Published in final edited form as:
Alcohol Clin Exp Res. 2009 March ; 33(3): 383–393. doi:10.1111/j.1530-0277.2008.00846.x.

OPRM1 Asn40Asp predicts response to naltrexone treatment; a haplotype-based approach

Gabor Oroszi, MD, PhD[1], **Raymond F. Anton, MD**[1], **Stephanie O'Malley, PhD**[2], **Robert Swift, MD, PhD**[3], **Helen Pettinati, PhD**[4], **David Couper, PhD**[5], **Qiaoping Yuan, PhD**[6], and **David Goldman, MD**[6]

[1]Center for Drug and Alcohol Programs, Medical University of South Carolina, Charleston, SC

[2]Substance Abuse Treatment Unit, Yale University School of Medicine, New Haven, CT

[3]Roger Williams Medical Center and Providence VA Medical Center, Brown University, Providence, RI

[4]Treatment Research Center, University of Pennsylvania School of Medicine, Philadelphia, PA

[5]Collaborative Studies Coordinating Center, University of North Carolina, Chapel Hill, NC

[6]Laboratory of Neurogenetics, NIAAA, NIH, Rockville, MD

Abstract

Background—Individualized pharmacotherapy requires identification of genetic variants predictive of treatment response. In *OPRM1*, Asn40Asp has been reported to be predictive of response to naltrexone treatment. Nevertheless, the *in vitro* function of the polymorphism remains elusive and over 300 *OPRM1* sequence variants have been identified to date. Therefore we used a haplotype-based approach to capture information of other genetic variants that might predict treatment response to naltrexone in the COMBINE Study.

Methods—5' nuclease genotyping assays (TaqMan®) were applied for 10 SNPs. Five-locus haplotypes in two *OPRM1* haplotype blocks were assigned to Caucasian participants. The relationship of the haplotypes to medication reflected by "good clinical outcome" was analyzed in 306 Caucasians treated without Combined Behavioral Intervention and with either naltrexone or placebo.

Corresponding author and reprint requests: Raymond F. Anton MD, Center for Drug and Alcohol Programs, Medical University of South Carolina, 67 President St., PO Box 250861, Charleston, SC 29425. Telephone: 843-792-1226 Fax: 843-792-17241 Email: antonr@musc.edu.

Disclosure/Conflict of Interest

Dr. Anton has reported receiving consultation fees and honoraria from Forest Laboratories and Alkermes (the maker of long-acting injectable naltrexone); consultation fees and grants from Bristol-Myers Squibb and Hythiam; Consultation fees, honoraria, and grants from Contral Pharma/Biotie Pharmaceuticals and Johnson & Johnson/OrthoMcNeil; consultation fees and grant funding from Pfizer; and, consultation fees from AstraZeneca, Axis Sheild, Cephalon, Drug Abuse Sciences, and Sanofi Aventis. In the near future he anticipates receiving consulting fees from Solvay Pharmaceuticals and a grant from Eli Lilly. Dr. O'Malley has reported receiving research support (grant support or clinical supplies) from Alkermes, DuPont, GlaxoSmithKline, Forest Laboratories, Lipha Pharmaceuticals, Ortho-McNeil, Bristol-Myers Squibb, Pfizer, Sanofi-Aventis, and Mallinckrodt and anticipates receiving a contract from Eli Lilly; serving as a consultant to Alkermes, Forest Laboratories, GlaxoSmithKline, Ortho-McNeil, Pfizer, Johnson & Johnson; receiving travel reimbursement from Alkermes; and that she is an inventor on patents held by Yale University entitled: "Smoking Cessation Treatments Using Naltrexone and Related Compounds". Dr. Pettinati has reported receiving research support from Alkermes, AstraZeneca, Bristol-Myers Squibb, Cephalon, Forest Laboratories, Lipha-Merck-KGaA, Ortho- McNeil; and, serving as consultant/advisory board/speakers bureau for Alkermes, AstraZeneca, Cephalon, and Forest Laboratories. Dr. Swift has reported receiving grant funding from Ortho-McNeill and Pfizer, Inc.; serving as consultant/advisory board/speakers bureau to Alkermes, Inc., Cephalon, Ortho-McNeil, Pfizer, Inc., Transoral Pharmaceuticals, Forest Laboratories. Drs. Oroszi, Couper, Yuan and Goldman had nothing to disclose.

Results—A significant haplotype by medication interaction (P=0.03) was found in *OPRM1* block 1. Naltrexone-treated alcoholics with haplotype AGCCC, the single haplotype carrying the Asp40 allele had the highest percent of good clinical outcome. When interaction of genotypes at each of the five loci comprising block 1 with medication was examined, only the Asn40/Asp40 and Asp40/Asp40 genotypes were found to significantly interact with naltrexone treatment. No haplotype by medication interaction was documented in *OPRM1* block 2.

