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Association of genetic variation in pharmacodynamic factors with methadone dose required for effective treatment of opioid addiction

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Abstract

Aim—The interindividual variability in the dose required for effective methadone maintenance treatment (MMT) for opioid addiction may be influenced in part by genetic variations in genes encoding pharmacodynamic factors of methadone. This study was conducted to identify some of these variants.

Materials & methods—This study focused on 11 genes encoding components of the opioidergic (*OPRM1*, *POMC* and *ARRB2*), the dopaminergic (*ANKK1* and *DRD2*) and the glutamatergic pathways (*GRIN1* and *GRIN2A*), as well as the neurotrophin system (*NGFB*, *BDNF*, *NTRK1* and *NTRK2*). The study includes 227 Israeli patients undergoing stable MMT.

Results—Out of the 110 variants analyzed, 12 SNPs (in *BDNF*, *NTRK2*, *OPRM1*, *DRD2* and *ANKK1*) were associated with methadone dose (nominal $p < 0.05$). Of these SNPs, *ANKK1* rs7118900 and *DRD2* rs2283265 are known to affect gene expression. Logistic regression of five representative SNPs discriminated between individuals requiring a methadone dose of >120 mg/

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Financial & competing interests disclosure

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day and <120 mg/day ($p = 0.019$), and showed moderate sensitivity and specificity (AUC of 0.63 in receiver operating characteristic analysis).

Conclusion—This data should stimulate further research on the potential influence and clinical significance of these variants on MMT.

Keywords

ANKK1; *BDNF*; *DRD2*; methadone maintenance; *NTRK2*; *OPRM1*; pharmacogenetics

It is estimated that there are more than 14 million heroin users worldwide [101]. In addition, abuse of prescription opioids is a serious public health problem. Opioid addiction, which is associated with severe medical and social problems, is currently treated mainly with methadone or buprenorphine maintenance. It is estimated that approximately 1 million individuals are currently undergoing methadone maintenance treatment (MMT) for opioid addiction worldwide. The challenges of MMT include the large interindividual variability in the dose required for optimal effective treatment, as well as the less than optimal retention and success rates. Individual responses to methadone may be influenced by a combination of pharmacodynamic and pharmacokinetic factors that can be regulated in part by genetic variants [1]. The identification of the association between drug therapeutic effects and genotype is a promising new aspect in current medicine (personalized medicine) that aims for modification of treatments for individuals using genetic tools [2].

This study was conducted to advance the understanding of potential genetic factors underlying methadone dose requirements for successful treatment outcome. Methadone is a synthetic μ -opioid receptor agonist and a moderate noncompetitive *N*-methyl-D-aspartate antagonist used for MMT of opioid addiction and for pain treatment [3,4]. The primary used form of methadone includes a racemic mix of its two enantiomers (*d/l* or *S/R*). Methadone has a narrow therapeutic index, complex pharmacokinetic profile with substantial interindividual variability, and concentration-dependent toxicity, making it challenging to manage. For a given dose, methadone plasma levels can vary extensively among individuals [5]. Methadone is metabolized slowly and reaches peak plasma levels within 2–4 h, with a half-life of approximately 24 h (range of 13–50 h) [6]. Patients in stable treatment can usually take the medication once a day without abstinence reactions with appropriate treatment.

We examined variants in genes encoding potential pharmacodynamic factors of methadone, including components of the opioidergic (*OPRM1*, *POMC* and *ARRB2*), the dopaminergic (*ANKK1* and *DRD2*) and the glutamatergic pathways (*GRIN1* and *GRIN2A*), as well as members of the neurotrophins family and their receptors (*NGFB*, *BDNF*, *NTRK1* and *NTRK2*). Some of these genes may have an indirect or modulatory effect on methadone action. Genotype data were analyzed separately for each variant as well as in aggregate, since it is assumed that a polygenic mode underlies the variability in methadone dose [7].

The μ -opioid receptors are the primary site of action of methadone. Opioid receptors are coupled via G-proteins to a variety of effectors. After activation, the receptor couples to *ARRB2*, which negatively regulates opioid receptor signaling [8–10]. There is evidence that stress and addictive behaviors are inter-related [11]. *POMC* is a polypeptide precursor of ten peptides including the endogenous opiates β -endorphin and ACTH, two principal components of the hypothalamic–pituitary adrenal axis. β -endorphin is an endogenous opioid peptide that exerts its effects through μ - and δ -opioid receptors [12].

The dopaminergic system mediates the rewarding effect of drugs of abuse, and is implicated in withdrawal and behavioral sensitization, as well as drug-related learning and

neuroadaptations [13]. Methadone was shown to increase dopamine levels in the mesolimbic dopaminergic system in rats in a dose-dependent manner [14]. ANKK1, a serine/threonine kinase, is a member of the family of the proteins involved with signal transduction and cellular responses to external stimuli. The genes encoding ANKK1 and DRD2 are located in close proximity on chromosome 11 and several of their variants are in linkage disequilibrium (LD). ANKK1 was shown to be activated by apomorphine, a dopaminergic agonist, also suggesting a functional connection between the two proteins [15,16].

