Nerve growth factor β polypeptide (NGFB) genetic variability: association with the methadone dose required for effective maintenance treatment

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Opioid addiction is a chronic disease with high genetic contribution and a large inter-individual variability in therapeutic response. The goal of this study was to identify pharmacodynamic factors that modulate methadone dose requirement. The neurotrophin family is involved in neural plasticity, learning, memory and behavior and deregulated neural plasticity may underlie the pathophysiology of drug addiction. Brain-derived neurotrophic factor (BDNF) was shown to affect the response to methadone maintenance treatment. This study explores the effects of polymorphisms in the nerve growth factor (β polypeptide) gene, NGFB, on the methadone doses required for successful maintenance treatment for heroin addiction. Genotypes of 14 NGFB polymorphisms were analyzed for association with the stabilizing methadone dose in 72 former severe heroin addicts with no major co-medications. There was significant difference in methadone doses required by subjects with different genotypes of the NGFB intronic single-nucleotide polymorphism rs2239622 (P = 0.0002). These results may have clinical importance.

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Introduction

Heroin addiction is a chronic disease characterized by compulsive drug seeking, drug abuse, physical dependence and tolerance.1 The genetic contribution to vulnerability to develop heroin addiction is estimated at 40–60%.2–4 Methadone, the major pharmacotherapy of opiate addiction, is a mu-opioid receptor full agonist and a moderate non-competitive N-methyl-D-aspartic acid receptor antagonist. Adequate doses of methadone are an important factor of successful treatment.5,6 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. The half-life of the racemic mixture in humans ranges from 16 to 28 h.7 Methadone is metabolized primarily by CYP3A4, CYP2B6 and CYP2D6.8 The large inter-individual variability in the therapeutic response to methadone may be influenced by genetic background (for example variants in the genes encoding metabolizing enzymes, drug transporters, drug targets and regulatory factors).9
The neutrophin family consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and neurotrophins 3–7 that are involved in neuron development and differentiation as well as maintenance of neuronal systems, modulation of neurotransmission and higher-order activities like learning, memory and behavior. They are synthesized as precursors that are proteolytically cleaved to active neurotrophins. The pathophysiology of drug addiction may be a result of a deregulation of synaptic plasticity that is caused by altered expression or binding affinity of neurotrophins. BDNF and its receptor, TrkB, induce glutamate release and also regulate GABAergic synapses. Recent studies have linked BDNF polymorphisms with memory impairments and susceptibility to psychiatric disorders and personality traits, as well as polysubstance abuse and heroin dependence in Asian males. In addition, BDNF was suggested to confer differential susceptibility to methadone maintenance treatment (MMT) response in opioid addicts. Animal studies demonstrated that BDNF regulates the mesolimbic dopamine pathway and is involved in the response to aversive social experiences. There are only a few studies of the effect of NGFB genetic variability in humans, mostly related to insensitivity to pain. NGFB variations were shown to be associated with anxiety, in a gender-dependent manner. In addition, mice with central nervous system deletion of neutrophin-3 have attenuated morphine withdrawal reaction that was restored by transgene-derived overexpression of neutrophin-3.

The goal of this pharmacogenetics study is to identify pharmacodynamic genetic factors involved in response to MMT. To explore the potential effects of NGFB variation on response to methadone, we have analyzed methadone dose data from a well-characterized sample of former heroin addicts successfully stabilized in a MMT clinic in Israel. In addition, we have reexamined the results of our previous hypothesis-driven case–control association studies with heroin addiction that included NGFB single-nucleotide polymorphisms (SNPs).

**Materials and methods**

**Subjects**

The sample consisted of 72 (33 females) unrelated former severe heroin addicts in MMT from the Dr Miriam and Sheldon G Adelson Clinic for Drug Abuse, Treatment and Research, Tel Aviv, Israel. The ages ranged from 18 to 65 years (mean 38 years). All subjects had one or more years of daily multiple uses of heroin and at least one withdrawal or failure in a detoxification center and at least 6 months in MMT with stable methadone dose for at least 4 weeks. Patients underwent repeated random and observed urine tests and had negative urine for illicit opiates, cocaine or benzodiazepines for at least 4 weeks before obtaining blood specimens for methadone plasma level. Patients with major co-medications were excluded. Patients with specific medications that are not known to affect methadone metabolism or drugs metabolized in a related way (for example acetylsalicylic acid, metformin, statin) and patients on prescribed benzodiazepines were included. All subjects signed informed consent for genetic studies. The studies were approved by the Helsinki Committee of Tel Aviv Sourasky Medical Center and the Institutional Review Board of The Rockefeller University Hospital.

The samples for the case–control association studies with heroin addiction are described in detail elsewhere. Briefly, the Caucasian sample consisted of 350 former severe heroin addicts in MMT and 184 controls, and the African American sample consisted of 207 cases and 167 controls. All subjects were from the United States.

**SNPs and ancestry informative markers genotyping**

Genomic DNA was extracted from whole-blood samples using standard techniques. Genotyping was performed on a 1536-plex GoldenGate Custom Panel (G50007064-OPA, Illumina, San Diego, CA, USA) as described and analyzed by Genome Studio software genotyping Module Version 1.0.10 (Illumina). The SNPs selected for this custom array are tag SNPs (based on HapMap data), non-synonymous SNPs and SNPs with the potential to alter splicing efficiency. Genotype data were filtered based on call rates and cluster separation. Ten percent of the sample was genotyped in duplicate.