Conclusions—Our haplotype-based approach confirms that the single *OPRM1* locus predictive of response to naltrexone treatment is Asn40Asp in exon 1. A substantial contribution of any other *OPRM1* genetic variant to interindividual variations in response to naltrexone treatment (at least in terms of good clinical outcome) is not supported by our findings.

Keywords

OPRM1 Asn40Asp; naltrexone; treatment response; haplotype; good clinical outcome; haplotype by medication interaction

Introduction

Effects of opioids are mediated through opioid receptors, mu, kappa, and delta, each a seven-transmembrane domain G-protein coupled receptor (Inturrisi, 2002). Of the three receptor subtypes, the opioid receptor mu 1 (OPRM1) is thought to account for the most of the opioidergic effects (Kieffer and Gaveriaux-Ruff, 2002; Sora et al., 2001; Uhl et al., 1999). OPRM1 is also the primary site of action of an endogenous opioid peptide, beta-endorphin, released in response to ethanol (Gianoulakis and Barcomb, 1987), and a μ opioid receptor antagonist, naltrexone (Volpicelli et al., 1992). Encoded by the *OPRM1* gene (6q24-q25; GeneID: 4988), OPRM1 is widely distributed in brain (Delfs et al., 1994). Over 300 *OPRM1* genetic variants have been identified to date as a result of several re-sequencing efforts that have included the 5’ and 3’ UTRs and flanking regions (Hoehe et al., 2000; Ikeda et al., 2005).

Most abundant among the missense variants is Asn40Asp, which results from an A118G transition (Bergen et al., 1997). The Asp40 allele frequency ranges from 0.10 to 0.15 in Caucasians but the allele frequency is population specific; the average frequency of Asp40 is 0.04, 0.25–0.45, 0.16, 0.14 and 0.21 in African-Americans, East Asians, SW American Indians, Hispanics and Ashkenazi Jews, respectively (Bergen et al., 1997; Bond et al., 1998; Crowley et al., 2003; Gelernter et al., 1999; Hernandez-Avila et al., 2003).

Beta endorphin was reported to have 3-fold higher binding affinity at the Asp40 mutated receptor than at the receptor encoded by the Asn40 allele. In addition, beta endorphin was three times more potent at the Asp40 receptor in activating GIRK channels compared to the receptor encoded by the Asn40 allele (Bond et al., 1998). By contrast, in two follow-up studies, binding affinity for the variant receptor was not found to be different from that for the normal receptor in COS cells (Befort et al., 2001), and no difference was documented either in binding affinity or potency of beta-endorphin for the variant receptor in mammalian HEK293 cells (Beyer et al., 2004). In a more recent study, the Asn40 mRNA was 1.5–2.5-fold more abundant than the Asp40 mRNA in human autopsy brain tissue. Additionally, in transfected CHO cells the Asp40 allele yielded 1.5-fold lower mRNA levels and more than 10-fold lower OPRM1 protein levels (Zhang et al., 2005).

The Asn40Asp polymorphism appears to have *in vivo* functional effects. Asp40 carriers were found to have altered HPA activation resulting in higher cortisol levels both at baseline and following infusion of the opioid receptor antagonist naloxone (Chong et al., 2006; Hernandez-Avila et al., 2003; Wand et al., 2002) but less cortisol response to a social

stressor (Chong et al., 2006). Due to its potential functional significance the polymorphism has been extensively studied for association with addictions, with inconclusive results (Bergen et al., 1997; Gelernter et al., 1999; Rommelspacher et al., 2001; Town et al., 1999). Based on a recent meta-analysis the OPRM1 Asn40Asp polymorphism does not appear to affect risk for substance dependence (Arias et al., 2006).

The utility of Asn40Asp as a predictor of treatment response in addictions has been examined in four studies. Among smokers on short-term nicotine replacement therapy, Asp40 carriers had higher rate of abstinence compared to Asn40/Asn40 homozygotes (Lerman et al., 2004). Of treatment-seeking alcoholics prescribed naltrexone, those carrying the Asp40 allele had significantly lower rates of relapse and took longer to resume heavy drinking than Asn40/Asn40 homozygotes (Oslin et al., 2003). By contrast, in a *post hoc* subgroup analysis no significant interactions were documented between any single nucleotide polymorphisms (SNP) (including Asn40Asp) in any of three opioid receptor genes and response to naltrexone treatment in the Veterans Affairs Cooperative Study (Gelernter et al., 2007), a study in which naltrexone was not efficacious in the primary analysis (Krystal et al., 2001). Finally, in a pre-planned pharmacogenetic ancillary study (Goldman et al., 2005) within the larger COMBINE Study, alcoholics on naltrexone carrying the Asp40 allele had increased percent days abstinent (PDA), decreased percent heavy drinking days (PHDD) and higher rates of good clinical outcomes (GCO) compared to Asn40/Asn40 alcoholics (Anton et al., 2008).