The glutamatergic pathway has been shown to be involved in long-term potentiation, learning and memory. R-methadone showed very high potency for ionotropic glutamate NR1/NR2A (encoded by *GRIN1* and *GRIN2A*, respectively) [17]. *GRIN2A* variants were shown by our laboratory to be associated with heroin addiction in African-Americans [18].

NGF and BDNF are signaling molecules responsible for neuron growth, axon targeting and synaptic plasticity, and have an important role in reward-related processes and opioid-induced plasticity [19,20]. They signal through their NTRK1 and NTRK2 receptors, respectively, and activate intracellular signaling cascades. There is growing evidence that *BDNF* is involved in the pathophysiology of affective disorders and in the mechanism of action of their therapeutic agents [21]. Chronic morphine exposure was shown to decrease BDNF expression in the ventral tegmental area in mice [22].

Several pharmacogenetic studies of MMT have been reported to date. These studies were conducted in diverse populations, used different treatment protocols and various phenotypes and produced mixed results. The pharmacodynamic factors analyzed in these studies were *OPRM1*, *DRD2/ANKK1*, *NGFB*, *BDNF* and *ARRB2* [23–30].

In the current study, we have analyzed a well-characterized sample of former heroin addicts from a MMT clinic in Israel who were all stabilized with methadone, to identify genetic variants in genes encoding pharmacodynamic factors that may be associated with effective methadone dose requirements.

Materials & methods

Subjects

The sample consisted of 227 (27% female and 73% male) unrelated former heroin addicts undergoing MMT who were admitted to the Adelson Clinic for Drug Abuse Treatment and Research in Tel-Aviv, Israel, since 1993. Of these subjects, 88 were included in our *ABCB1* study [31], and 66 were included in our two other studies [23,32], including 22 subjects who were included in the three studies. The sample consisted of 93% Jewish patients who include Ashkenazi (24%), non-Ashkenazi (65%) and Jewish patients of mixed or unknown origin (4%). The rest of the patients were Arabic non-Jewish European (Supplementary Table 1; www.futuremedicine.com/doi/suppl/10.2217/pgs.13.58).

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. The inclusion criteria for this specific study were:

- At least 6 months undergoing MMT;
- Negative urine for illicit opiates, cocaine and benzodiazepine for at least 4 weeks;
- Stable methadone dose for at least 2 weeks;
- HIV negative;

- No comedication known to affect methadone metabolism 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.

Genes/SNPs selection & array design

Eleven genes were chosen for analysis based on their methadone-related functions or previous reports of association with methadone response (Table 1 & Supplementary Table 2). A 1536-plex GoldenGate Custom Panel (GS0013101-OPA, Illumina, CA, USA) was designed on the basis of the addiction-focused array (GS0007064-OPA, designed by Hodgkinson *et al.*, National Institute on Alcohol Abuse and Alcoholism [NIAAA]) [33]. The addiction-focused array included 112 SNPs from these genes. Ten SNPs were excluded from the new array because of ambiguous clustering or low variability in the population of this study based on our previous study [34]. In addition to the remaining 102 SNPs, an additional 24 SNPs were selected for the new array based on potential functionality, suitability to the genotyping platform, as well as previous reports of association with related phenotypes (see Supplementary Table 2 for additional references). Of these 24 SNPs, three SNPs were not included since they failed the design criteria.

Of the original 186 ancestry informative markers (AIMs) from the GS0007064 panel [33,35], 171 SNPs with adequate quality were included in the new panel.

Notably, 12 *OPRM1* SNPs are also part of the *OPRM1* overlapping gene *IPCEF1* that encodes a scaffold protein [36]. One *ARRB2* SNP is located at the flanking *RPS12P29* gene that encodes ribosomal protein and is also in the 5' flanking region of *PELP1*. One *POMC* SNP is located at the 3'-UTR of the flanking *EFR3B* gene. Six *BDNF* SNPs are also located in the overlapping noncoding antisense RNA transcript encoded by the nonprotein coding *BDNF-AS1* (Supplementary Table 2) [37].

Genotyping

Genomic DNA was extracted from whole-blood samples using standard techniques. Genotyping was performed on a GoldenGate Custom Panel at the Rockefeller University Genomics Resource Center according to the manufacturer's protocol (Illumina). Analysis was performed using BeadStudio software v2.3.43 (Illumina), which provides automated genotype calling, cluster separation score and quality check. The data were also visually inspected to confirm calling and to resolve questionable data. Duplicate samples with mismatched genotype were excluded. Genotype data were filtered based on SNP call rates, minor allele frequency (MAF) and deviation from Hardy-Weinberg equilibrium (HWE).

Sequencing analysis was performed for the *NGFB* SNP rs2239622, for which the BeadStudio clustering was not optimal. Primers for PCR amplification and sequencing were designed using NCBI/Primer-BLAST (using Primer3 and BLAST) [38] (forward-AGAGGTCC T TGC TACC TCCCC A, reverse: GGAGAGCCAGGAAGAGCCAGGT). PCR conditions were as described elsewhere [31].

Statistical analysis

Genotype deviation from HWE was evaluated by exact testing using PLINK [39,102]. Pairwise LD (r^2) were estimated using Haploview 4.2 [40].

