The μ -opioid receptor nonsynonymous variant 118A>G is associated with prolonged abstinence from heroin without agonist treatment

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Aim: This study assesses whether opioid-related gene variants contribute to reduced vulnerability to relapse to heroin in persons who are not treated with μ -opioid receptor agonist. **Methods:** Genotypes of 71 SNPs, in nine genes, were analyzed for association with long-term abstinence in former heroin-dependents of European/Middle Eastern ancestry, either without agonist treatment ($n = 129$) or in methadone maintenance treatment ($n = 922$). **Results:** The functional *OPRM1* nonsynonymous SNP rs1799971 (118A>G) showed significant association with long-term abstinence ($P_{\text{permutation}} = 0.03$, dominant model, OR: 2.2; 95% CI: 1.5–3.3). **Conclusion:** Since the stress axis is regulated in part by β -endorphin, this functional *OPRM1* SNP may blunt the endogenous stress response and contribute to reduced vulnerability for relapse.

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Drug addiction is a chronic brain disease characterized by compulsive use of drugs and high incidence of relapse episodes even following years of abstinence. Drug addiction is caused by a combination of genetic and environmental factors and it is estimated that about a quarter of individuals who use heroin develop opioid addiction [1]. The brain changes that occur in persons with addiction remain after long-term abstinence and may challenge their resistance ability (e.g., [2]).

Opioids are a class of drugs that include the illicit drug heroin as well as prescription pain relievers, oxycodone, morphine and others. Endogenous opioids (β -endorphin, prodynorphin, enkephalin and orphanin/nociceptin, encoded by *POMC*, *PDYN*, *PENK* and *PNOC*, respectively), as well as exogenous opioids (e.g., heroin, methadone) bind to opioid receptors μ , κ , δ and receptor-like, encoded by *OPRM1*, *OPRK1*, *OPRD1* and *OPRL1*, respectively. These receptors are G-protein-coupled receptors that alter neural signal transmission. The endogenous opioid system plays a key role in drug addiction and mediates the analgesic and rewarding properties of drugs. The effects of heroin, methadone and nonopioid drugs are primarily mediated by the μ -opioid receptor (*OPRM1*). Binding of β -endorphin in the striatum results in disinhibition of dopaminergic neurons and the dopamine increase is associated with reward.

Dysregulation of the stress response is one of the factors affecting relapse to addictive behaviors. There is a high interindividual variability in stress response that depends on a combination and interaction of genetic and nongenetic factors [1,3]. Endogenous and exogenous opioids modulate the hypothalamic-pituitary-adrenal (HPA)

axis that is responsible for the adrenal secretion of glucocorticoids [4]. Genetic variations in the opioid pathway may modulate opioid effects on the stress response.

Only a small percentage of individuals meeting criteria for opiate dependence are able to maintain long-term abstinence without μ -opioid receptor agonist treatment (e.g., methadone or buprenorphine). This study was designed to assess whether specific SNPs in the opioid pathway (opioid receptors and their endogenous ligands, as well as receptor-interacting protein arrestin- β 2) are associated with this capability. Toward this goal, the genotype frequencies of selected polymorphisms were compared between former opiate-dependent individuals either in long-term abstinence (>10 years) without μ -opioid receptor agonist treatment [5] or in methadone maintenance treatment (MMT).

Subjects & methods

Study population

Subjects in long-term abstinence without agonist treatment

The abstinence sample ($n = 129$, 79% male) included subjects with a history of at least one year of using heroin multiple times per day. All subjects were previously treated in diverse addiction facilities, had several relapses and had maintained abstinence from heroin without medication treatment for >10 years at the time of the study. None of them was in MMT for more than 5 months in their lifetime so their abstinence was not achieved by agonist treatment. All subjects stopped using other drugs (e.g., cocaine) and the majority stopped drinking alcohol and smoking tobacco.

Subjects were recruited at the Dr Miriam and Sheldon G Adelson Clinic for Drug Abuse Treatment and Research, Tel Aviv, as described [5]. All subjects had personal interviews in which they were asked about their medications, rehabilitation processes, psychiatric diagnoses and hospitalizations, as well as demographic details. Although there is no objective confirmation of their past abstinence, their abstinence histories were verified by a senior-counselor from another addiction treatment institute that was familiar with the subjects. The Kreek–McHugh–Schluger–Kellogg scale (KMSK) [6], an instrument for the assessment of a lifetime diagnosis of opiate, cocaine, alcohol and other drug dependence, was administered. Subjects were also tested for current drugs of abuse in urine.