OPRM1 haplotypes were associated with substance dependence (Hoehe et al., 2000; Luo et al., 2003) but not with severe opiod dependence (Crowley et al., 2003). More recently, haplotypes composed of Asn40Asp and predominantly intronic SNPs were associated with substance dependence in case/control studies (Zhang et al., 2006a; Zhang et al., 2006b) but not in a family-based association study (Xuei et al., 2007). Considering the over 300 variants in *OPRM1*, the paucity of clinical studies addressing *OPRM1* haplotypes, the inconsistent results regarding the *in vitro* function of Asn40Asp, and the inconclusive associations of Asn40Asp with substance dependence and treatment response, we decided to use a haplotype-based approach to capture more information of genetic variants that might predict treatment response to naltrexone in the COMBINE Study population (Anton et al., 2006; Goldman et al., 2005).

Materials and Methods

Subject Population

The subjects for this report were drawn from those 1383 individuals participating in the federally funded COMBINE Study. Specific screening, selection and assessment methods, detailed treatments, outcome measures and overall results can be found in a previous report (Anton et al., 2006) Specific details of those that participated in the genetic sub-study of the parent COMBINE Study can be found in a report focusing only on the relationship of the Asp40 variant to naltrexone treatment response (Anton et al., 2008).

In essence, all subjects met DSM-IV criteria for alcohol dependence. After screening and 4 days of abstinence, subjects were randomized to receive naltrexone (100 mg/daily), acamprosate (3 grams daily), both drugs, or their matching placebos for 16 weeks. All subjects received medical management provided by health care professionals and half the subjects received Combined Behavioral Intervention, a specialized counseling given by addiction professionals. The subjects for this report are those that received naltrexone (with or without acamprosate) or naltrexone placebo (with acamprosate or acamprosate placebo). We limited the analysis to those who received the medical management condition without Combined Behavioral Intervention since in our intent to treat analysis (Anton et al., 2006)

and in our previous report of the Asp40 prediction of naltrexone response (Anton et al., 2008) it was only this group of subjects in which naltrexone effects were observed. The rationale for this, as detailed in our original report, was based on the interpretation of the original finding that the CBI-treated subjects had no added benefit from naltrexone likely due to a maximal treatment effect in this study population. We reasoned then that the naltrexone (pharmacological) effect and subsequently the gene by naltrexone interaction could only be observed in those that did not have the confound of receiving treatment (CBI) that would obscure the observation of these effects. We utilized the same rationale in this report to specifically examine the impact of other OPRM1 SNP's and their combinations (haplotypes) on the already established naltrexone effect in the medical management (MM) group. The numbers in each condition, the population demographics and pre-study drinking data and alcohol measures are given in Table 1.

Drinking was assessed over the course of the study with the timeline calendar follow-back method (Sobell et al., 1972) which captures a daily estimate of drinking between visits. The other outcome measure relevant for this report is the DrInC scale (Miller *et a*l, 1995) which captured the kind and level of alcohol related problems at the end of the study. Both drinking quantity and frequency and level of alcohol problems were combined to construct an a priori "good clinical outcome" measure based on previously published work (Zweben and Cisler, 2003) and which captured a positive effect for naltrexone in the intent to treat analysis (Anton et al., 2006) and in our original evaluation of the interaction of Asp40 allele with naltrexone treatment (Anton et al., 2008). In this report we limit ourselves to this one, easily interpretable, and consistent outcome measure. A good clinical outcome (GCO) is defined as abstinent or moderate drinking without problems, a maximum of 11 (women) or 14 (men) drinks per week, with no more than 2 days on which more than 3 drinks (women) or 4 drinks (men) were consumed and 3 or less alcohol-related problems endorsed on the DrInC scale during the last 8 weeks of treatment. Subjects with missing values for this variable were deemed as having experienced a poor (not good) clinical outcome.

SNP selection and genotyping

To date, ten splice variants of human *OPRM1* have been identified with exons 1,2 and 3 as constituent exons and differing by alternative splicing downstream from exon 3 (Bare et al., 1994; Pan et al., 2005; Pasternak, 2004). Of these, the original version of the human *OPRM1* gene (GenBank NM_000914.2) (Benson et al., 2007) containing four exons and spanning 79,864 bp has been the most extensively investigated, and was studied in the present work. A schematic diagram of *OPRM1* displaying the lengths of the exons and introns is shown in Figure 1. Ten SNPs were selected from public databases including; the SNP database of the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/SNP/), the Applied Biosystems SNP database (http://www.appliedbiosystems.com) and the International HapMap Project (http://www.hapmap.org/). SNPs had minor allele frequencies of >0.05 in Caucasians and were chosen to haplotype-tag the *OPRM1* gene including 5' regulatory and 3' flanking regions. The positions of the SNPs in *OPRM1* and relative to the translation initiation site (den Dunnen and Antonarakis, 2001) are given in Figure 1. To estimate the extent to which the selected (tag) SNPs are representative (proxies) of non-genotyped *OPRM1* SNPs recorded in HapMap the program Tagger (http://www.broad.mit.edu/mpg/tagger/) was employed (de Bakker et al., 2005).

