haploview version 4.2. Analysis of variance (ANOVA) was performed to determine if the mean levels of the daily methadone doses were significantly different among subjects with different genotypes for each of the SNPs. ANOVA was also

performed with sex, age and the *ABCB1* SNP rs1128503, as covariates in the model, for *CYP2B6* SNPs rs3745274 and rs2279343. The *P*-values presented are not corrected for multiple testing.

Results

The stabilizing daily methadone dose in the sample ranges from 13-260 mg with a mean of 140±52 mg and is normally distributed (Fig. 1, Supplementary Table S2). There is no significant gender difference in methadone daily dose although the mean dose for females (n=33) is higher than that of males (n=41) (151±50.7 mg and 131±51.0 mg, respectively, $P = 0.08$, F(3.12,1,72)). The mean trough plasma (*R/S*) methadone level is 498±269 ng/ml (range 100-1220 ng/ml) (Supplementary Table S2). The correlation between trough plasma levels and methadone dose is moderate (r = 0.40, Supplementary Fig. S1).

Ancestry informative markers (AIMs)

Ancestry Biographic Scores were estimated for 71 of the subjects based on genotypes of 168 AIMs (Supplementary Fig. 2, Supplementary Table S3). There is very low contribution of African, Far East Asian, Oceanian and/or Native American populations in the entire sample. The majority of the Ashkenazi Jewish subjects in this sample were shown to have a large European contribution and a few subjects also have some Middle Eastern and Asian contribution. The non-Ashkenazi group in this sample includes subjects with primarily a Middle Eastern or European contribution, as well as subjects with mixed Middle Eastern/ European, and Asian contribution. Of note, the European and Middle Eastern clusters are relatively close (population differentiation $F_{st} = 0.005$) and are not distinguished by STRUCTURE analysis with six major clusters (K=6).

CYP2B6*6—Our first goal was to look for association of the *CYP2B6*6* genotype and methadone dose required for effective treatment. The *CYP2B6*6* allele corresponds to the combination of allele *4 (785A>G, rs2279343) and allele *9 (516G>T, rs3745274). Pairwise LD analysis confirmed a strong LD between the two SNPs ($D' = 1$, $r^2 = .9$) in this sample population (Supplementary Fig. S3a, b).

There were significant differences in the daily methadone doses required by subjects with different genotype groups of *CYP2B6* SNPs 785A>G (rs2279343) and 516G>T (rs3745274). The mean methadone doses required by subjects homozygous for the variant allele (88, 96 mg, respectively) were significantly lower than those of the heterozygotes (133, 129 mg, respectively) and the non-carriers (150, 151 mg, respectively) (nominal $P = 0.012$, F(4.73,2,65), 0.048, F(3.18,2,65)), respectively for association with genotypes) (Table 1, Fig. 2). Fig. 3 shows the distribution of the three genotype group of SNP rs2279343 in two methadone dose groups: <150 and ≥150 mg/day. No subject homozygote for the variant allele required methadone dose above 135 mg/day. Association test of the *CYP2B6*6* haplotype T-G (516G>T, 785A>G, respectively) was also significant ($P = 0.019$, F(3.54,3,64)). There were no significant differences in trough plasma (*R/S*) methadone levels between *CYP2B6*6* genotypes.

We previously reported an association of homozygosity for the *ABCB1* SNP 1236C>T (rs1128503) with high methadone doses (Levran et al., 2008). Based on the current results, an opposite effect on methadone dose is expected for the 1236T/T genotype (increase) and the carriers of the *CYP2B6*6* allele (decrease). Analysis of each of the two *CYP2B6* SNPs with the *ABCB1* 1236T/T genotype, as well as sex and age, as covariates in the model, substantiated the results [rs2279343: $P = 0.0063$, F(5.50,2,62); rs3745274: $P = 0.0298$, F(3.72,2,62)].