From the 157 subjects for which DNA samples were available, eight subjects were excluded since they had been in MMT for more than 5 months in their lifetime, 13 subjects were excluded based on their different ancestry contribution (calculated by Structure analysis, see later), one subject was excluded because he was related to another subject in the study, and six subjects were excluded because of high genotyping failure rate.

MMT sample

The MMT sample ($n = 922$, 67% male), that serves as a control sample in this study, included subjects with a history of at least 1 year of daily multiple uses of heroin, who were treated at a methadone maintenance treatment program. Ascertainment for the study was made by interview using the KMSK scale, the Addiction Severity Index [7], and the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition (DSM-IV). Subjects with active DSM-IV axis I disorder were excluded. The participating sites were the Adelson Clinics in Tel Aviv ($n = 363$) and Las Vegas ($n = 276$), the Rockefeller University Hospital ($n = 227$), and the Manhattan Campus of the VA NY Harbor Healthcare System ($n = 56$). This sample is similar to the sample used in our previous association studies (e.g., [8]).

Consent & IRB approval

All subjects signed informed consent for genetic studies. The study was carried out in accordance with the latest version of the declaration of Helsinki. The Institutional Review Boards of the Rockefeller University Hospital (for Rockefeller University and the Las Vegas clinic), the VA New York Harbor Healthcare System and the Tel Aviv Sourasky Medical Center (Helsinki Committee) approved the study.

SNP genotyping

DNA was extracted and precipitated as described [9]. Genotyping was performed on two Illumina® (CA, USA) 1536-plex GoldenGate custom panels (GS0013101-OPA and GS0014419-OPA) at the Rockefeller University Genomics Resource Center according to the manufacturer's protocol. The panels were designed based on the 'Addiction array', a hypothesis-based array that is based on linkage disequilibrium (LD) – tagging SNPs and capture a substantial proportion of common genetic variation [10]. GS0013101-OPA was described elsewhere [8], and

GS0014419-OPA was a modification of this panel. Random samples were genotyped in duplicate. Analysis was performed with BeadStudio software v2.3.43 (Illumina). The genotype data were visually inspected to verify and correct automatic calling. Genotype data were filtered based on SNP call rates (<99%), minor allele frequency (MAF) <0.05 and deviation from Hardy–Weinberg equilibrium.

The analysis in the current study was limited *a priori* to SNPs in nine selected opioid-system genes (Table 1). Of the 111 variants genotyped from these genes in the two arrays, 32 SNPs were not included in this analysis since genotype data was only available for the part of the sample (GS0014419-OPA), and six SNPs were removed because of inadequate cluster separation. A total of 71 SNPs were included in the analysis after removal of two SNPs based on low frequency (MAF <0.05; Table 1).

Structure analysis

Structure analysis of 155 Ancestry Informative Markers was used to estimate ancestry contributions with seven-factor solution (e.g., the fractions of genetic affiliation of the individual in seven clusters) [10,11]. Each subject was anchored against 1051 samples representing 51 worldwide populations, as described [12]. A total of 13 subjects with less than 50% estimated proportion of European and/or Middle Eastern ancestry were excluded. The European and Middle-Eastern clusters were combined for calculating estimation proportions based on their low population differentiation [13].

Statistical analysis

Exact tests for deviation from Hardy–Weinberg equilibrium were performed with PLINK (version 1.9). Pairwise LD (D' and r^2) was estimated using Haploview v4.2. LD blocks were identified using the D' CI bound of 0.7–0.98 [14]. Single SNP association analyses were conducted by logistic regression, under dominant or recessive model assumptions, using PLINK. Sex and the estimated proportions of contribution of five major ancestries were included as covariates. Correction for multiple testing was performed by permutation test ($n = 100,000$) for each model of inheritance, using PLINK. A maximum test statistic was applied to account for the dominant and the recessive model tests, using Sumstat [15]. A secondary analysis using only the Israeli MMT subsample ($n = 363$) was carried out for the top signal in a similar manner, for validation purposes.