In all, 186 ancestry informative markers were genotyped as described and 168 SNPs with adequate quality were selected for further analysis. Biographic Ancestry Scores (fractions of genetic affiliation of the individual in each of a predetermined number of clusters) were estimated by Structure 2.0 with K = 7, using 1051 CEPH subjects represented in the HGDP-CEPH (Human Genome Diversity Cell Line Panel) as reference.

**Statistical analyses**

Linkage disequilibrium (LD) (D' and r2) was estimated using R and Haploview version 4.2. Analysis of variance was performed to determine if the mean levels of the daily methadone doses were significantly different among genotypes for each of the SNPs. Analysis was also performed with ethnicity, age and co-medication as co-variates in the model. In a separate analysis, analysis of variance was also performed with the homozygosity for the ABCB1 SNP rs1236T allele, and the two CYP2B6 SNPs as co-variates in the model.

**Results**

The NGFB gene (NM_002506.2) is located on chromosome 1p13.1 and consists of three exons encoding the preproNGF transcript. The complete proNGF protein is encoded by exon 3. Ten SNPs are described in the coding region (National Center for Biotechnology Information), of which three are synonymous and only one non-synonymous SNP (rs6330) is common. A total of 15 polymorphisms were genotyped for this study, of which three are non-synonymous, and the rest are intronic tag SNPs and SNPs from the 5’ region of the gene. One SNP (rs10776799)
had a low cluster separation score and was excluded from further analysis (Table 1; Supplementary Table S1). Three additional SNPs were excluded from analysis: two non-synonymous SNPs (rs11466110 and rs11466112) were monomorphic, and one SNP (rs6326) had very low minor allele frequency (MAF < 0.01). Observed genotype distributions were consistent with Hardy–Weinberg equilibrium.

The sample details for this study are described in Table 2 (see also Materials and methods). The stabilizing daily methadone dose ranges from 12.5 to 260 mg, with a mean of 140 ± 52 mg and normal distribution. There is no significant gender difference in the methadone level (P = 0.32) and the non-carriers (153 and 140 mg, respectively) (81.7 mg) were lower than those of the heterozygotes and doses in subjects homozygous for the variant A allele of SNP rs2239622 (Figure 1). The mean daily methadone dose in subjects with different genotype groups was 119 ± 51 mg (P = 0.015). The subjects that are homozygous (AA) for SNP rs2239622 had a lower methadone dose (115 ± 49 mg) than those of the heterozygotes and non-carriers (153 and 140 mg, respectively) (P = 0.0002 for genotype test with recessive mode). The range of the daily methadone doses in this group was 25–135 mg. Analysis of the data for SNP rs2239622 by three dose groups (< 80 mg, 80–149, > 149) resulted in similar results (P = 0.0015). The subjects that are homozygous (AA) for SNP rs2239622 include two Ashkenazi Jews, a non-Jewish Caucasian and six non-Ashkenazi Jews, so this genotype group is not limited to a specific subgroup. The results were comparable when ethnicity, age and co-medication were used as co-variates in the model (P = 0.0004). No significant differences in the mean trough plasma levels between the genotype groups were found (data not shown).

We have previously reported an association of homozygosity T/T for SNP 1236C>T (rs1128503) in the P-glycoprotein encoding gene, ABCB1, with high methadone doses (> 150 mg per day). In addition, we have recently found that carriers of two CYP2B6 SNPs require relatively lower methadone doses (Levran et al., manuscript in preparation). To account for a potential multiple gene effect, we have analyzed the combined data of NGFB SNP rs2239622, CYP2B6 SNPs and ABCB1 SNP rs1128503, and this analysis substantiated the original result (P = 0.0001).

### Table 1 NGFB SNPs details

| No. | SNP ID     | Alleles | Position (build 37.1) | Location | Protein |
|-----|------------|---------|-----------------------|----------|---------|
| 1   | rs3811014  | A/G     | 115882503             | 5' Near gene |
| 2   | rs4332358  | G/A     | 115876546             | 5' Near gene |
| 3   | rs6537860  | C/T     | 115856344             | Intron 1  |
| 4   | rs4529705  | C/T     | 115851491             | Intron 1  |
| 5   | rs6678788  | G/A     | 115839671             | Intron 1  |
| 6   | rs2856813  | G/A     | 115837919             | Intron 1  |
| 7   | rs2239622  | C/T     | 115837709             | Intron 1  |
| 8   | rs910330   | C/A     | 115835500             | Intron 2  |
| 9   | rs2268793  | G/A     | 115831783             | Intron 2  |
| 10  | rs6326     | C/G     | 115830461             | Intron 2  |
| 11  | rs6328     | G/T     | 115829943             | Intron 2  |
| 12  | rs6330     | C/T     | 115829313             | Exon 3    A35V |
| 13  | rs11466110 | G/A     | 115829203             | Exon 3    V72M |
| 14  | rs11466112 | C/T     | 115828756             | Exon 3    R221W |