Genotyping was performed with, 5' nuclease (TaqMan®), a rapid and accurate method for high throughput genotyping of SNPs (Livak, 1999; Shi et al., 1999). Genomic DNA was extracted from peripheral blood mononuclear cells. The 5' nuclease genotyping assay (TaqMan®) combines polymerase chain reaction (PCR) amplification and sequence variant detection into a single step (Livak, 1999; Shi et al., 1999). Locus-specific primers and

fluorogenic allele-specific probes were designed and manufactured by Applied Biosystems (ABI) (Foster City, CA, USA). Probes were fluorescently labeled either with 6-FAM or VIC reporter dyes at the 5' end. At the 3' end was a non-fluorescent quencher (NFQ). The 5µl reaction mixture consisted of 2.5µl of Taqman Universal Master Mix (ABI), 0.125µl of 40X Assay Mix (ABI) (8µM detection probe for each allele, 36µM forward and reverse primer each), and 10 ng of genomic DNA diluted in 2.375µl of Tris EDTA (TE) pH 8.0 (Quality Biological, Inc; Gaithersburg, MD). Amplification was performed with an ABI Gene Amp® PCR System 9700 using 384-well plates and the following amplification profile: 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. After amplification, endpoint fluorescence intensity was measured directly in the reaction plates, by means of 7900 ABI Sequence Detector. Genotypes were determined using Sequence Detection System Software Version 2.0 (ABI). Four genotyping signal clusters were identified representing allele1/allele1 homozygotes, allele1/allele2 heterozygotes, allele2/allele2 homozygotes and no-DNA-template control for each locus.

Genotyping accuracy was determined by replicate genotyping of at least 20% of the DNA samples at each locus. Nucleotide substitution, discrepancy rate (ratio of the number of discordant genotypes to the number of duplicates), genotyping completion rate (ratio of the number of valid genotypes to the number of subjects genotyped (915)) at each locus and the number of the duplicates at each locus are provided in Table 2 for the whole cohort.

HWE, LD and haplotype analysis

The program Haploview v3.32 (Barrett et al., 2005) was used to compute Hardy-Weinberg equilibrium (HWE) for each locus, to estimate the extent of linkage disequilibrium (LD) between each pair of markers and to determine the haplotype block structure. Haplotype blocks were defined according to criteria proposed by Gabriel (Gabriel et al., 2002). Genotype distributions for all SNPs were in HWE in the whole cohort and in the Caucasian subgroup (Table 3). Haplotype pairs were assigned to each Caucasian participant using PHASE v2.01 (Stephens and Donnelly, 2003). PHASE estimates the probabilities of all likely pairs of haplotypes (diplotypes) assigned to each individual from genotype data. Of these, diplotypes assigned with a probability of ≥ 0.80 were selected for further analysis. In COMBINE *OPRM1* block 1 and block 2, 668 and 667 Caucasian subjects respectively had a diplotype assigned with a probability of ≥ 0.80. Non-Caucasian participants were excluded from haplotype-based analysis. All genotyping, construction and assignment of haplotypes, and data reporting were done blind to treatment assignment and outcome variables.

Statistical analysis

The statistical analysis was restricted to those 306 Caucasians (of the 687 Caucasians genotyped) who received medical management without Combined Behavioral Intervention and had haplotype data in at least one of the haplotype blocks. Baseline characteristics were compared between the naltrexone and naltrexone placebo groups (Table 1). Categorical variables are expressed as frequencies and percentages and equality tested using chi-squared tests. Continuous variables are expressed as means and standard deviations and equality of the means tested using two-sample t-tests. The baseline characteristics did not differ significantly between the naltrexone and naltrexone placebo groups in any variables (all P values were > 0.05).

The relationship of combinations of haplotypes with GCO was investigated separately for each treatment group using a chi-squared test. Logistic regression was used to test whether the relationships differed between the two treatment groups. In these models the GCO indicator was used as the dependent variable and indicator variables for the haplotype combinations, for treatment group and for combinations of treatment group by haplotype

combinations as the independent variables. Because of the relatively small number of participants, the models were not adjusted for baseline variables. The models were run separately for block 1 and block 2 haplotype combinations (Table 7 and Table 10).

For block 1, each of the five SNPs in the block was investigated separately using a similar approach to that described above for haplotypes. For each SNP, the proportion of participants with a GCO was estimated separately for each combination of genotype and treatment group. The interaction of genotype by treatment group on the GCO was tested using logistic regression (Table 8). These analyses were then repeated for the four SNPs other than the Asn40Asp locus in the group of participants who were Asn40/Asn40 homozygotes.

