

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. *The Pharmacogenomics Journal* (2012) 12, 319–327; doi:10.1038/tpj.2011.6; published online 1 March 2011

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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

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The neurotrophin 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.^{10,11} 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.¹² BDNF and its receptor, TrkB, induce glutamate release and also regulate GABAergic synapses.¹¹ 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.^{10,13–15} In addition, *BDNF* was suggested to confer differential susceptibility to methadone maintenance treatment (MMT) response in opioid addicts.¹⁶ Animal studies demonstrated that *BDNF* regulates the mesolimbic dopamine pathway and is involved in the response to aversive social experiences.¹⁷ There are only a few studies of the effect of *NGFB* genetic variability in humans, mostly related to insensitivity to pain.^{18–20} *NGFB* variations were shown to be associated with anxiety, in a gender-dependent manner.²¹ In addition, mice with central nervous system deletion of neurotrophin-3 have attenuated morphine withdrawal reaction that was restored by transgene-derived overexpression of neurotrophin-3.²²

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).^{23,24}

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.²⁵ 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.^{23,24} 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 (GS0007064-OPA, Illumina, San Diego, CA, USA) as described^{23,26} 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.²⁶ 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.^{23,26,27} 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 2.0²⁸ 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 r^2) was estimated using R and Haploview version 4.2.²⁹ 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-variables 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-variables 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 near the gene. One SNP (rs10776799)

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	115875645	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.

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 daily dose. The mean trough plasma (R/S) methadone level is 498 ± 269 ng ml⁻¹ (range 100–1220 ng ml⁻¹), with a moderate correlation between trough plasma levels and methadone dose ($r = 0.40$). The sample consists of the 62 Jewish subjects and 10 subjects that are either Caucasians or Arabs. For simplicity, the Jewish subjects are divided into two main groups: Ashkenazi and 'non-Ashkenazi'. The 'non-Ashkenazi' group includes Moroccan, Yemenite, Iraqi, Turkish, Iranian, Libyan, Syrian, Greek subjects and subjects with mixed origin or unknown origin. Five subjects are Jewish of mixed or unknown origin. Ancestry Biographic Scores (for example fractions of genetic affiliation of the individual in each of a predetermined number of clusters) were estimated based on genotypes of 168 ancestry informative markers with data from 1051 individuals representing 51 worldwide populations as a reference.^{26,27} There is a very low contribution of African, Far East Asian, Oceanian and/or Native American populations in this Israeli sample (data not shown). The Ashkenazi Jewish group has a mainly European contribution and some Middle Eastern contribution and the non-Ashkenazi group has a Middle Eastern or European contribution (Table 2).

Listed in Table 3 are the MAF of the SNPs genotyped in this study, our previous association study in Caucasians,²³

and in three HapMap populations (<http://www.hapmap.org>). HapMap data reveal major differences in the MAF of most of the SNPs between Caucasian, Asian and African populations, out of which, for six SNPs, the minor allele in Caucasians is the major allele in the African and/or the Chinese population. The non-synonymous SNP rs6330 is less frequent in Africans, SNP rs6328 is more common in Chinese, SNP rs6326 is African-specific and rs2268793 is much more common in the Chinese population compared with the Caucasian and African populations. In general, the allele frequencies in our previous study of Caucasians are compatible with the HapMap data.

Interestingly, the MAF of several SNPs in the Israeli sample are higher than those found in the European American sample from our previous study and from those reported in the HapMap Caucasian population, but are not as high as those for the African and Asian populations (Table 3). Interestingly, the MAF of SNPs rs2239622 and rs910330 is higher in the Israeli sample than in both Caucasian and African HapMap samples and is closer to the MAF in the Chinese HapMap sample. No data are publicly available on the frequency of these SNPs in Middle Eastern populations and the significance of these findings is still in question because the sample is small and may not represent the general population because of the non-random selection.

Association of NGFB genetic variability with methadone dose

Individual SNPs were analyzed for association with stabilizing methadone dose (Table 4; Supplementary Table S1). There were significant differences in the mean daily methadone dose in subjects with different genotype groups of SNP rs2239622 (Figure 1). The mean daily methadone doses in subjects homozygous for the variant A allele (81.7 mg) were lower than those of the heterozygotes and the 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-variables 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).³⁰ 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 2 Sample details

No.	Methadone dose (mg per day)	Trough plasma levels (ng ml ⁻¹)	Ethnicity		European contribution ^a	Middle Eastern contribution ^a
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	Iraqi	0.95	0.01

Table 2 Continued

No.	Methadone dose (mg per day)	Trough plasma levels (ng ml ⁻¹)	Ethnicity		European contribution ^a	Middle Eastern contribution ^a
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	Iraqi	0.11	0.80
72	260	1070	Jewish	mixed	0.67	0.25

Abbreviation: AIMS, ancestry informative markers.