Results

Genotypes of 71 selected SNPs in nine selected opioid-system genes (*OPRM1*, *OPRK1*, *OPRD1*, *OPRL1*, *POMC*, *PDYN*, *PENK*, *PNOC* and *ARRB2*) were analyzed for association with long-term abstinence without μ agonist treatment (Table 1). Of these, 13 SNPs (one SNP triplet and five SNP pairs) were in complete or strong LD ($r^2 \geq 0.97$) with another SNP in the study. A total of 21 haplotype blocks that include 55 SNPs were detected, reducing the effective number of independent SNPs to 37 (Supplementary Figure 1).

Association analysis under a dominant or recessive model of inheritance was performed with the abstinence sample ($n = 129$, subjects with long-term abstinence) as ‘case’ and the MMT sample ($n = 922$) as ‘control’. The *OPRM1* functional nonsynonymous SNP rs1799971 (118A>G, Asn40Asp) was the only SNP that showed significant association after permutation analysis ($P_{perm} = 0.0016$, dominant model). The signal remained significant after correction for the two models tested, and accounting for sex and the estimated proportions of five major ancestries ($P_{final} = 0.03$). The proportion of carriers of the minor 118G allele in the abstinence sample was significantly higher (44%) than in the MMT sample (26%; OR: 2.2; 95% CI: 1.5–3.3). The frequency of the minor 118G allele in the MMT sample was similar to that described for European populations (~ 0.15).

A secondary analysis using only the Israeli MMT subsample ($n = 363$) was carried out in a similar manner for validation. The proportion of the carriers of the 118G allele was 31% in this subsample. The association signal was nominally significant after accounting for sex and ancestry with similar effect size ($p = 0.006$, OR: 1.8; 95% CI: 1.2–2.7, dominant model), but was not significant after permutation correction.

Discussion

The major finding in this report is that the proportion of the carriers of at least one copy of the 118G allele of the functional *OPRM1* SNP rs1799971 (118A>G, Asn40Asp) is significantly higher in subjects in long-term abstinence from heroin that are not treated with μ -opioid receptor agonist, than in subjects in methadone maintenance treatment. The association signal may also reflect associations of other SNPs in LD with the identified

Table 1. SNP list.

1	rs9895782	17	4608458	[A/G]	ARRB2
2	rs1045280	17	4622638	[A/G]	ARRB2
3	rs2036657	17	4625159	[A/G]	ARRB2
4	rs16837697	1	29134546	[A/G]	OPRD1
5	rs2236861	1	29139756	[A/G]	OPRD1
6	rs678849	1	29145188	[A/G]	OPRD1
7	rs204055	1	29159373	[T/C]	OPRD1
8	rs2236857	1	29161609	[T/C]	OPRD1
9	rs2298896	1	29166138	[T/G]	OPRD1
10	rs3766951	1	29169559	[A/G]	OPRD1
11	rs529520	1	29174946	[A/C]	OPRD1
12	rs581111	1	29175373	[T/C]	OPRD1
13	rs2234918	1	29189597	[T/C]	OPRD1
14	rs204076	1	29190390	[A/T]	OPRD1
15	rs3802280	8	54140999	[A/G]	OPRK1
16	rs3802281	8	54141163	[A/G]	OPRK1
17	rs7817710	8	54142815	[A/C]	OPRK1
18	rs16918909	8	54150034	[T/C]	OPRK1
19	rs7016778	8	54150105	[T/A]	OPRK1
20	rs6473797	8	54152982	[A/G]	OPRK1
21	rs7826614	8	54157426	[T/C]	OPRK1
22	rs10504151	8	54158240	[A/G]	OPRK1
23	rs3808627	8	54164802	[A/G]	OPRK1
24	rs6090041	20	62712676	[T/C]	OPRL1
25	rs6512305	20	62716270	[A/G]	OPRL1
26	rs6090043	20	62717930	[T/C]	OPRL1
27	rs7271530	20	62727915	[A/G]	OPRL1
28	rs6089789	20	62731992	[T/C]	OPRL1
29	rs1799971	6	154360797	[T/C]	OPRM1
30	rs510769	6	154362019	[A/G]	OPRM1
31	rs3778151	6	154393680	[A/G]	OPRM1
32	rs660756	6	154415434	[T/G]	OPRM1
33	rs558025	6	154441965	[A/G]	OPRM1
34	rs10485058	6	154445215	[T/C]	OPRM1
35	rs548339	6	154460799	[A/G]	OPRM1
36	rs483481	6	154463968	[T/C]	OPRM1
37	rs569284 [†]	6	154464330	[T/G]	OPRM1
38	rs2236256	6	154478440	[A/C]	OPRM1
39	rs1918760	6	154482167	[A/G]	OPRM1
40	rs2281617	6	154487421	[A/G]	OPRM1
41	rs6941251	6	154487977	[A/G]	OPRM1
42	rs1998220	6	154506428	[T/C]	OPRM1
43	rs9322451	6	154522661	[A/G]	OPRM1
44	rs9479791	6	154529514	[A/G]	OPRM1
45	rs790266	6	154541903	[T/C]	OPRM1
46	rs2272381	6	154542491	[A/G]	OPRM1
47	rs9371781	6	154552207	[A/C]	OPRM1
48	rs6935927	6	154560337	[A/G]	OPRM1
49	rs4314511	6	154565335	[A/G]	OPRM1