Results

Extent of SNP informativeness and haplotype blocks

The program Tagger (http://www.broad.mit.edu/mpg/tagger/) (de Bakker et al., 2005) provided evidence that the 10 SNPs genotyped in COMBINE captured all the common SNPs (MAF ≥ 5%) in *OPRM1* (NM_000914.2) in HapMap (CEU) with an average r^2 of 0.78 and 79% are captured with high r^2 (≥ 0.8) using the aggressive (multimarker) approach. LD analyses of the Caucasians subjects employing the program Haploview v3.32 demonstrated that the ten SNPs were distributed in two separate haplotype blocks (Figure 2). SNPs 1–5 (SNP1 in the 5'regulatory region, SNP2 (Asn40Asp) in exon 1 and SNP3-5 in intron 1) were in block 1 and SNP 6–10 (SNP6 in intron 2, SNP7-9 in intron 3 and SNP 10 in the downstream region) were in block 2.

Comparison of haplotypes in COMBINE and HapMap

We used a haplotype tagging approach and therefore expected that the configuration and frequency of haplotypes constructed in the COMBINE dataset would be similar to those available in HapMap. Indeed, the configurations of the major haplotypes (frequency ≥ 3%) both in block 1 (Figure 3a) and block 2 (Figure 3b) are congruent with those of the major haplotypes in the corresponding blocks of HapMap (Figure 3a and 3b). Likewise, the frequencies of the major haplotypes in block 1 (Table 4) and block 2 (Table 5) are close to the frequencies in HapMap Caucasians. Because many of HapMap haplotypes are minor variations (low frequency daughter haplotypes) differing from a major haplotype by only one or a few SNPs we grouped them on an objective basis. The five major haplotypes both in the COMBINE Study block 1 and block 2 represent the collapse of HapMap daughter haplotypes with mother haplotypes on a cladistic basis (Figure 3a and Figure 3b). The haplotype cladograms in Figures 3a and 3b were constructed using HapCluster (available from Qiaoping Yuan, (Zhou et al., submitted)) by hierarchical clustering of HapMap SNPs within block regions. For the clustering, distances based on linkage disequilibrium (r^2) are used to group SNPs in linkage disequilibrium, and these distances are modified by the frequencies of SNPs to give greater weight to abundant SNPs. Five tag SNPs in each block were sufficient to detect the major HapMap haplotypes which can be derived from 44 and 49 SNPs in blocks one and two, respectively. Of note, the five major haplotypes in block 1 account for 96.9% of all detected haplotypes. Similarly, the five major haplotypes in block 2 account for 96.9% of all detected haplotypes. These findings are consistent with observations that within human haplotype blocks most chromosomes are usually represented by a relatively small number (three to five) of haplotypes (Gabriel et al., 2002). The grouping of major haplotypes in the COMBINE Study block 1 and block 2 for haplotype-based analyses is shown in Table 6.

Relationship of haplotypes to medication in OPRM1 *block 1*

A significant haplotype by medication interaction (P=0.03) was found in patients treated with naltrexone (Table 7). One haplotype combination (diplotype) accounted for most individuals carrying the Asp40 allele, and this combination was AB. Only interactions of the three most common diplotypes (AA, AB and AC) with medication were explored. Rare diplotypes (BB, BC, CC, AO, BO, CO, OO) were omitted from the analysis. A higher percentage (90.0%) of naltrexone-treated patients with the AB diplotype (one copy of haplotype A plus the Asp40-carrying haplotype B) had GCO compared to naltrexone-treated patients who did not carry haplotype B or placebo-treated patients of whatever diplotype (Table 7). The percentage of placebo-treated patients with GCO did not differ significantly among the diplotypes (Table 7). As a secondary analysis we also evaluated the effect on the diplotypes on the percent of heavy drinking days over the course of the study. Those with diplotypes AA, AB, AC treated with placebo had 15+/−3%, 16+/−4%, 17+/−3% heavy drinking days (mean+/−SE) respectively compared to those same diplotypes treated with naltrexone who had 11+/−2.0%, 5+/−3%, 9+/−2% heavy drinking days respectively. While in the same direction as the GCO variable, with the Asp-40-containing diplotype (AB) showing 45–55% less heavy drinking days compared to the other diplotypes when treated with naltrexone, the interaction of medication and diplotype was not significant.

Relationship of each SNP comprising block 1 haplotypes to medication

Based on the significant haplotype by medication interaction in *OPRM1* block 1, the intent of this analysis was to evaluate whether any of the genotypes at the SNPs genotyped in block 1 would show a naltrexone response over that expected by placebo (interaction of medication with genotypes as shown in Table 8). A higher percent of patients had a GCO among naltrexone-treated Asp40 carriers (87.1%) compared to naltrexone-treated Asn40 homozygotes (54.4%), placebo-treated Asp40 carriers (50%) and placebo treated Asn40 homozygotes (54.1%), a significant genotype by medication interaction (p=0.008). Comparing naltrexone-treated Asp40 carriers to naltrexone-treated Asn40 homozygotes, the odds ratio of having GCO was 6.28 (CI: 1.94–20.34) which is consistent with our previously reported data (Anton et al., 2008). No significant genotype by medication interaction was found at any of the other four SNPs (Table 8).