Abbreviations: NGFB, nerve growth factor β polypeptide; SNP, single-nucleotide polymorphism.
| No. | Methadone dose (mg per day) | Trough plasma levels (ng ml⁻¹) | Ethnicity | European contribution | Middle Eastern contribution |
|-----|-----------------------------|--------------------------------|-----------|-----------------------|---------------------------|
| 1   | 12.5                        | 100                            | Non-Ashkenazi Jewish Moroccan | 0.44                  | 0.48                      |
| 2   | 25                          | 450                            | Non-Ashkenazi Jewish Moroccan | 0.04                  | 0.94                      |
| 3   | 50                          | 230                            | Non-Ashkenazi Jewish Moroccan | 0.93                  | 0.03                      |
| 4   | 55                          | 250                            | Ashkenazi Jewish               | 0.95                  | 0.02                      |
| 5   | 55                          | 280                            | Non-Ashkenazi Jewish Moroccan | 0.97                  | 0.01                      |
| 6   | 55                          | 750                            | Non-Ashkenazi Jewish Syrian, Turkish | 0.06                  | 0.85                      |
| 7   | 60                          | 180                            | Non-Ashkenazi Jewish Greek     | 0.72                  | 0.19                      |
| 8   | 60                          | 140                            | Non-Ashkenazi Jewish Moroccan, Spain | 0.03                  | 0.92                      |
| 9   | 70                          | 280                            | Ashkenazi Jewish               | 0.90                  | 0.08                      |
| 10  | 75                          | 310                            | Caucasian (non-Jewish)         | 0.02                  | 0.82                      |
| 11  | 78                          | 220                            | Non-Ashkenazi Jewish Yemenite  | 0.02                  | 0.89                      |
| 12  | 90                          | 260                            | Ashkenazi Jewish               | 0.86                  | 0.04                      |
| 13  | 90                          | 650                            | Ashkenazi Jewish               | 0.91                  | 0.05                      |
| 14  | 95                          | 260                            | Non-Ashkenazi Jewish           | 0.14                  | 0.83                      |
| 15  | 95                          | 210                            | Non-Ashkenazi Jewish Turkish   | 0.48                  | 0.40                      |
| 16  | 100                         | 840                            | Non-Ashkenazi Jewish Turkish   | 0.06                  | 0.86                      |
| 17  | 110                         | 820                            | Non-Ashkenazi Jewish Iraqi     | 0.01                  | 0.89                      |
| 18  | 110                         | 300                            | Caucasian (non-Jewish)         | 0.89                  | 0.08                      |
| 19  | 115                         | 890                            | Non-Ashkenazi Jewish Moroccan  | 0.03                  | 0.91                      |
| 20  | 115                         | 270                            | Jewish mixed                   | 0.87                  | 0.02                      |
| 21  | 115                         | 660                            | Ashkenazi Jewish               | 0.76                  | 0.02                      |
| 22  | 120                         | 500                            | Non-Ashkenazi Jewish           | 0.95                  | 0.01                      |
| 23  | 120                         | 330                            | Arab                           | 0.01                  | 0.96                      |
| 24  | 120                         | 230                            | Caucasian (non-Jewish)         | 0.93                  | 0.04                      |
| 25  | 120                         | 900                            | Non-Ashkenazi Jewish Moroccan  | 0.07                  | 0.92                      |
| 26  | 125                         | 230                            | Arab                           | 0.04                  | 0.81                      |
| 27  | 125                         | 200                            | Non-Ashkenazi Jewish Yemenite  | 0.19                  | 0.77                      |
| 28  | 130                         | 580                            | Non-Ashkenazi Jewish Iranian   | 0.76                  | 0.19                      |
| 29  | 130                         | 390                            | Non-Ashkenazi Jewish           | 0.01                  | 0.03                      |
| 30  | 130                         | 340                            | Non-Ashkenazi Jewish           | 0.08                  | 0.77                      |
| 31  | 130                         | 290                            | Caucasian (non-Jewish)         | 0.93                  | 0.03                      |
| 32  | 135                         | 330                            | Ashkenazi Jewish               | 0.92                  | 0.05                      |
| 33  | 135                         | 340                            | Non-Ashkenazi Jewish Yemenite  | 0.73                  | 0.26                      |
| 34  | 135                         | 100                            | Non-Ashkenazi Jewish Moroccan  | 0.02                  | 0.07                      |
| 35  | 135                         | 940                            | Non-Ashkenazi Jewish Libyan     | 0.39                  | 0.41                      |
| 36  | 140                         | 1220                           | Ashkenazi Jewish               | 0.37                  | 0.56                      |
| 37  | 140                         | 630                            | Non-Ashkenazi Jewish Iraqi     | 0.97                  | 0.02                      |
| 38  | 140                         | 630                            | Non-Ashkenazi Jewish Moroccan  | 0.03                  | 0.89                      |
| 39  | 140                         | 450                            | Ashkenazi Jewish               | 0.98                  | 0.01                      |
| 40  | 140                         | 230                            | Jewish mixed                   | 0.28                  | 0.17                      |
| 41  | 140                         | 600                            | Ashkenazi Jewish               | 0.96                  | 0.02                      |
| 42  | 140                         | 850                            | Ashkenazi Jewish               | 0.80                  | 0.14                      |
| 43  | 145                         | 780                            | Jewish mixed                   | 0.93                  | 0.04                      |
| 44  | 150                         | 250                            | Non-Ashkenazi Jewish Turkish, Iraqi | 0.01                  | 0.96                      |
| 45  | 150                         | 450                            | Arab                           | 0.58                  | 0.33                      |
| 46  | 150                         | 340                            | Arab                           | 0.01                  | 0.85                      |
| 47  | 160                         | 110                            | Non-Ashkenazi Jewish Moroccan  | 0.57                  | 0.30                      |
| 48  | 160                         | 1000                           | Non-Ashkenazi Jewish Yemenite  | 0.34                  | 0.54                      |
| 49  | 165                         | 540                            | Non-Ashkenazi Jewish           | 0.08                  | 0.66                      |
| 50  | 165                         | 640                            | Ashkenazi Jewish               | 0.03                  | 0.90                      |
| 51  | 170                         | 400                            | Caucasian (non-Jewish)         | 0.39                  | 0.44                      |
| 52  | 170                         | 500                            | Ashkenazi Jewish               | 0.91                  | 0.02                      |
| 53  | 175                         | 290                            | Non-Ashkenazi Jewish Turkish   | 0.66                  | 0.27                      |
| 54  | 180                         | 430                            | Caucasian (non-Jewish)         | 0.22                  | 0.68                      |
| 55  | 183                         | 600                            | Ashkenazi Jewish               | 0.54                  | 0.10                      |
| 56  | 185                         | 490                            | Jewish unknown                 | 0.01                  | 0.87                      |
| 57  | 187.5                       | 440                            | Ashkenazi Jewish               | 0.50                  | 0.45                      |
| 58  | 190                         | 820                            | Non-Ashkenazi Jewish           | 0.95                  | 0.01                      |
The list is sorted by ascending methadone dose.