[†]SNPs were excluded from analysis due to minor allele frequency <0.05.

Table 1. SNP list (cont.).

50	rs2235749	20	1959939	[T/C]	<i>PDYN</i>
51	rs10485703	20	1960313	[A/G]	<i>PDYN</i>
52	rs6045824	20	1961977	[T/C]	<i>PDYN</i>
53	rs6035222	20	1963413	[A/G]	<i>PDYN</i>
54	rs2235751	20	1969934	[A/G]	<i>PDYN</i>
55	rs2281285	20	1972460	[T/C]	<i>PDYN</i>
56	rs1997794	20	1974858	[T/C]	<i>PDYN</i>
57	rs6136667	20	1978301	[G/C]	<i>PDYN</i>
58	rs4738501 [†]	8	57356272	[T/C]	<i>PENK</i>
59	rs1437277	8	57356734	[T/C]	<i>PENK</i>
60	rs1975285	8	57358682	[C/G]	<i>PENK</i>
61	rs2722897	8	28173197	[A/G]	<i>PNOC</i>
62	rs1563945	8	28174886	[T/G]	<i>PNOC</i>
63	rs2614095	8	28184991	[T/C]	<i>PNOC</i>
64	rs351776	8	28191306	[T/G]	<i>PNOC</i>
65	rs351784	8	28197747	[G/C]	<i>PNOC</i>
66	rs4732850	8	28198716	[T/C]	<i>PNOC</i>
67	rs1866146	2	25380573	[T/C]	<i>POMC</i>
68	rs6713532	2	25384833	[A/G]	<i>POMC</i>
69	rs934778	2	25389224	[A/G]	<i>POMC</i>
70	rs6545976	2	25394650	[T/G]	<i>POMC</i>
71	rs6719226	2	25396012	[C/G]	<i>POMC</i>
72	rs874401	2	25402280	[T/C]	<i>POMC</i>
73	rs4665775	2	25407121	[A/G]	<i>POMC</i>

[†] SNPs were excluded from analysis due to minor allele frequency <0.05.

SNP that were not included in this study. Several variants in the *OPRM1* 5' upstream and intronic regions are in strong LD with SNP rs1799971 in a population-specific manner [16].

The μ -opioid receptor modulates a diverse range of physiological systems, including stress responsivity, analgesia, immune function and reward. The receptor is encoded by the gene *OPRM1* on chromosome 6. The Asn40Asp variant is located in exon 1 in the N-terminal extracellular domain of the receptor. It removes a putative N-glycosylation site and was suggested to introduce an extra DNA methylation site [17]. Previous studies showed several functions of SNP rs1799971 and its mouse equivalent (*Oprm1* 112A>G), including modulation of receptor binding, downstream signaling, gene expression, N-glycosylation and brain protein stability (e.g., [18–22]). There is also evidence that the 118G allele modulates sensitivity to both physical pain and social rejection (e.g., [23,24]). This variant also showed modulation of therapeutic response to μ -opioid receptor antagonist treatment for alcohol dependence (e.g., naltrexone), where 118G carriers had lower relapse rate than non-carriers [25].