When only Asn40/Asn40 homozygotes were considered, thereby eliminating any main effect of the Asp40 allele, no significant genotype by medication interaction was documented for any of the other four SNPs (Table 9). Also, when Asp40/Asp40 homozygotes and Asp40/Asn40 heterozygotes were separately evaluated, there was again no additive effect of any other Block 1 SNP (data not shown). Thus there is no significant genotype by medication interaction of the Block 1 SNPs other than Asn40Asp.

Relationship of haplotypes to medication in OPRM1 block 2

The percentage of patients with GCO either in naltrexone- or placebo-treated groups did not differ significantly among the three most common block 2 diplotypes (AA, AB and BB) (Table 10). Other diplotypes with low frequencies (AO, BO, and OO) were omitted from the analysis. Of note, differences in the percentage of patients with GCO in the naltrexone group that are apparently significant (Table 10) are again due to the Asp40 allele, found in block 1. This is probably due to extended linkage disequilibrium across the whole OPRM1 region, with a tendency of the Asp40 allele to occur on the Block 2 “A” haplotype background. Of the 64 subjects on naltrexone with the block 2 AA diplotype, 34.4% had at least one copy of the Asp40 allele whereas among 70 subjects with the AB and BB diplotypes only 10.0% carried the Asp40 allele. These percentages differ significantly (p<0.0008).

Discussion

The configuration and frequency of *OPRM1* haplotypes in 685 Caucasians studied in the COMBINE Study clinical trial are consistent with HapMap reference haplotypes derived from 90 Caucasian subjects (CEU, Utah residents with a Northern and Western European ancestry). In addition, evidence has been provided for a haplotype by medication interaction in alcoholics treated with naltrexone. A significantly higher percentage of natrexone-treated patients carrying the Block 1 haplotype AGCCC had GCO as compared to patients with other diplotypes (haplotype combinations) or patients treated with placebo. The distinguishing feature of this haplotype is the Asp40 allele, at position 2. No significant interaction was found between any of the other major block 1 haplotypes (frequency ≥ 3%) and medication. Also, in *OPRM1* block 2 none of the five major haplotypes had any significant effect on percentage of patients with GCO in either treatment group.

Despite the diversity of haplotypes representing Block 1, with five major haplotypes having frequency ≥ 3%, only the AGCCC haplotype carries the Asp40 allele. As mentioned above Asp40 has been shown to be predictive of response to naltrexone treatment in alcoholics by ourselves and at least one other group, (Anton et al., 2008; Oslin et al., 2003) and evidence has been provided for the in vitro and in vivo functionality of this missense variant. It should also be noted that in our original report (Anton et. al, 2008) we presented data showing that the Asp40 allele did not confer different medication compliance and/or adverse event profiles, strengthening the specific primary therapeutic significance of this specific polymorphism. We did not repeat that analysis for the haplotypes or diplotypes since no other alleleic SNP or combination of SNPs was associated with good treatment response. Hence, we hypothesize that Asp40 is likely to account for all of haplotype by medication effect observed for block 1 haplotypes. In support of this hypothesis, when interaction of genotypes at each of the five loci comprising block 1 with medication was examined, only the Asn40/Asp40 and Asp40/Asp40 genotypes were found to significantly interact with naltrexone treatment resulting in higher percent of patients with GCO. No significant genotype by medication interaction was seen at any other locus in *OPRM1* block 1 in our study. Of importance, the OPRM1 block 1 haplotype is at least 44-kb in size, extending up to 11.6 kb upstream of the 5’ end of *OPRM1* and including the human *OPRM1* promoter region (Borner et al., 2002; Borner et al., 2004; Kraus et al., 2001). At least 37 genetic variants (mainly SNPs) have been identified in the 5’ flanking region of the human *OPRM1* to date (Hoehe et al., 2000; Ikeda et al., 2005) and a few of them (−1320A/G, −995C/A and −554G/A) have been reported to be functional at least *in vitro* (Bayerer et al., 2007; Kraus et al., 2001). Despite these reports, none of the major haplotypes in block 1 covering the promoter region was found to interact with medication in the COMBINE Study sample except the one carrying Asp40. Therefore it remains possible, but seems unlikely, that any of these other genetic variants in the promoter or 5’UTR of *OPRM1* would be predictive of, or would have significant impact on, naltrexone treatment response.