| No. | Methadone dose (mg per day) | Trough plasma levels (ng ml⁻¹) | Ethnicity | European contribution* | Middle Eastern contribution* |
|-----|-----------------------------|-------------------------------|-----------|------------------------|-----------------------------|
| 59  | 190                         | 350                           | Non-Ashkenazi Jewish Syrian | 0.02                  | 0.92                        |
| 60  | 190                         | 1210                          | Non-Ashkenazi Jewish       | 0.96                  | 0.01                        |
| 61  | 190                         | 380                           | Non-Ashkenazi Jewish       | 0.88                  | 0.09                        |
| 62  | 190                         | 940                           | Ashkenazi Jewish           | 0.37                  | 0.56                        |
| 63  | 190                         | 520                           | Non-Ashkenazi Jewish Yemenite | 0.09                 | 0.85                        |
| 64  | 195                         | 490                           | Non-Ashkenazi Jewish Moroccan | 0.96                | 0.02                        |
| 65  | 200                         | 750                           | Non-Ashkenazi Jewish Yemenite | 0.05                 | 0.93                        |
| 66  | 205                         | 390                           | Ashkenazi Jewish           | 0.43                  | 0.29                        |
| 67  | 220                         | 600                           | Ashkenazi Jewish           | 0.94                  | 0.01                        |
| 68  | 220                         | 700                           | Ashkenazi Jewish           | 0.96                  | 0.03                        |
| 69  | 225                         | 670                           | Non-Ashkenazi Jewish Moroccan | 0.95                | 0.01                        |
| 70  | 230                         | 580                           | Non-Ashkenazi Jewish       | 0.12                  | 0.78                        |
| 71  | 240                         | 680                           | Non-Ashkenazi Jewish Yemenite | 0.11                | 0.80                        |
| 72  | 260                         | 1070                          | Jewish mixed              | 0.67                  | 0.25                        |

Abbreviation: AIMs, ancestry informative markers.

*The proportion of ancestral contribution was calculated with AIMs data (see Materials and methods). Only the two major contributors (European and Middle East), out of seven calculated, are shown.

The list is sorted by ascending methadone dose.

Table 3 Allele frequencies of the NGFB SNPs in different populations

| No. SNP ID | Israeli sample* | European Americans 23 | HapMap |
|------------|-----------------|------------------------|--------|
|            | n = 72 Cases n = 350h | Controls n = 184 CEU YRI CHB |
| 1 rs3811014 | 0.16 0.18 0.21 0.16 | 0.60f 0.16 |
| 2 rs4332358 | 0.45 0.28 0.36 0.23 | 0.70f 0.57f |
| 3 rs6537860 | 0.41 0.31 0.34 0.30 | 0.65f 0.81 |
| 4 rs4529705 | 0.42 0.31 0.34 0.40 | 0.63f 0.80f |
| 5 rs6678788 | 0.39 0.30 0.31 0.29 | 0.43 0.49 |
| 6 rs2856813 | 0.42 0.46 0.48 0.48 | 0.89f 0.70f |
| 7 rs2239622 | 0.38 0.29 0.30 0.28 | 0.19 0.46 |
| 8 rs910330 | 0.39 0.30 0.31 0.29 | 0.25 0.49 |
| 9 rs2268793 | 0.12 0.07 0.10 0.07 | 0.02 0.30 |
| 10 rs6326 | 0.01 0.01 0.01 0.01 | 0.31h 0.00 |
| 11 rs6328 | 0.42 0.37 0.36 0.34 | 0.29 0.53f |
| 12 rs6330 | 0.37 0.43 0.40 0.40 | 0.16 0.13 |
| 13 rs11466110 | 0.00 0.00 0.00 0.01 | 0.04 0.00 |
| 14 rs11466112 | 0.00 0.00 0.00 0.00 | 0.00 0.00 |

Abbreviations: NGFB, nerve growth factor β polypeptide; SNP, single-nucleotide polymorphism.