Biographic Ancestry Scores (e.g., fractions of genetic affiliation of the individual in each cluster) were estimated by Structure based on genotypes of 155 AIMs, using 1051 Centre

d'Etude du Polymorphisme Humain [CEPH] subjects represented in the Human Genome Diversity Cell Line Panel as reference, with $K = 7$, as described [41]. From the 171 AIM SNPs genotyped, 16 SNPs were excluded from analysis because of ambiguous clustering ($n = 12$) or because they were from genes that may be related to the addiction phenotype ($n = 4$).

To look at population stratification, multidimensional scaling (MDS) was carried out [42], with genotype data from 110 SNPs using PLINK. Such MDS components are based, for each pair of individuals, on the average identity by state (IBS) over all SNPs. For example, if two individuals have the same genotype in all SNPs then their average IBS is equal to 1. The IBS distance between two individuals is then 1 minus their average IBS and forms the basis for MDS. We computed the two largest MDS components.

One-way analysis of variance (ANOVA) was performed to determine if the mean levels of the daily stabilized methadone doses were significantly different among subjects with different genotypes for each of the SNPs. For each SNP, this resulted in an F value and a corresponding nominal (point wise) p-value. Permutation analysis (40,000 replicates) was carried out to adjust for testing three modes of inheritance (codominant, dominant and recessive) for each SNP. For a given SNP, we carried out each of the three tests and retained that result furnishing the smallest p-value, but then imposed permutation analysis to correct for these three tests. A modification of the Sumstat program was used [103].

For a dichotomous variable representing higher methadone levels (>120 mg/day) versus lower levels (≤ 120 mg/day), logistic regression analysis was carried out with five SNPs that were selected based on the associations identified in the individual SNP analyses and taking into account the LD data. Receiver operating characteristic (ROC) [43] calculations were carried out with IBM SPSS version 20. Methadone doses >120 were scored 1 (higher) and anything else was scored 0 (lower).

Results

Sample characteristics

The sample consisted of 227 (27% female and 73% male) unrelated former severe heroin addicts undergoing effective MMT who were admitted to the Adelson Clinic for Drug Abuse Treatment and Research, since 1993. The stable daily methadone dose range in this sample was 10–280 mg (normal distribution, mean 141 ± 51 mg; Figure 1). The sample demographic details are shown in Supplementary Table 1. The sample consisted of 93% Jewish patients who include Ashkenazi (24%), non-Ashkenazi (65%) and mixed or unknown (4%). The rest of the patients were Arabic or non-Jewish European. The majority of the sample (91%) did not administer other medications and 9% administered medications that are not known to affect methadone metabolism.

The sample shows relative homogeneity based on MDS (Supplementary Figure 1). Structure analysis of 155 AIMs with seven clusters, where each subject is anchored against 1051 CEPH subjects as reference, revealed that the majority of patients have a European, Middle Eastern or mixed European/Middle Eastern ancestry contribution (Supplementary Table 1 & Supplementary Figure 2). There was a very low contribution of African, Far East Asian, Oceanian or Native American populations. There was some Central Asian contribution in several patients.

Association analyses

A total of 123 SNPs from 11 genes (*OPRM1*, *ARRB2*, *POMC*, *NGFB*, *BDNF*, *NTRK1*, *NTRK2*, *DRD2*, *ANKK1*, *GRIN1* and *GRIN2A*) were genotyped using the Illumina

GoldenGate array (Table 1 & Supplementary Table 2). Thirteen SNPs were excluded from analysis because of ambiguous clustering ($n = 8$), very low MAF ($n = 2$) or complete LD with another SNP in the study ($n = 3$). Five SNPs showed nominal significant deviations from HWE ($p < 0.05$) that were not significant after Bonferroni correction. The final statistical analysis was performed with 110 SNPs (Supplementary Table 2).

Pairwise LD (r^2) was estimated for each gene (Supplementary Figure 3) and the relevant findings are discussed below.

A total of 110 variants were analyzed for association with methadone doses. One-way ANOVA (recessive, dominant and additive) showed point-wise significant association with methadone dose of 12 SNPs in five genes: *OPRM1*, *BDNF*, *NTRK2*, *DRD2* and *ANKK1* (nominal $p < 0.05$, Tables 2 & 3). The association of *NGFB* SNP rs2239622 reached borderline nominal significance ($p = 0.057$). Seven of these associations remained significant after permutation analysis and none remained significant after adjusting for testing multiple SNPs (Bonferroni correction). The results are discussed below in more detail for each gene.

BDNF

Out of 13 *BDNF* SNPs analyzed, three noncoding *BDNF* SNPs were associated with methadone dose (Tables 2 & 3). LD analysis revealed relatively strong LD ($r^2 = 0.71$) between SNPs rs10835210 and rs7934165, and a moderate LD ($r^2 = 0.56$) between SNPs rs1491850 and rs7934165 (Supplementary Figure 3A). None of these SNPs are in strong LD with *BDNF* SNP rs6265 (Val66Met), which was not associated with methadone dose in this sample. These findings are in agreement with data from the HapMap population of European origin (CEU) [104].