Of note, though the most extensively investigated nonsynonymous variant in exon 1 is generally (including our study) referred to as Asn40Asp (A118G) as described originally (Bergen et al., 1997) this designation is no longer available in any of the public databases (NCBI, HapMap, ABI), because of an understanding that the OPRM1 protein can contain an additional 62 amino acids. The new designation of the SNP (rs1799971) based on the NCBI Human Genome Assembly 36 is Asn102Asp (A355G) transition (http://www.ncbi.nlm.nih.gov/SNP/).

This study has limitations. *OPRM1* was defined (as in all other studies) according to transcript variant NM_000914.2 (GenBank) containing four exons, encoding isoform MOR-1 and spanning 79,864 bp. Therefore we selected markers to cover this region and up

to 11.6 kb of the 5’ flanking region. However, another transcript variant that is publicly available is MOR-10 (NM_001008503.1) (GenBank). MOR-10 lacks the 3' exon present in MOR-1, but has an alternate downstream exon, as compared to variant MOR-1. Based on this transcript the length of the gene is 207,558 bp spanning 127,694 bp downstream of the 3' end of transcript MOR-1. Exons 1,2,3 are identical in both transcript variants. Accordingly, our markers and coverage are adequate in block 1 (covering up to 11.6 kb of the 5’ flanking region and exon 1) but in block 2 (covering exon 3 and 4 based based on MOR-1) the markers are not informative of the additional 127,694 bp downstream sequence including the alternate downstream exon (175 bp) based on transcript variant MOR-10. In addition, the statistical analysis was restricted to the three most common combinations of haplotypes both in block 1 and block 2, omitting certain combinations of major haplotypes (frequency ≥ 3%) and haplotypes with a frequency < 3%.

In this paper we attempted to evaluate the use of a single functional SNP against haplotypes that did, and did not, contain that SNP. While it is beyond the scope of this paper, the issue of a single SNP versus a haplotype approach could be generally raised. In general, haplotype approaches are likely to be better when a single SNP has not been previously identified as important or functional. This more exploratory approach, covering a larger genomic region, could provide direction for further discovery that might include a more detailed evaluation of whether any particular SNP in the haplotype carried a disproportionate weight of the prediction or association. In the particular case of the OPRM1 gene, it appeared that this was the case for the Asp40 SNP but we started from this premise and worked backward to arrive at that conclusion. Other discovery might proceed in the other direction from haplotype identification to specific SNP identification. It is also recognized that ancestry-specific haplotype differences pose a particular challenge to this approach. A recent article on alcohol expectancy effects in American Indians (Ehlers et al., 2008) that implicated OPRM1 SNP’s, other than that coding for Asp40, is an example of a number of these challenges.

In summary, in the present work, a haplotype-based approach was applied to identify effects of unknown genetic variants in *OPRM1* which might predict response to naltrexone treatment. The ten SNPs genotyped were distributed in two haplotype blocks, a 44-kb block covering up to 11.6 kb of the 5’regulatory region and exon 1, and a 28-kb block covering exons 3 and 4, each represented by five major haplotypes. The only significant haplotype by medication interaction found was in *OPRM1* block 1 evidenced by the highest percent of GCO among naltrexone-treated alcoholics with one copy of haplotype AGCCC, the single haplotype carrying the Asp40 allele. No haplotype by medication interaction was observed in block 2. Therefore, the haplotype-based approach confirms that the single locus predictive of response to naltrexone treatment is Asn40Asp in exon 1. Contribution of any other genetic variant of *OPRM1* to interindividual variation in response to naltrexone treatment (at least in terms of good clinical outcome) is not supported by our findings.

Acknowledgments

This work was funded by the National Institute on Alcohol Abuse and Alcoholism (NIAAA) Cooperative Agreements U10AA11715, 11716, 11721, 11727, 11756, 11768, 11773, 11776, 11777, 11783, 11787, 11799, and 11773 and the NIAAA Intramural Research Program (Dr. Goldman). Lipha Pharmaceutical supplied the medication for the study.

The authors wish to acknowledge the work of all of the COMBINE Study investigators and staff whose names are listed on the COMBINE Study website (http://www.cscc.unc.edu/combine). The authors also wish to acknowledge the work of James D. Hosking, who, prior to his untimely death, served as principal investigator of the COMBINE Study Coordinating Center (University of North Carolina).