*This study.

hThe proportion of ancestral contribution was calculated with AIMs data (see Materials and methods). Only the two major contributors (European and Middle East), out of seven calculated, are shown.

fThe minor allele in CEU is the major allele in YRI or CHB.

gOther NCBI Caucasian population (no data in HapMap).

eHan Chinese.

dAfricans from Yoruba.

Revisiting the hypothesis-driven case–control association study with heroin addiction

We have previously reported a hypothesis-driven case–control association study of 130 genes.21,24 The finding of association of the NGFB variant with stabilizing methadone dose prompted us to reexamine the results of these studies, since only a limited number of SNPs that gave the lowest P-values in the association test were originally reported. These studies were performed on the same array as the current study, so the same NGFB SNPs were genotyped. Intriguingly, the result for NGFB SNP rs4332358 was just slightly above the cutoff chosen in the original study. The frequency of subjects with the G/G genotype among the heroin addicts was nominally significantly higher than that of controls (P = 0.003, odds ratio = 1.73, 95% confidence interval = 1.18, 2.53, for a genotype test with a recessive mode; see also Table 3 for the differences in allele frequency between cases and controls). The minor allele ‘A’ may be considered a protective allele. Notably, this allele is the major allele in the African population (Table 3). The results for the rest of the NGFB SNPs were not significant.

No significant associations of any NGFB SNPs were found in African Americans. Of importance, ancestry informative markers data revealed that only 2% of the Caucasian subjects and none of the African American subjects in the two previous studies have a major Middle Eastern contribution (> 0.75) (Levran et al., manuscript in preparation).

LD analysis

Most of the SNPs genotyped in this study were chosen as tagging SNPs26 and are expected to show low LD and represent different haplotypes. Analysis of the genotype data revealed high LD between the following SNPs: rs910330 and rs6678788 (D’ = 0.97, r² = 0.94), rs2239622 and rs6678788 (D’ = 0.81, r² = 0.64), rs910330 and rs2239622 (D’ = 0.81, r² = 0.64), and rs4529705 and rs6537860 (D’ = 0.10, r² = 0.97). These results are compatible with those of the HapMap Caucasian population. Listed in Supplementary Table S2 are HapMap SNPs that are tagged by SNPs from...
this study (D’>0.8, r²>0.6) in the HapMap Caucasian population. There are no data available for SNPs rs4529705 and rs6326 in HapMap. Notably, the non-synonymous SNP rs6330 is in complete LD with SNP rs6327 located at a distance of 673 bp in intron 2 that was not analyzed in this study. SNPs rs2239622 and rs4332358, indicated in this study for association with methadone dose or heroin addiction, respectively, are in high LD with several SNPs in intron 1 and 2.

### Discussion

One of the mechanisms underlying drug addiction may be due to synaptic plasticity changes, which may be a result of alterations in the expression of genes encoding neurotrophins and their receptors, among other genes.31 The NGFB gene consists of three exons encoding the preproNGF transcript. After removal of the signal peptide, the precursor generates proNGF. The complete proNGF protein is encoded by exon 3 and undergoes further post-translational processing to generate a mature product. It was demonstrated that proNGF is the predominant form of NGF in human and rodent brain tissue, suggesting that proNGF may have independent biological activity.32,33 The effect of NGF is mediated by tyrosine kinase receptor NTRK1 (TrkA) and the cytokine p75 neurotrophin receptor through activation of intracellular signaling cascades that regulate several processes including gene expression. Numerous stimuli including stress cause dynamic modulation of NGF and NGF receptor expression.34 NGF also modulates the neuronal and inflammatory component of pain.33 NGF was suggested to function as a molecular switch that redirects the δ-opioid receptor (OPRD1) to the surface membrane of central synaptic terminals under chronic opioid conditions.35 OPRD1 has an important role in pain control36 and is also interacting with OPRM1.37 An NGF-responsive

| No. | SNP ID     | Genotype | n  | Mean methadone dose | SE  | A/A | n  | Mean methadone dose | SE  | B/B |
|-----|------------|----------|----|---------------------|-----|-----|----|---------------------|-----|-----|
| 1   | rs3811014  |          | 49 | 138.3               | 8.3 |     | 23 | 141.5               | 7.6 |     |
| 2   | rs4332358  |          | 19 | 115.5               | 12.3|     | 40 | 151.3               | 7.2 |     |
| 3   | rs6537680  |          | 23 | 127.7               | 11.3|     | 39 | 151.9               | 7.6 |     |
| 4   | rs4529705  |          | 22 | 127.1               | 11.9|     | 40 | 151.6               | 7.4 | 12  |
| 5   | rs6678788  |          | 24 | 138.5               | 10.7|     | 40 | 147.1               | 7.6 | 10  |
| 6   | rs2856813  |          | 22 | 123.0               | 11.6|     | 39 | 154.3               | 6.7 | 11  |
| 7   | rs2239622  |          | 26 | 139.7               | 9.9 | 37  | 153.1               | 7.9 |     |
| 8   | rs910330   |          | 25 | 139.3               | 10.3|     | 38 | 145.6               | 7.9 | 9   |
| 9   | rs2268793  |          | 57 | 137.4               | 6.9 | 13  | 149.6               | 6.2 | 2   |
| 10  | rs6326     |          | 70 | 123.0               | 11.6|     | 2  | 127.5               | 7.5 | NA  |
| 11  | rs6328     |          | 25 | 134.9               | 12.0|     | 34 | 153.4               | 6.4 | 13  |
| 12  | rs6330     |          | 29 | 134.8               | 9.3 | 33  | 144.1               | 8.5 | 10  |
| 13  | rs11466110 |          | 0  | 0                   |     | 0   | 0  | 0                   |     | 0   |
| 14  | rs11466112 |          | 0  | 0                   |     | 0   | 0  | 0                   |     | 0   |