Additional SNPs

CYP2B6—Eleven additional *CYP2B6* SNPs were polymorphic in our sample, out of which two SNPs (rs4803418 and rs36079186) were excluded from analysis because of low call rate (Table 2). Listed in Supplementary Table S4 are the 10 SNPs that were monomorphic in our sample. The frequencies of all variants in our sample are not significantly different from those reported in Caucasians and other studies of Jewish communities (ALFRED, http://alfred.med.yale.edu/alfred/index.asp). There is no significant difference in allele frequencies between the Ashkenazi and the non-Ashkenazi group.

Although the *CYP2B6**6 allele generally refers only to the non-synonymous coding SNPs 516G>T and 785A>G, LD analysis supports the inclusion of the intronic SNP rs8192719 in this allele (haplotype), at least for this population (D'=1, r^2 =1 with rs3745274) (Supplementary Fig. S3a, b). Other block of high LD were identified including SNPs 64C>T (rs8192709) and 216G>C (rs2279341) (D'=1, r^2 =1), which corresponds to allele *CYP2B6**2B, as well as SNPs −82T>C (rs34223104) and 777C>A (rs45482602, *CYP2B6**3) (D'=1, r^2 =.8) (Supplementary Fig. S3a, b).

CYP3A4—Three of the four *CYP3A4* polymorphic SNPs are rare and one SNP, the intronic rs2242480, is more frequent (MAF=.11) (Table 2). 73% of the sample was monomorphic for all 4 *CYP3A4* polymorphic SNPs genotyped. Listed in Supplementary Table S4 are the 21 SNPs that were monomorphic in our sample.

CYP2D6—Twelve *CYP2D6* polymorphic SNPs were identified in this study and one SNP (rs5030655) was excluded from analysis because of low call rate (Table 2). The allele frequencies in our sample were concordant with those reported in Caucasians and Jewish communities (Luo et al., 2004; Scott et al., 2007). One subject was homozygous for SNP 2988G>A (rs28371725) that leads to a non-functional enzyme.

Three subjects were homozygous for the 5′ region SNP −1584G>C (rs1080985) that is associated with fast metabolism. Three blocks of high LD were observed: 1) SNPs −2182G>A (rs28360521), 100C>T (rs1065852) and 506-1G>A (rs3892097, also known as 1846G>A) (D'=1, r^2>.97) corresponds to allele*4; 2) SNPs 1661G>C (rs1058164) and 4180C>G (rs1135840) (D'=1, r^2=.97); and 3) SNPs −1774A>G (rs1080983) and 2850T>C (rs16947) (D'=1, r^2=.79) (Supplementary Fig. S3c, d).

Allele *CYP2D6**4 results in defective splicing and a non-functional enzyme and is responsible for the majority of the poor metabolizers in Caucasians. The three subjects with genotype *4/*4 and the subject homozygous for the 2988G>A (rs28371725) require relatively high daily methadone doses (125-220 mg), the opposite of what would be expected of a non-functional enzyme with a major role in methadone metabolism.

No association between methadone dose requirement and the additional *CYP2B6* SNPs**,** or SNPs in *CYP3A4 or CYP2D6* has been identified (Supplementary Table S5).

Discussion

Adequate methadone dosing is critical for therapeutic success. One of the aims of pharmacogenomics is to explain the inter-individual variability in drug response and to facilitate individualized therapy (Ingelman-Sundberg et al., 2007). Previous studies reported slower methadone metabolism in carriers of the *CYP2B6**6 allele, but did not find significant influence on methadone dose requirements (Crettol et al., 2005; Crettol et al., 2006), or did not investigate methadone dose requirements (Bunten et al., 2010). This study supports the finding of a slower methadone metabolism in carriers of the *CYP2B6*6* allele

and further suggests that carriers of two *CYP2B6*6* alleles require relatively low methadone doses (<100 mg/day).

The *CYP2B6**6 allele was associated with low hepatic expression and decreased enzymatic activity *in vitro* and *in vivo.* Although it involves changes of two amino acids, the functional cause is suggested to be aberrant splicing and reduced mRNA expression (Zanger et al., 2007). This allele occurs in high frequencies across different populations and is associated with the response to several drugs including efavirenz in HIV-1 patients and bupropion in smoking cessation (Zanger et al., 2007; Zhou et al., 2009). There were no significant differences in trough plasma (*R/S*) methadone levels between subjects with different *CYP2B6*6* genotypes in our sample. This study cannot address specific effect on *R*- or *S*-methadone because it was limited to plasma racemic (*R/S*) methadone.