Carriers of two C alleles (homozygotes) of rs10835210 require relatively higher methadone doses (mean 159 mg) than heterozygotes or non-carriers (139 and 134 mg, respectively). Similarly, carriers of two T alleles of rs7934165 require higher doses (mean 153 mg) than carriers of one or two copies of the C allele (mean 137 mg). Carriers of two C alleles of the rs1491850 SNP require relatively lower methadone doses (mean 125 mg) than heterozygotes or non-carriers (139 and 149 mg, respectively; Table 3).

Multilocus genotype patterns (MLGPs) (Supplementary Table 3), in concordance with the LD data, suggest that the association signals of the rs10835210 and rs7934165 SNPs are not independent of each other but are independent of the association signal of rs1491850. Most (44 out of 45) of the homozygotes rs10835210 CC genotype are also homozygotes for the rs7934165 TT genotype, both associated with higher dose, and the rs1491850 TT genotype, which has no effect on dose, creates the MLGP 'CC–TT–TT' (rs10835210–rs7934165–rs1491850) haplotype with a frequency of 19%, which requires relatively high doses (mean 157 mg). There is no overlapping between carriers of the rs1491850 CC genotype, associated with lower doses, and carriers of the CC–TT (rs10835210–rs7934165) genotype associated with a higher dose.

The MAFs of SNPs rs10835210 and rs7934165 were slightly higher (5%) and the MAF of rs1491850 and rs1519480 were slightly lower (5%) than that of the HapMap CEU. These MAFs are similar to those of Middle Eastern populations [105].

NTRK2 (BDNF receptor)

Out of 15 SNPs analyzed, six intronic *NTRK2* SNPs were associated with methadone dose (Tables 2 & 3), out of which four associations remain significant after permutation testing. Two of the SNPs (rs1948308 and rs2378676) are in almost complete LD ($r^2 = 0.99$;

Supplementary Figure 3B). Two SNPs (rs4358872 and rs4877900) are in relatively strong LD with each other and with the SNPs rs1948308/rs2378676 ($r^2 > 0.73$), all of which are located in intron 21, in agreement with the HapMap data for populations of European origin. The rs2289658 SNP, located at a splice site of intron 19/exon 20 junction [44], is less frequent and is not in strong LD with the rest of the SNPs. There are no LD data in HapMap for the rs2289658 SNP. The rs2120266 SNP is not in LD with the other SNPs.

Carriers of one or two of the variant C alleles of the rs2289658 SNP require relatively higher methadone doses (168 mg) than noncarriers (143 mg) suggesting a dominant effect (Table 3). Carriers of two A alleles of rs2120266 require relatively higher methadone doses (191 mg) than noncarriers, suggesting a recessive effect.

MLGP analysis revealed three major groups (Supplementary Table 4): 21 subjects (9%) carry one or two alleles associated with higher dose (rs2289658 and rs2120266) and no allele associated with lower dose (mean dose 180 mg), 51 subjects (22%) carry up to four alleles associated with lower dose (rs4358872, rs1948308, rs2378676 and rs4877900) and no allele associated with higher dose (mean dose 123 mg). Only five subjects (2%) carry alleles associated with both higher and lower dose (mean dose 133 mg). The rest of the subjects (66%) do not carry any of the associated alleles (mean dose 142 mg).

DRD2 & ANKK1

The adjacent *ANKK1* and *DRD2* genes are located on chromosome 11 with proximity of 9.4 kb and were analyzed together. Out of the 20 SNPs analyzed, five SNPs were excluded from analysis (Supplementary Table 2). The *DRD2* rs4350392 SNP and *ANKK1* rs1800497 SNP (previously termed '*DRD2* Taq1A') were excluded because of ambiguous clustering, and the *DRD2* rs7109897 SNP was monomorphic in this sample, as expected for population without African ancestry. LD analysis (Supplementary Figure 3C) revealed complete LD between two *DRD2* SNP pairs; rs2587548/rs1076563 and rs1125394/rs2471857. The rs2587548 and rs2471857 SNPs were not included in the analysis. Strong LD ($r^2 > 0.94$) was found between four *DRD2* SNPs rs2283265/rs1079596/ rs1125394/rs2471857. This four-SNP block is also in moderate LD ($r^2 > 0.5$) with the *DRD2* rs1076560 SNP and the *ANKK1* rs7118900 SNP. These data are mostly compatible with the HapMap data in European originated populations although there are no data available for four SNPs (rs2587548, rs2283265, rs1125394 and rs1079596).

Two SNPs (the nonsynonymous *ANKK1* rs7118900 and the intronic *DRD2* rs2283265 SNP) were associated with lower methadone dose (Tables 2 & 3). Carriers of the variant A allele of *ANKK1* rs7118900 or the variant T allele of *DRD2* rs2283265 required lower methadone doses than noncarriers. MLGP analysis revealed an overlap in the data such that the subgroup of *DRD2* rs2283265 carriers is included in the *ANKK1* rs7118900 carriers group, so the two signals may not be independent of each other, possibly representing a single causal allele (Supplementary Table 5).

OPRM1 & NGFB

Out of 23 *OPRM1* SNPs analyzed, one SNP (rs558025) in the 3' flanking region was associated with methadone dose (Tables 2, 3 & Supplementary Table 2). Carriers of one or two of the variant G alleles of rs558025 require relatively lower methadone doses (132 mg) than noncarriers (147 mg). This SNP is in relatively strong LD ($r^2 = 0.70$) with SNP rs660756 in intron 3 and their MAF is compatible with that of other populations of European origin (Supplementary Figure 3D).