^aThe 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 ^a	European Americans ²³		HapMap		
		n = 72	Cases n = 350 ^b	Controls n = 184	CEU ^c	YRI ^d	CHB ^e
1	rs3811014	0.16	0.18	0.21	0.16	0.60^f	0.16
2	rs4332358	0.45	0.28	0.36	0.23	0.70^f	0.57^f
3	rs6537860	0.41	0.31	0.34	0.30	0.65^f	0.81^f
4	rs4529705	0.42	0.31	0.34	0.40 ^g	0.63^f	0.80^f
5	rs6678788	0.39	0.30	0.31	0.29	0.43	0.49
6	rs2856813	0.42	0.46	0.48	0.48	0.89^f	0.70^f
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	0.31^h	0.00
11	rs6328	0.42	0.37	0.36	0.34	0.29	0.53^f
12	rs6330	0.37	0.43	0.40	0.44	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	0.003	0.00

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

^aThis study.

^bSample does not include the Israeli subjects.

^cCaucasians.

^dAfricans from Yoruba.

^eHan Chinese.

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

^gOther NCBI Caucasian population (no data in HapMap).

^hAfrican specific.

Numbers in bold are significantly different than those of HapMap Caucasians.

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.^{23,24} 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) (Levrán *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 ($D'=0.97$, $r^2=0.94$), rs2239622 and rs6678788 ($D'=0.81$, $r^2=0.64$), rs910330 and rs2239622 ($D'=0.81$, $r^2=0.64$), and rs4529705 and rs6537860 ($D'=0.10$, $r^2=0.97$). 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

Table 4 Association of individual SNPs with daily methadone dose

No.	SNP ID	Genotype ^a									P-value ^b
		A/A			A/B			B/B			
		n	Mean methadone dose	SE	n	Mean methadone dose	SE	n	Mean methadone dose	SE	
1	rs3811014	49	138.3	8.3	23	141.5	7.6	0	—	—	0.8087
2	rs4332358	19	115.5	12.3	40	151.3	7.2	12	128.8	15.6	0.0311
3	rs6537860	23	127.7	11.3	39	151.9	7.6	10	117.0	18.0	0.0702
4	rs4529705	22	127.1	11.9	40	151.6	7.4	10	117.0	18.0	0.0699
5	rs6678788	24	138.5	10.7	40	147.1	7.6	8	103.1	22.1	0.0920
6	rs2856813	22	123.0	11.6	39	154.3	6.7	11	118.9	20.4	0.0268
7	rs2239622	26	139.7	9.9	37	153.1	7.9	9	81.7	11.8	0.0007^c
8	rs910330	25	139.3	10.3	38	145.6	7.9	9	112.8	21.7	0.2392
9	rs2268793	57	137.4	6.9	13	149.6	16.2	2	127.5	7.5	0.7152
10	rs6326	70			2			0			NA
11	rs6328	25	134.9	12.0	34	153.4	6.4	13	111.0	16.7	0.0367
12	rs6330	29	134.8	9.3	33	144.1	8.5	10	136.8	22.3	0.7750
13	rs11466110	0			0			0			NA
14	rs11466112	0			0			0			NA

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

^a 'A' represents the more common allele and 'B' represents the less common variant

^bGenotype test with co-dominant mode.

^cP = 0.0002 for genotype test with recessive mode.

The data in bold is for the SNP with the most significant results.

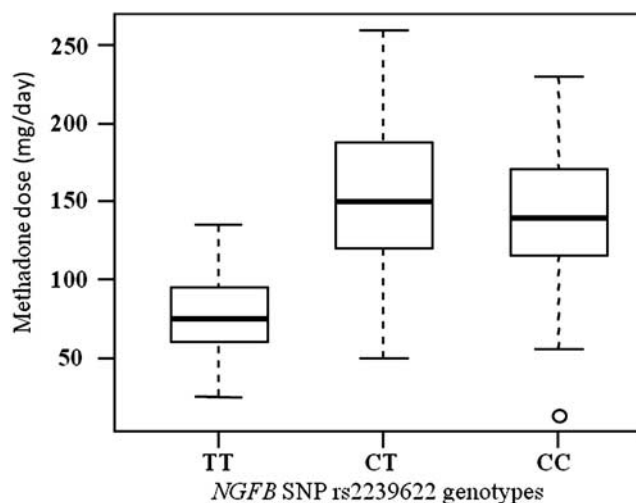


Figure 1 Association of *NGFB* SNP rs2239622 with daily methadone doses required for successful treatment. The results are shown as box plots where the box represents the middle 50% of the data and the whiskers represent the spread of the remaining data. The median is represented by the line in the center. Stable methadone doses are plotted against the genotypes.

this study ($D' > 0.8$, $r^2 \geq 0.6$) 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 673bp 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 *NGFB* 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

region was identified in the rodent *oprd1* promoter.^{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).⁴⁰ Chronic alcohol exposure has been shown to downregulate human plasma NGF with a greater decrease in patients with family history of alcohol dependency.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.^{30,42–50} *BDNF* was suggested to confer differential susceptibility to MMT response in opioid addicts.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.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.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 (Levrán *et al.*, manuscript in preparation). An analysis of *NGFB* SNP rs2239622 with the three SNPs mentioned above as co-variables 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).^{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.^{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 neurotrophins 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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