CYP2B6 is highly inducible by various drugs, including methadone. Two recent studies, using an acute intravenous single methadone dose have suggested a prominent role for CYP2B6 in its metabolism (Kharasch et al., 2008; Totah et al., 2008). CYP2B6 expression has been shown to differ between sexes and ethnicities (Lamba et al., 2003). Another important factor may be brain-specific drug metabolism and CYP induction. CYP enzymes were shown to have brain-specific expression that may differ from the hepatic forms (Miksys and Tyndale, 2002; Miksys and Tyndale, 2009) suggesting that a patients' response to a centrally-acting drug may not be predicted by plasma drug level. For example, brain CYP2B6 (and not liver CYP2B6) was detected at higher levels in alcoholics and smokers (Miksys and Tyndale, 2009).

The additional SNPs in *CYP2B6, CYP3A4* and *CYP2D6* identified in this sample did not show a significant association with methadone dose. These results should be interpreted with caution given that this study had a limited power to detect association with rare SNPs because of the relatively small sample size.

The relatively low variability of *CYP3A4* in our sample is in line with the suggestion that CYP3A4 alleles may not have significant clinical importance (Shiran et al., 2009; Zhou et al., 2009). Several studies showed an effect of CYP2D6 on methadone metabolism but there is no consensus on its level of involvement relative to the other CYP enzymes (Shiran et al., 2003; Wang and DeVane, 2003; Crettol et al., 2006; Lotsch et al., 2006). Since many drugs are CYP2D6 inhibitors, co-medication may affect methadone therapy.

Methadone dose may explain only part of the variation in plasma methadone level (Pond et al., 1985; Li et al., 2008; Shiran et al., 2009). Studies from our and other laboratories have showed correlation between methadone dose and plasma levels, especially for higher doses (Foster et al., 2000; Adelson et al., 2007), however poor correlation was also reported in one study in which methadone doses were not specified (Charlier et al., 2001). There is an important contribution of the reservoir of tissue-bound methadone to steady-state plasma levels; therefore binding to plasma proteins may also be a factor in methadone pharmacokinetics (Kreek, 1973). In addition, the total plasma methadone may not reflect the amount of the active opioid (*R*) because the *R/S* ratio in plasma has been reported to vary widely among individuals (Buchard et al., 2010).

Methadone is a substrate of the efflux transporter p-glycoprotein (encoded by the *ABCB1* gene). We previously reported that homozygosity to the *ABCB1* SNP 1236C>T(rs1128503) is associated with high methadone dose requirement (Levran et al., 2008). It was therefore of importance to take the *ABCB1* SNP genotype into account in the analysis. In this case, the association of the *CYP2B6**6 SNPs with low methadone doses was substantiated when controlling for the *ABCB1* 1236 T/T genotype. It serves as an example of the importance of multivariate analysis that reflects more realistically the simultaneous contribution of several

genetic factors to methadone pharmacokinetics and pharmacodynamics, and therefore dose requirement. The potential contribution of alleles in additional genes is currently under investigation.

The effect of drug-drug interaction is important in MMT patients, since the efficacy of methadone is significantly altered by several medications that are often consumed by these patients (Kharasch et al., 2008; McCance-Katz et al.). For example, methadone is often co-administered with HIV/AIDS treatment and also medication for affective disorders. Recently, methadone was shown to induce hepatic expression of CYP2B6 through activation of pregnane X receptor (PXR) and constitutive androstane receptor (CAR) *in vitro* (Tolson et al., 2009). The effect of co-administered drugs was largely eliminated in the current study by including only subjects who were for the most part not co-administered drugs. In the future, it will be important to assess the effects of these polymorphisms in patients treated by other medications.