**Abbreviations:** n, number of subjects; NA, not applicable; SE, standard error; SNP, single-nucleotide polymorphism.

- **A’** represents the more common allele and ‘B’ represents the less common variant
- Genotype test with co-dominant mode.
- P = 0.0002 for genotype test with recessive mode.

The data in bold is for the SNP with the most significant results.
region was identified in the rodent oprd1 promoter.\textsuperscript{38,39} Perinatal methadone exposure was shown to reduce rat striatal NGF content, but not mRNA levels, suggesting regulation on other levels (for example processing, stability or release).\textsuperscript{40} Chronic alcohol exposure has been shown to downregulate human plasma NGF with a greater decrease in patients with family history of alcohol dependency.\textsuperscript{41}

Individual methadone dosage optimization is one of the key factors in effective MMT, since methadone has large inter-individual variability in response and a narrow therapeutic index. Several pharmacogenetics studies aimed to identify pharmacodynamic genetic factors that modulate response to methadone maintenance were reported.\textsuperscript{30,42–50} BDNF was suggested to confer differential susceptibility to MMT response in opioid addicts.\textsuperscript{16} The main findings of these earlier studies are that variants in ABCB1, OPRM1, dopamine D2 receptor (DRD2), BDNF and potassium inwardly-rectifying channel KCNJ6 may be related to MMT response.

The major finding of this study is that the NGFB intronic variant rs2239622 is associated with relatively low methadone doses required in some patients for successful treatment of opiate addiction, in an Israeli population with Caucasian and Middle Eastern ancestry. The mean daily methadone doses in subjects homozygous for the variant A allele was 81.7 mg (range 25–135 mg) compared with 148 mg in heterozygotes and non-carriers. This is the first study associating NGFB variation with methadone dose requirement. The effect of NGFB on MMT response is not known and may be related to alteration in NGFB expression and/or function that leads to alteration in neural plasticity. The biological effect of the specific intronic variant is not currently known and it may be a marker for another functional SNP or a haplotype. This is the first step toward identifying specific gene variants associated with dose requirements that may possibly allow prediction of individual response to MMT, which is of clinical significance.

NGFB variants are unlikely to be acting alone in modulating the response to methadone, and multivariate analysis reflects more realistically the potential contribution of several genetic factors to dose requirement. For example, an NGFB variant was shown to modify the risk for eating disorders conferred by the risk genotype of a SNP in the neurotrophin receptor gene NTRK3, suggesting epistatic interaction.\textsuperscript{51} Methadone is a substrate of the efflux transporter P-glycoprotein that is encoded by the ABCB1 gene. We have previously reported that homozygosity to the T allele of ABCB1 SNP 1236C>T is associated with higher methadone doses.\textsuperscript{30} In addition, we have recently found that carriers of two variants in the gene encoding cytochrome P450 methadone metabolizing enzyme CYP2B6 require relatively lower methadone doses (Levran et al., manuscript in preparation). An analysis of NGFB SNP rs2239622 with the three SNPs mentioned above as co-variates substantiated the significance of the results obtained in the single SNP analysis.

The efficacy of methadone is significantly altered by several medications that are often consumed by MMT patients (for example treatments for HIV/AIDS and affective disorders).\textsuperscript{52,53} To eliminate the effect of other drugs on the results, subjects selected for this study were, for the most part, not receiving other prescribed medication and none had evidence of ongoing drug abuse.

The significant variation in allele frequency of NGFB SNPs among different populations, as demonstrated in HapMap, and elucidated to a limited extent in this study, may have clinical relevance and may reflect an evolutionary dynamic process of selective advantage or genetic drift. It is especially relevant for admixed populations such as these in Israel. There is high genetic similarity between the Jewish and the Caucasian populations, but also some differences that may be explained by the complex demographic history of the Jewish people.\textsuperscript{54,55} If the results from this small non-randomly-selected sample reflect the allele frequencies of this population, the NGFB gene is one of the genes in which Jewish people and/or Middle Eastern populations differ from Caucasians.

This study has several limitations: (1) small sample size; (2) a relatively large number of SNPs have been analyzed, increasing the risk for type I error; (3) all the patients are recruited from one clinic in one country; and (4) the sample ethnicity is a quite unique admixture of Caucasian and Middle Eastern. To address these limitations, larger studies in other populations and clinics are warranted.

In summary, the NGFB SNP rs2239622 is shown to be associated with relatively low stabilizing methadone doses in patients in MMT in Israel, and NGFB SNP rs4332358 has been associated with heroin addiction in Caucasians. Further studies are necessary to confirm these results, to determine the functionality of these SNPs (or linked SNPs) and the mechanism by which they may affect methadone response. An improved understanding of the role of neuromodulators in drug addiction and its treatment may facilitate the search for effective treatment and prevention.