In our previous study with a subset of this sample [23], an association was detected between the *NGFB* rs2239622 SNP and low methadone dose. In this study, homozygosity for rs2239622 resulted in a 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 ($p = 0.057$; Table 3).

Multi-SNP analysis

Five SNPs (*NTRK1* rs2120266, rs2289658 and rs4358872, *BDNF* rs10835210 and *ANKK1* rs7118900) were selected based on the top ANOVA association signals and taking into account the LD data to reduce redundancy. Logistic regression was performed to evaluate the cumulative predictive value of these SNPs for high stable methadone doses (>120 mg). Genotypes were dichotomized based on the mean doses of each genotype group and the predicted mode of inheritance (Table 3). Methadone doses (the dependent variable) were dichotomized to high (>120 mg; $n = 142$) and low (≤ 120 mg; $n = 85$). Logistic regression significantly discriminated between high and low doses. While the 95% CI for the odds ratio in each SNP contained unity, the combined SNP analysis was significant ($p = 0.019$; Table 4). ROC analysis was performed to consider the effect size of this prediction using estimates of the coefficient (Figure 2). The sensitivity of the test (true-positive rate) was 0.72. The specificity was 0.48 (false-positive rate = 0.52) and the area under the ROC curve was 0.632. Thus, this analysis has a probability of 72% to correctly predict that an individual is in the high methadone level group (>120 mg). However, it also has a chance of 52% of mistakenly predicting an individual in the high group as belonging to the low group.

Discussion

In this study we have focused on a limited number of SNPs in 11 genes within systems related to methadone function. Since it is likely that genetic influences on variability in dose requirements are polygenic, we have also examined the joint effect of SNPs that were identified in the individual SNP analyses. Nominal significant associations of 12 SNPs, in five genes, with methadone dose were identified. It is too early to say if these statistically significant differences are also clinically significant. The majority of these variants were reported to be associated with related phenotypes and/or to have a functional effect. These associations may also be through LD with other SNPs in these genes or nearby genes. A deep resequencing strategy may be helpful to identify the causal variants in these cases.

The ancestry make up of this Israeli sample had predominantly variable degrees of Middle Eastern and European ancestry, consistent with recent reports [32,45,46]. The evidence that these two main ancestries are relatively close (population differentiation, $F_{st} = 0.005$) [47] indicate that this sample is relatively homogeneous and resembles other European populations. In addition, the allele frequencies of most of the SNPs indicated in this study were compatible with those of Europeans; with the exception of *BDNF* SNPs, which is discussed below. Therefore, no correction for ancestry was used in the study.

This study suggests association of methadone dose with SNPs in *BDNF* and *NTRK2*. These results may be explained by alteration of brain plasticity resulting from altered expression and functionality of the neurotropic system. The *BDNF* gene, located at chromosome 11p13, contains ten 5' noncoding exons and one coding exon, and spans approximately 70 kb. It displays a complex structure of alternative promoters, splice sites and polyadenylation sites. An overlapping nonprotein coding gene *BDNF-AS1*, in a reverse orientation, produces alternatively spliced antisense transcripts [37] that were shown to inhibit *BDNF* transcription [48]. Two *BDNF* polymorphisms have been reported to have a functional impact; a microsatellite located upstream of the translation start site [49], and rs6265 (Val66Met), which was shown to alter *BDNF* mRNA and protein trafficking, and has been implicated in

substance abuse, psychiatric disorders, cognitive deficits and anxiety-related behavior [50–54]. The rs6265 SNP was not associated with methadone dose in this study. Although most previous association studies were limited to the Val66Met polymorphism, other *BDNF* variants have been associated with vulnerability to polysubstance abuse and bipolar disorder [49,55–58].

There are several studies of the three *BDNF* SNPs that are indicated in this study to be associated with methadone dose. The rs10835210 SNP was reported to be associated with bipolar disorder, schizophrenia, internalizing disorders and phobic disorders [59–61]. Interestingly, the rs10835210 SNP is located in a noncoding region of overlap between *BDNF* and *BDNF-AS1* and may have a regulatory function. The rs1491850 SNP, located at the 5' flanking region, was associated with treatment response phenotypes in major depressive disorder and with obsessive–compulsive disorder [62–64]. The rs7934165 SNP was associated with eating disorders and nicotine dependence measures in a population-specific or a gender-specific manner [65,66]. A study of 30 *BDNF* SNPs in a Spanish sample reported that *BDNF* variability confers a differential susceptibility to MMT response [24]. The investigators identified a rare *BDNF* haplotype that includes the rs7934165 T allele to be associated with poorer response to MMT. The comparison of this study to our study is difficult since the studies are designed differently. Although the authors of the Spanish study stated that there was no upper limit on methadone dosage and all subjects received stable methadone doses, they compared responders to nonresponders with a similar range of methadone doses. This nonresponder group would have been defined as unstable in our study and would have been excluded from the analysis.