The Israeli population is comprised of distinct groups with complex demographic history and there is clinical importance to assessing the differences between these groups. Recent studies revealed that Jewish communities show a high degree of sharing in their genome-wide patterns (Atzmon et al., 2010; Behar et al., 2010). Our AIMs data support this finding. The majority of the Ashkenazi Jewish group has a major European contribution but some also have a significant Middle Eastern contribution. The non-Ashkenazi group is divided between subjects with a major Middle Eastern contribution, a major European contribution and a mixed contribution. Of note, the European and Middle Eastern clusters are relatively close (population differentiation $F_{st} = 0.005$) and are not distinguished by STRUCTURE analysis with six major clusters (K=6). The data reflect the resemblance of different Jewish groups and the resemblance of the Jewish Israeli population to Middle Eastern groups.

In summary, the functional *CYP2B6**6 allele was shown to be associated with relatively low stabilizing methadone doses in patients treated for heroin addiction in an MMT clinic in Israel. This study was limited to a relatively small sample from one clinic and it will be necessary to reproduce it in a larger sample and in subjects from other populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank and acknowledge the contribution of Dr. Pei-Hong Shen, Dr. David Goldman, Dr. Connie Zhao, Ms. Bin Zhang, Ann Ho, Susan Russo and Anat Sason. This work was supported in part by NIDA-P60-05130 (M.J.K.) and the Dr. Miriam and Sheldon G. Adelson Medical Research Foundation.

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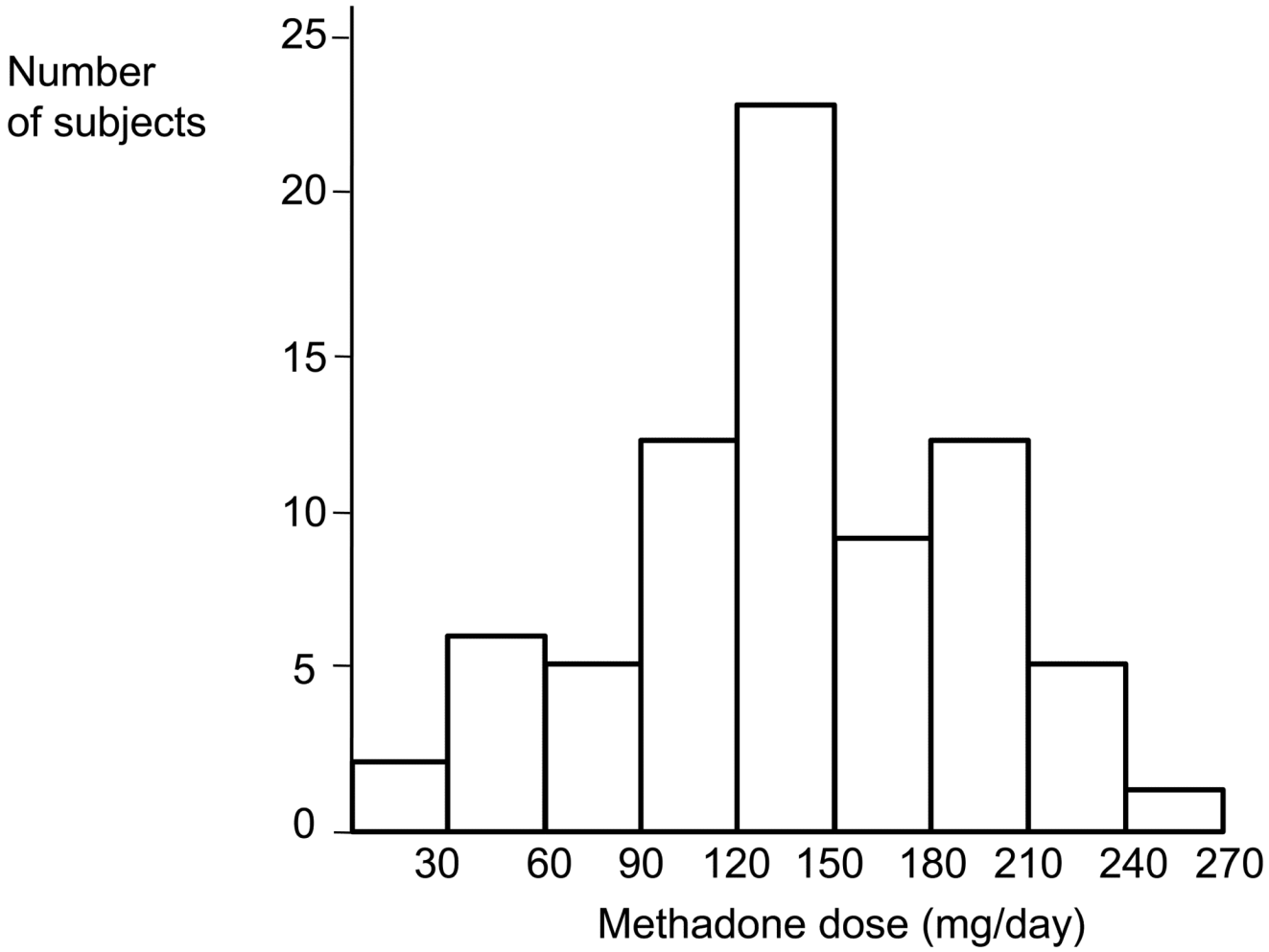