Conflict of interest
The authors declare no conflict of interest.

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References
1. Kreek MJ. Methadone-related opioid agonist pharmacotherapy for heroin addiction. History, recent molecular and neurochemical research and future in mainstream medicine. Ann N Y Acad Sci 2000; 909: 186–216.
2. Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. Am J Psychiatry 2003; 160: 687–695.
3. Tsuang MT, Lyons MJ, Meyer JM, Doyle T, Eisen SA, Goldberg J et al. Co-occurrence of abuse of different drugs in men: the role of

---

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drug-specific and shared vulnerabilities. Arch Gen Psychiatry 1998; 55: 967–972.

4 Tsuang MT, Lyons MJ, Eisen SA, Goldberg J, True W, Lin N et al. Genetic influences on DSM-III-R drug abuse and dependence: a study of 3,572 twin pairs. Am J Med Genet 1996; 67: 473–477.

5 Amato L, Davoli M, Perucci CA, Feni M, Faggiano F, Mattick RP. An overview of systematic reviews of the effectiveness of opiate maintenance treatments: available evidence to inform clinical practice and research. J Subst Abuse Treat 2005; 28: 321–329.

6 Kreek MJ, LaForge KS, Butelman E. Pharmacotherapy of addictions. Nat Rev Drug Discov 2002; 1: 710–726.

7 Kreek MJ, Voci F. History and current status of opioid maintenance treatments: blinding conference session. J Subst Abuse Treat 2002; 23: 93–105.

8 Zhou SF, Liu JP, Chowbay B. Polypharmacism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009; 41: 89–295.

9 Li Y, Kantelip JP, Gerritsen-van Schiepen P, Davani S. Interindividual variability of methadone response: impact of genetic polymorphism. Mol Diagn Ther 2008; 12: 109–124.

10 Chao MV, Rajagopal R, Lee FS. Neurotrophin signalling in health and disease. Clin Sci (Lond) 2006; 110: 167–173.

11 Carvalho AL, Caldeira MV, Santos SD, Duarte CB. Role of the brain-derived neurotrophic factor at glutamatergic synapses. Br J Pharmacol 2008; 153(Suppl 1): S510–S524.

12 Bolanos CA, Nestler EJ. Neurotrophic mechanisms in drug addiction. Neuroulemolecular Med 2004; 5: 69–83.

13 Cheng CY, Hong CJ, Yu YW, Chen TJ, Wu HC, Tsai SJ. Brain-derived neurotrophic factor (Val66Met) genetic polymorphism is associated with substance abuse in males. Brain Res Mol Brain Res 2005; 140: 86–90.

14 de Cid R, Fonseca F, Gratacos M, Gutierrez F, Martinez-Santos R, Estivill X et al. BDNF variability in opioid addicts and response to methadone treatment: preliminary findings. Genes Brain Behav 2008; 7: 515–522.

15 Berton O, McClung CA, Delicorne RJ, Krishnan V, Renthal W, Russo SJ et al. Essential role of BDNF in the mesolimbic dopaminergic pathway in social defeat stress. Science 2006; 311: 864–868.

16 Einarsdottir E, Carlsson A, Minde J, Toolanen G, Svensson O, Solders G et al. A mutation in the neurotrophin factor beta gene (NGFB) causes loss of pain perception. Hum Mol Genet 2004; 13: 799–805.

17 Larsson E, Kuma R, Norberg A, Minde J, Holmberg M. Nerve growth factor (R221W) responsible for insensitivity to pain is defectively processed and accumulates as proNGF. Neurobiol Dis 2009; 33: 221–228.

18 Fitzgibbon Cg, Kingston H, Needham M, Gaunt L. Haploinsufficiency of the nerve growth factor beta gene in a 1p13 deleted female child with an insensitivity to pain. Dev Med Child Neurol 2009; 51: 833–837.

19 Lang UE, Hellweg R, Bajbouj M, Lenzken KP, Sander T et al. Essential role of BDNF in the mesolimbic dopaminergic pathway in social defeat stress. Science 2006; 311: 864–868.

20 Enoch MA, Shen PH, Xu K, Hodgkinson C, Goldman D. Using ancestry-informative markers to define populations and detect population stratification. J Psychopharmacol 2006; 20: 19–26.

21 Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics 2000; 155: 945–959.

22 Barrett JC, Fry B, Maller J, Daly MJ. Haplview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263–265.

23 Levrin O, O’Hara K, Peles E, Li D, Barral S, Ray B et al. ABCB1 (MDR1) genetic variants are associated with methadone doses required for effective treatment of heroin dependence. Hum Mol Genet 2008; 17: 2219–2227.

24 McClung CA, Nestler EJ. Neuroplasticity mediated by altered gene expression. Neuropsychopharmacology 2008; 33: 3–17.

25 Fahnestock M, Michalski B, Xu B, Coughlin MD. The precursor pro-nerve growth factor is the predominant form of nerve growth factor in brain and is increased in Alzheimer’s disease. Mol Cell Neurosci 2001; 18: 210–220.

26 Pezet S, McMahone SB. Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci 2006; 29: 507–538.

27 Fiore M, Chalidakos GN, Aloe L. Nerve growth factor as a signaling molecule for nerve cells and also for the neuroendocrine-immune systems. Rev Neurosci 2009; 20: 133–145.