Unlike the majority of the SNPs analyzed, where the MAF in this Israeli sample resembles that of European originated populations, the MAF of the *BDNF* SNPs were slightly different ($\pm 0.5\%$) from that of HapMap European-originated samples and most probably reflect the contribution of Middle Eastern ancestry in this sample. This is in concordance with a global study of *BDNF* [67] that reported high *BDNF* allele and haplotype diversity among populations and evidence for positive selection at this locus. Interestingly, similar variability between Middle Eastern and European samples were found in our recent study of another gene from the neurotrophins family, *NGFB* [23].

Several *NTRK2* variants have been associated with drug dependence and affective disorders, including suicide attempts, suicidal ideation during antidepressant treatment, obsessive–compulsive disorder, autism and nicotine and alcohol dependencies [68–73]. The *NTRK2*, mapped to chromosome 9q22.1, spans 353 kb and is composed of 21 exons. At least three protein isoforms of TrkB are produced by alternative splicing. From the SNPs indicated in this study, a *NTRK2* haplotype that includes the rs2289658 SNP was associated with susceptibility to major depressive disorder [74]. This SNP is located within 6 bp of the intron 19/exon 20 junction and may affect splicing [44]. There are no reports of association or functionality of the other three intronic *NTRK2* SNPs indicated in this study, and their potential correlation with altered functional activity of the protein remains to be clarified.

The study also suggests the association of SNPs in the adjacent *DRD2* and *ANKK1* genes with methadone dose. The ANKK1 kinase was shown to be expressed exclusively in astrocytes and has a potential relationship with the dopaminergic system [15]. The two SNPs indicated in this study (*DRD2* rs2283265 and *ANKK1* rs7118900) are in moderate LD ($r^2 > 0.6$), suggesting that their association signals may not be independent of each other. The nonsynonymous *ANKK1* rs7118900 SNP (Ala239Thr) was shown to change ANKK1 expression levels at baseline and after stimulation with dopamine agonist in HEK293T cells [16]. This SNP is predicted to create an additional phosphorylation site in the ANKK1 kinase domain [15].

The D2 receptors exist in two alternatively spliced isoforms, the D2 long and the D2 short that have distinct functions [75]. *DRD2* intronic rs2283265 SNP, indicated in this study, was shown to modulate a D2S/L splicing shift by modulating putative splice factor binding sites and was associated with greater activity in the striatum and prefrontal cortex during working memory [76,77]. This SNP was also associated with cocaine abuse [78].

The most studied *ANKK1* SNP, rs1800497 (originally identified as *DRD2* TaqIA RFLP [79]) has been associated with various phenotypes including substance dependence and pharmacological responses (e.g., [80–83]). Several studies explored the effects of rs1800497 on MMT. Lawford *et al.* reported association with treatment outcomes and the amount of heroin consumption before treatment, but not with methadone dose [29]. This result was not replicated by other studies [27,28,30]. An *ANKK1-DRD2* haplotype that includes rs1800497 was associated with methadone dose in Han Chinese patients [26]. Unfortunately, the cluster separation of this SNP was not optimal in this platform and it was excluded from analysis. In addition, Doehring *et al.* reported that the *DRD2* rs6275 SNP modulated methadone treatment response in Caucasians. This SNP was not included in this array [30].

This study suggests an association of the rs558025 SNP, in the 3' flanking region of *OPRM1*, with methadone dose. Preliminary evidence for association between this SNP and anxious attachment and aggression scores in patients with borderline personality disorder was reported [84]. The major form of the μ -opioid receptor, the primary site of action of methadone, is encoded by four constitutive exons but several alternatively spliced forms have been described in rodents and humans [85,86]. The SNP indicated in this study, rs558025, is in strong LD ($r^2 = 0.7$) with the rs660756 SNP that is located in intron 3 in the major form of the gene and in the alternative exon Y [85,87]. Genetic variability in alternative exons that were found to be expressed in a tissue-specific manner under specific conditions may lead to phenotypic differences.

No association of methadone dose with the *OPRM1* nonsynonymous variant rs1799971 (118A>G) has been found in this study, in concordance with other studies of MMT [26,27]. This variant was shown to remove a potential *N*-glycosylation site in the extracellular domain, to be more potent in β -endorphin binding and receptor activity, and to reduce receptor signaling efficacy [88–90]. A large number of association studies of this SNP across various phenotypes were reported, including association with opioid addiction, alcohol dependence, stress responsivity and treatment responses (for recent reviews see [90,91]). One of these studies reported association of 118A>G with methadone dose only in a multigene analysis [26]. Barratt *et al.* reported a significant association of this SNP with high methadone doses only when controlling for opposing effects of *ABCB1* SNPs [92].

We previously reported that homozygosity of the *NGFB* rs2239622 SNP is associated with a lower methadone dose requirement [23]. In this study, homozygosity for the rs2239622 results in a mean dose that was lower than those of heterozygotes and noncarriers, but the difference only reached borderline significance. Additional studies will be necessary to further evaluate the importance of this association. This study did not replicate a report indicating that homozygosity for the variant alleles of the *ARRB2* rs3786047, rs1045280 and rs2036657 SNPs confers a nonresponding phenotype in a European population [25], but the two studies are differently designed.