Fig. 1.

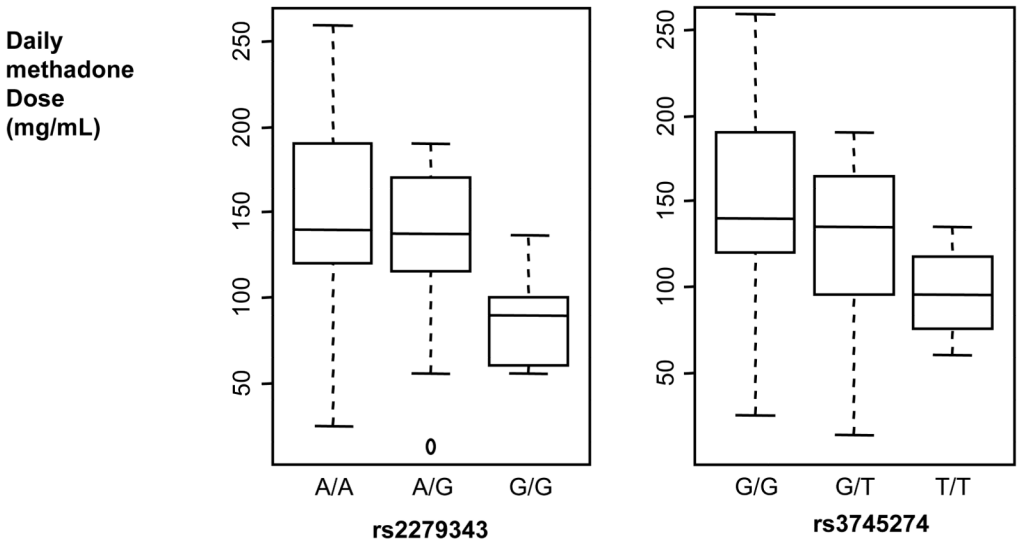

Fig. 2.

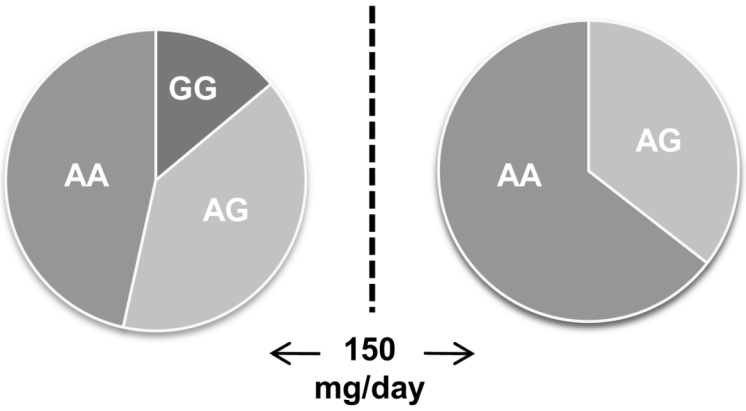

Fig. 3.