28 Bie B, Zhang Z, Cai YQ, Zhu W, Zhang Y, Dai J et al. Nerve growth factor regulates emergence of functional delta-opioid receptors. J Neurosci 2010; 30: 5617–5628.

29 Narita M, Kuzumaki N, Miyatake M, Sato F, Wachi H, Seyama Y et al. Role of delta-opioid receptor function in neurogenesis and neuroprotection. J Neurochem 2006; 97: 1494–1505.

30 Kieffer BL, Covarrubias-Ruff C. Exploring the opioid system by gene knockout. Prog Neurobiol 2002; 66: 285–306.

31 Chen YL, Monteith N, Law PY, Loh HH. Dynamic association of p300 with the promoter of the G protein-coupled rat delta opioid receptor gene during NGF-induced neuronal differentiation. Biochem Biophys Res Commun 2010; 396: 294–298.

32 Chen YL, Law PY, Loh HH. Action of NF-kappaB on the delta opioid receptor gene promoter. Biochem Biophys Res Commun 2007; 352: 818–822.

33 Wu VW, Mo Q, Yabe T, Schwartz JP, Robinson SE. Perinatal opioids reduce striatal nerve growth factor content in rat striatum. Eur J Pharmacol 2001; 414: 211–214.

34 Yoon SJ, Roh S, Lee H, Lee JY, Lee BH, Kim YJ et al. Possible role of nerve growth factor in the pathogenesis of alcohol dependence. Alcohol Clin Exp Res 2005; 30: 1060–1065.

35 Doehring A, Hentig N, Krause R, Blasko A, Schröder R et al. Genetic variants altering dopamine D2 receptor expression or function modulate the risk of opiate addiction and the dosage requirements of methadone substitution. Pharmacogenet Genomics 2009; 19: 407–414.

36 Crettol S, Besson J, Croquette-Krokar M, Haggmig R, Goutheyre I, Monnat M et al. Association of dopamine and opioid receptor genetic polymorphisms with response to methadone maintenance treatment. Prog Neuropsychopharmacol Biol Psychiatry 2008; 32: 1722–1727.

37 Lafwood BR, Young RM, Noble EP, Sargent J, Rowell J, Shadforth S et al. The D(2) dopamine receptor A(1) allele and opioid dependence: association with heroin use and response to methadone treatment. Am J Med Genet 2000; 96: 592–598.

38 Lutsch J, Skarke C, Wieting J, Oertel BG, Schmidt H, Brockmoller J et al. Modulation of the central nervous effects of levomethadone by genetic polymorphisms potentially affecting its metabolism, distribution, and drug action. Clin Pharmacol Ther 2006; 79: 72–89.

39 Crettol S, Deglon JJ, Besson J, Croquette-Krokar M, Haggmig R, Goutheyre I et al. ABCB1 and cytochrome P450 genotypes and phenotypes: influence on methadone plasma levels and response to treatment. Clin Pharmacol Ther 2006; 80: 668–681.

40 Callier JK, Barratt DT, Dahlen K, Loennechen MH, Somogyi AA. ABCB1 genetic variability and methadone dosage requirements in opioid-dependent individuals. Clin Pharmacol Ther 2006; 80: 682–690.

41 Fonseca F, Gratacos M, Escaramis G, De Cid R, Martin-Santos R, Fernandez-Espejo E et al. Response to methadone maintenance treatment is associated with the MYOCD and GMR6 genes. Mol Diagn Ther 2010; 14: 171–188.
Barratt DT, Coller JK, Somogyi AA. Association between the DRD2 A1 allele and response to methadone and buprenorphine maintenance treatments. *Am J Med Genet B Neuropsychiatr Genet* 2006; **141**: 323–331.

Lotsch J, Pruss H, Veh RW, Doehring A. A KCNJ6 (Kir3.2, GIRK2) gene polymorphism modulates opioid effects on analgesia and addiction but not on pupil size. *Pharmacogenet Genomics* 2010; **20**: 291–297.

Mercader JM, Saus E, Aguera Z, Bayes M, Boni C, Carreras A et al. Association of NTRK3 and its interaction with NGF suggest an altered cross-regulation of the neurotrophin signaling pathway in eating disorders. *Hum Mol Genet* 2008; **17**: 1234–1244.

Kharasch ED, Bedynek PS, Walker A, Whittington D, Hoffer C. Mechanism of ritonavir changes in methadone pharmacokinetics and pharmacodynamics: II. Ritonavir effects on CYP 3A and P glycoprotein activities. *Clin Pharmacol Ther* 2008; **84**: 506–512.

McCance-Katz EF, Sullivan LE, Nallani S. Drug interactions of clinical importance among the opioids, methadone and buprenorphine, and other frequently prescribed medications: a review. *Am J Addict* 2010; **19**: 4–16.

Need AC, Kasperaviciute D, Cirulli ET, Goldstein DB. A genome-wide genetic signature of Jewish ancestry perfectly separates individuals with and without full Jewish ancestry in a large random sample of European Americans. *Genome Biol* 2009; **10**: R7.

Behar DM, Yunusbayev B, Metspalu M, Metspalu E, Rosset S, Parik J et al. The genome-wide structure of the Jewish people. *Nature* 2010; **466**: 238–242.

Supplementary Information accompanies the paper on the The Pharmacogenomics Journal website (http://www.nature.com/tpj)