Adequate methadone dosing in MMT for opioid addiction is critical for therapeutic success, and high doses may be required for optimal treatment of some patients. Currently, methadone is administered by trial and error, and a large proportion of patients do not benefit from the protocols still used in many clinics that limit the maximum methadone doses. Knowledge of a patient's genotype and the frequency of specific variants in a specific

population may be helpful to the treating physician. As indicated by previous studies (e.g., [26,92]), a combination of additive and opposing pharmacogenetic effects may exist in the same individual and it may be necessary to consider them in a combined analysis when assessing their effect. This may explain some of the differences in the results in different studies using individual SNP analyses. To assess the clinical relevance of these data to the clinic [93], we asked whether five SNPs with the most significant association results can cumulatively predict if a patient will require a dose >120 mg. This cutoff was selected since most early guidelines suggest 80–120 mg/day to achieve optimum effect, and in Israel, permission is still required for the administration of dose >120 mg/day. We showed that logistic regression can significantly discriminate between high (>120 mg) and low doses with a sensitivity of 0.72 and a specificity of 0.48. For this assessment we have used ROC analysis that has been widely applied to assessing the accuracy of diagnostic tests and to assess treatment effect size [94,95]. This preliminary analysis demonstrates some of the promises and limitations of this tool. The modest results also enforce the fact that pharmacogenetic markers alone may not achieve high sensitivity and specificity but they can still be useful for the clinician. For example, the label of warfarin (Coumadin®) now recommends lower initial doses in patients with certain SNPs in *CYP2C9* or *VKORC1* [96].

Although this study focused on MMT for opioid addiction, its findings may be relevant to other fields. Methadone is also widely used for severe acute and chronic pain, and individual differences in the effectiveness of methadone and its side effects limit its therapeutic success [97]. However, the major difference in side effects may depend on whether the patient is opioid dependent owing to chronic treatment or is opioid naive. In addition, some of the SNPs indicated in this study may be associated with other phenotypes and response to other medications that are modulated by similar factors.

Conclusion

This study found nominal associations of several variants in genes encoding potential pharmacodynamic factors of methadone with methadone dose required for effective MMT. The results should be treated as preliminary, as none of the signals survive correction for multiple testing. The results may also be population specific. Further studies will be required to replicate these results in diverse populations and clinics using different protocols, to verify the nature of the effect and the relative and aggregate contribution of these variants to methadone dose and response to methadone, and to assess the potential clinical applications of these findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Papers of special note have been highlighted as:

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Executive summary

Background

- A major challenge of methadone maintenance treatment for opioid addiction is the large interindividual variability in the dose required for optimal effective treatment.
- This interindividual variability may be influenced by genetic variations.
- This study was designed to identify variants in genes encoding potential pharmacodynamic factors of methadone that may explain some of this interindividual variability.

Materials & methods

- The sample consisted of 227 former heroin addicts from a methadone maintenance treatment clinic in Israel who were stabilized with methadone.
- Eleven genes (110 SNPs) were analyzed based on their methadone-related functions or previous reports of association with methadone response.
- One-way analysis of variance was performed to determine if the mean levels of the daily doses are significantly different among subjects with different genotypes.

Results

- Point-wise significant associations with methadone dose were found for 12 SNPs in five genes: *OPRM1*, *BDNF*, *NTRK2*, *DRD2* and *ANKK1* (nominal $p < 0.05$), including two SNPs with known functionality (*ANKK1* rs7118900 and *DRD2* rs2283265).
- Logistic regression of a set of five SNPs significantly discriminated between high (>120 mg) and low doses with a sensitivity of 0.72 and a specificity of 0.48.

Conclusion

- Further studies will be required to replicate these results and to verify the nature of the effect and the aggregate contribution of these variants for effective methadone dose, and to assess the potential clinical application of these findings.

Figure 1. Distribution of the methadone doses
Arrow represents the mean.

Figure 2. Receiver operating characteristic curve showing the predictive power of the five selected SNPs (AUC = 0.63)

The diagonal line represents no predictive ability (AUC = 0.5).

ROC: Receiver operating characteristic.

Table 1

Genes analyzed in the study.

Gene symbol	Gene name	SNPs (n)
<i>Opioidergic pathway</i>		
<i>OPRM1</i>	μ -opioid receptor	23
<i>POMC</i>	Proopiomelanocortin	7
<i>ARRB2</i>	β -arrestin 2	5
<i>Glutamatergic pathway</i>		
<i>GRIN1</i>	Glutamate receptor, ionotropic, <i>N</i> -methyl D-aspartate 1	3
<i>GRIN2A</i>	Glutamate receptor, ionotropic, <i>N</i> -methyl D-aspartate 2A	16
<i>Dopaminergic pathway</i>		
<i>DRD2</i>	Dopamine receptor D2	12
<i>ANKK1</i>	Ankyrin repeat and kinase domain containing 1	3
<i>Neurotrophins family</i>		
<i>NGFB</i>	Nerve growth factor, β polypeptide	10
<i>BDNF</i>	Brain-derived neurotrophic factor	13
<i>NTRK1</i>	Neurotrophic tyrosine kinase, receptor, type 1	3
<i>NTRK2</i>	Neurotrophic tyrosine kinase, receptor, type 2	15

Table 2

Details of SNPs associated with methadone dose.