TABLE 1

Association of *CYP2B6* SNPs with methadone dose and trough plasma levels

			Methadone dose			Trough methadone plasma levels		
SNP	**Genotype**	**n**	**Mean (mg/day)**	**SE**	***p* value**	**Mean (ng/mL)**	**SE**	***p* value**
rs3745274 (516G>T, *9)	GG	40	150.3	8.1	0.048[a]*	503.5	39.9	0.45
	GT	29	128.6	9.1		464.1	52.7	
	TT	4	96.3	15.5		642.5	178.0	
rs2279343 (785A>G, *4)	AA	39	151.4	8.4	0.012[b]*	514.9	40.8	0.45
	AG	28	132.6	8.9		456.8	53.3	
	GG	6	88.3	11.9		596.7	132.3	

[a] 0.0298

[b] 0.0063, after controlling for age, sex and *ABCB1* SNP rs1128503.

TABLE 2

SNPs polymorphic in this study population

	SNP	Position[a]	Exon/ intron	Position[b]	Protein	MAF[c]	MAF (Cau.)[d]	Allele[e]	Other name
CYP2B6									
1	rs34223104	4618896	5′	−82T>C		0.03	0.02	*22	
2	rs8192709	4618911	ex 1	64C>T	R22C	0.08	0.06	*2	
3	rs35303484	4618918	ex 1	136A>G	M46V	0.01	0.01	*11	
4	rs2279341	4620179	ex 2	216G>C	P72P	0.08	0.07	*2B	
5	rs4803419	4620463	i 3	485-18C>T		0.36	0.30	*13B	15582
6	**rs3745274**	4620468	ex 4	516G>T	Q172H	0.25	0.25	*9,	
7	rs45482602	4620709	ex 5	777C>A	S259R	0.02	0.007	*3	
8	**rs2279343**	4620710	ex 5	785A>G	K262R	0.27	0.20	*4,	rs28399497
9	rs2279344	4620732	i 5	822+183G>		0.26[f]	0.40[f]	many	18273
10	rs8192719	4621061	i 8	1294+53C>		0.22	0.27	*9	21563
11	rs28399500	4621455	ex 9	1459C>T	R487C	0.07	0.10	*5, *7	rs3211371
CYP3A4									
1	rs2740574	9922003	5′	−392A>G		0.04	0.05	*1B	290A>G
2	rs2242480	9919940	i 10	1026+12G>		0.11	0.08	*1G	24595742
3	rs4986910	9919646	ex 12	1334T>C	M445T	0.01	0.008	*3	
4	rs67666821	9919374	ex 13	1461_1462i	488fs	0.007	nd[g]	*20	
CYP2D6									
1	rs28360521	4085892	5′	−2182G>A		0.15	0.22		
2	rs1080983	4085851	5′	−1774A>G		0.32	nd		
3	rs1080985	4085832	5′	−1584C>G		0.20	0.25	*2A	
4	rs1065852	4085663	ex 1	100C>T	P34S	0.15	0.30	*4, *10	
5	rs28371706	4085571	ex 2	320C>T	T107I	0.01	0.01	*17	1023
6	rs1058164	4085507	ex 3	408G>C	V136V	0.47	0.30	*19	1661
7	rs3892097	4085489	i 3	506-1G>A		0.15	0.30	*4	1846
8	rs5030656	4085412	ex 5	841-843del	K281de	0.01	nd	*9	2615_2617
9	rs16947	4085388	ex 5	886T>C	C296R	0.38	0.45	*17	2850
10	rs28371725	4085374	i 6	985+39G>A		0.14	0.15	*41	2988

	SNP	Position[a]	Exon/ intron	Position[b]	Protein	MAF[c]	MAF (Cau.)[d]	Allele[e]	Other name
11	rs1135840	4085255	ex 8	1457G>C	S486T	0.47	0.45	*19	4180

[a] Build 36.3.

[b] The mRNA accession numbers are: NM_017460.3 (*CYP3A4*) NM_000767.4 (*CYP2B6*), *and* NM_000106.4 (*CYP2D6*).

[c] minor allele frequency.

[d] Caucasians (NCBI).

[e] according to the human Cytochrome P450 allele nomenclature committee.

[f] G is the minor allele.

[g] not detected. The SNPs in bold are part of the *CYP2B6*6* allele.