The 118G allele is the minor allele across various populations, with frequencies ranging from 0.0 in African samples, to 0.16 in European-ancestry samples, to >0.40 in Asian samples. Since, the 118G allele is rare in African populations, it is most probably a relatively new variant that arose after the out-of-Africa migration of modern humans. This high variability in the frequency of this variant across population makes the study vulnerable to a potential effect of population substructure. Several steps were taken to limit the effect of population substructure on the results. We accounted for the major ancestry contributions detected by Structure analysis of AIMs and ensured that there is very low 'far-Asian' ancestry contribution in this cohort. Furthermore, we showed that the higher frequency of the 118G allele in the abstinence sample was not affected by the proportion of contribution of European ancestry. Nevertheless, it is possible that an undetected population substructure contributed to the result.

Association studies of the 118A>G SNP with drug dependence and related phenotypes, including large meta-analyses, revealed mixed results and there is currently no clear consensus as to the effect of this SNP on addiction vulnerability and on the direction of its potential effect (e.g., [26–28]). A population-specific LD pattern may contribute to the mixed results of different studies of this variant. Our laboratory has previously reported an

association of the 118G allele with heroin addiction in a Swedish sample [29]. In a later study of subjects with predominantly European ancestry, which included a sample similar to the MMT sample used in the current study, we have identified associations of heroin addiction with SNPs in several opioid pathway genes, including *OPRM1*, but no association of *OPRM1* SNP rs1799971 was detected [9]. This information suggests that the finding of association of SNP rs1799971 with long-term abstinence in the current study may not be related to the vulnerability to develop the addiction, at least in this sample.

One of the possible explanations for the finding is that the variation in the μ -opioid receptor modulates the HPA axis response to stress and could impact the vulnerability to relapse. Genetic factors were shown to modulate the HPA axis [30]. The HPA axis is the central stress response system that is regulated, in part, by the endogenous opioid, β -endorphin, acting on μ -opioid receptors. In response to stressors, CRH and vasopressin are released and stimulate ACTH secretion which, in turn, stimulates cortisol secretion from the adrenal gland. Cortisol negative feedback inhibits both the hypothalamus and the pituitary gland and also reduces the cleavage of proopiomelanocortin into ACTH and β -endorphins. Opioid antagonists were shown to activate the HPA axis (e.g., [31]) and high levels of stress-related drug craving and dysfunctional HPA axis responses were shown to increase vulnerability to relapse [3].

Our group reported that healthy volunteer carriers of 118G have blunted response to metyrapone [32]. Carriers of the 118G allele may have greater endogenous opioid restraint of the stress axis compared with non-carriers, resulting in blunted response to stress. Previous studies of healthy volunteers reported greater cortisol response to naloxone in 118G carriers that may be population-specific (e.g., [33,34]).

Conclusion

Relapse is a major problem for treatment of drug addiction. Understanding the factors that lead to or protect from relapse may help in providing effective interventions. This study suggests that the common functional polymorphisms (118A>G, N40D) within the *OPRM1* gene, and/or variants in strong LD with it in the studied population, may contribute to the ability of particular subjects with a history of heroin addiction to remain abstinent for a long period without μ -opioid receptor agonist treatment. The effect of this polymorphism may be related to stress resilience. Genetic testing of this variant may help to identify patients that are likely to benefit from specific intervention. Further studies are warranted to support these findings and to understand the mechanisms mediating the potential reduced vulnerability for relapse by this variant. The clinical significance of these findings to drug addiction and other stress-related diseases remains to be determined.

Summary points

- Relapse is a major problem for treatment of heroin addiction.
- Understanding the factors that lead to or protect from relapse may help in providing effective interventions.
- This study assesses whether 71 opioid-related genetic variants contribute to reduced vulnerability to relapse.
- The functional *OPRM1* nonsynonymous SNP rs1799971 (118A>G) showed significant association with long-term abstinence.
- The *OPRM1* SNP may blunt the endogenous stress response.

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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 human experimental investigations. In addition, informed consent has been obtained from the participants involved.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/pgs-2017-0092

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