Gene	SNP	Chr.	Position build 37.3	MAF	Gene location	Protein change
<i>BDNF</i>	rs10835210	11	27695910	0.44 [†]	Intron 8	
	rs7934165	11	27731983	0.49 [†]	Intron 3	
	rs1491850	11	27749725	0.37 [†]	5' upstream	
<i>NTRK2</i>	rs2120266	9	87456351	0.14	Intron 15	
	rs2289658	9	87563370	0.05	Intron 19	
	rs4358872	9	87600969	0.46	Intron 21	
	rs1948308	9	87616257	0.45	Intron 21	
	rs2378676	9	87619423	0.46	Intron 21	
	rs4877900	9	87632318	0.43	Intron 21	
<i>OPRM1</i>	rs558025	6	154441965	0.24	3' downstream	
<i>ANKK1</i>	rs7118900	11	113266821	0.15	Exon 5	Ala239Thr
<i>DRD2</i>	rs2283265	11	113285536	0.11	Intron 5	

[†]The MAF of these SNPs is slightly different than those of European populations (see text).

Chr.: Chromosome; MAF: Minor allele frequency.

Table 3

Mean methadone doses by genotype groups.

Gene	SNP	Genotype [‡]	n	Freq.	Mean	SD	p-value	Adj. p-value	r ² >0.7 with SNP in the study
<i>BDNF</i>	rs1491850	CC	31	0.14	125.1↓	44.9	0.049 [‡]	0.049	
		TC	104	0.46	138.8	52.0			
		TT	92	0.41	149.2	51.0			
	rs7934165	CC	57	0.25	133.8	43.3	0.036 [‡]	0.077	rs10835210
		TC	108	0.48	138.4	57.2			
		TT	62	0.27	152.7↑	45.0			
	rs10835210	CC	45	0.20	158.7↑	47.8	0.010 [‡]	0.023	rs7934165
		AC	111	0.49	138.5	55.2			
		AA	71	0.31	134.1	44.3			
<i>NTRK2</i>	rs2120266	AA	5	0.02	191.0↑	95.6	0.027 [‡]	0.060	
		GA	55	0.24	132.5	40.5			
		GG	167	0.74	142.5	52.0			
	rs2289658	CC	1	0.004	165.0↑	0.0	0.009 [§]	0.015	
		TC	22	0.10	167.7↑	52.6			
		TT	204	0.90	138.2	50.4			
	rs4358872	GG	49	0.22	123.9↓	53.4	0.008 [‡]	0.017	rs1948308
		TG	113	0.50	145.0	44.6			rs2378676
		TT	65	0.29	147.4	57.6			rs4877900
	rs1948308	GG	51	0.22	126.3↓	50.5	0.019 [‡]	0.041	rs4358872
		AG	104	0.46	145.0	46.5			rs2378676
		AA	72	0.32	146.0	56.4			rs4877900
	rs2378676	TT	51	0.22	126.3↓	50.5	0.019 [‡]	0.041	rs4358872
		GT	105	0.46	145.4	46.5			rs1948308
		GG	71	0.31	145.4	56.6			rs4877900
	rs4877900	CC	46	0.20	125.9↓	52.7	0.024 [‡]	0.052	rs4358872
		TC	105	0.46	144.8	46.7			rs1948308

Gene	SNP	Genotype [†]	n	Freq.	Mean	SD	p-value	Adj. p-value	r ² >0.7 with SNP in the study
OPRM1	rs558025	TT	76	0.33	145.3	54.8			rs2378676
		GG	14	0.06	131.6↓	61.4	0.028§	0.060	rs660756
		AG	80	0.35	132.4↓	50.7			
		AA	133	0.59	147.4	49.7			
DRD2	rs2283265	TT	4	0.02	121.2↓	51.4	0.0496§	0.102	
		GT	40	0.18	128.2↓	53.2			
		GG	183	0.81	144.4	50.4			
ANKK1	rs7118900	AA	7	0.03	119.0↓	57.1	0.014§	0.028	
		GA	54	0.24	128.5↓	51.7			
		GG	166	0.73	146.2	50.1			
NGFB	rs2239622	TT	26	0.12	123.5↓	52.4	0.057 [‡]		
		CT	99	0.44	146.1	51.6			
		CC	101	0.45	141.4	50.0			

Adjusted 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 (codominant, dominant and recessive).

[†]The genotype of homozygosity for the minor (less frequent) allele in this sample is listed first.

[‡]Recessive model.

[§]Dominant model.

↓: Decrease; ↑: Increase; Adj.: Adjusted; Freq.: Frequency; SD: Standard deviation.

Table 4

Odds ratio estimates of predictive factors by logistic regression.

SNP	Gene	OR	SE	95% CI	
				Lower	Upper
rs2120266	NTRK2	1.917	2.223	0.198	18.611
rs2289658	NTRK2	1.592	0.813	0.586	4.329
rs4358872	NTRK2	1.695	0.567	0.880	3.266
rs10835210	BDNF	2.142	0.848	0.986	4.655
rs7118900	ANKK1	1.755	0.548	0.951	3.236

Combined SNP analysis data: $\chi^2 = 13.5$; degrees of freedom = 5; $p = 0.019$.
OR: Odds ratio; SE: Standard error.