References

Anton RF, O'Malley SS, Ciraulo DA, Cisler RA, Couper D, Donovan DM, Gastfriend DR, Hosking JD, Johnson BA, LoCastro JS, Longabaugh R, Mason BJ, Mattson ME, Miller WR, Pettinati HM, Randall CL, Swift R, Weiss RD, Williams LD, Zweben A. Combined pharmacotherapies and behavioral interventions for alcohol dependence: the COMBINE study: a randomized controlled trial. JAMA. 2006; 295(17):2003–2017. [PubMed: 16670409]

Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D. An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Arch Gen Psychiatry. 2008; 65(2):135–144. [PubMed: 18250251]

Arias A, Feinn R, Kranzler HR. Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. Drug Alcohol Depend. 2006; 83(3):262–268. [PubMed: 16387451]

Bare LA, Mansson E, Yang D. Expression of two variants of the human mu opioid receptor mRNA in SK-N-SH cells and human brain. FEBS Lett. 1994; 354(2):213–216. [PubMed: 7957926]

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]

Bayerer B, Stamer U, Hoeft A, Stuber F. Genomic variations and transcriptional regulation of the human mu-opioid receptor gene. Eur J Pain. 2007; 11(4):421–427. [PubMed: 16843022]

Befort K, Filliol D, Decaillot FM, Gaveriaux-Ruff C, Hoehe MR, Kieffer BL. A single nucleotide polymorphic mutation in the human mu-opioid receptor severely impairs receptor signaling. J Biol Chem. 2001; 276(5):3130–3137. [PubMed: 11067846]

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. GenBank. Nucleic Acids Res. 2007; 35(Database issue):D21–D25. [PubMed: 17202161]

Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, Goldman D. Mu opioid receptor gene variants: lack of association with alcohol dependence. Mol Psychiatry. 1997; 2(6): 490–494. [PubMed: 9399694]

Beyer A, Koch T, Schroder H, Schulz S, Hollt V. Effect of the A118G polymorphism on binding affinity, potency and agonist-mediated endocytosis, desensitization, and resensitization of the human mu-opioid receptor. J Neurochem. 2004; 89(3):553–560. [PubMed: 15086512]

Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek MJ, Yu L. Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci U S A. 1998; 95(16):9608–9613. [PubMed: 9689128]

Borner C, Hollt V, Kraus J. Involvement of activator protein-1 in transcriptional regulation of the human mu-opioid receptor gene. Mol Pharmacol. 2002; 61(4):800–805. [PubMed: 11901219]

Borner C, Kraus J, Schroder H, Ammer H, Hollt V. Transcriptional regulation of the human mu-opioid receptor gene by interleukin-6. Mol Pharmacol. 2004; 66(6):1719–1726. [PubMed: 15448191]

Chong RY, Oswald L, Yang X, Uhart M, Lin PI, Wand GS. The Micro-opioid receptor polymorphism A118G predicts cortisol responses to naloxone and stress. Neuropsychopharmacology. 2006; 31(1):204–211. [PubMed: 16123758]

Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA Jr, O'Brien CP, Berrettini WH, Grice DE. A genetic association study of the mu opioid receptor and severe opioid dependence. Psychiatr Genet. 2003; 13(3):169–173. [PubMed: 12960749]

de Bakker PI, Yelensky R, Pe'er I, Gabriel SB, Daly MJ, Altshuler D. Efficiency and power in genetic association studies. Nat Genet. 2005; 37(11):1217–1223. [PubMed: 16244653]

Delfs JM, Kong H, Mestek A, Chen Y, Yu L, Reisine T, Chesselet MF. Expression of mu opioid receptor mRNA in rat brain: an in situ hybridization study at the single cell level. J Comp Neurol. 1994; 345(1):46–68. [PubMed: 8089277]

den Dunnen JT, Antonarakis SE. Nomenclature for the description of human sequence variations. Hum Genet. 2001; 109(1):121–124. [PubMed: 11479744]

Ehlers CL, Lind PA, Wilhelmsen KC. Association between single nucleotide polymorphisms in the mu opioid receptor gene (OPRM1) and self-reported responses to alcohol in American Indians. BioMed Central Medical Genetics. 2008; 9(35)

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. The structure of haplotype blocks in the human genome. Science. 2002; 296(5576):2225–2229. [PubMed: 12029063]

Gelernter J, Gueorguieva R, Kranzler HR, Zhang H, Cramer J, Rosenheck R, Krystal JH. Opioid receptor gene (OPRM1, OPRK1, and OPRD1) variants and response to naltrexone treatment for alcohol dependence: results from the VA Cooperative Study. Alcohol Clin Exp Res. 2007; 31(4): 555–563. [PubMed: 17374034]

Gelernter J, Kranzler H, Cubells J. Genetics of two mu opioid receptor gene (OPRM1) exon I polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. Mol Psychiatry. 1999; 4(5):476–483. [PubMed: 10523821]

Gianoulakis C, Barcomb A. Effect of acute ethanol in vivo and in vitro on the beta-endorphin system in the rat. Life Sci. 1987; 40(1):19–28. [PubMed: 2948090]

Goldman D, Oroszi G, O'Malley S, Anton R. COMBINE genetics study: the pharmacogenetics of alcoholism treatment response: genes and mechanisms. J Stud Alcohol Suppl. 2005; (15):56–64. discussion 33. [PubMed: 16223057]

Hernandez-Avila CA, Wand G, Luo X, Gelernter J, Kranzler HR. Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1). Am J Med Genet. 2003; 118B(1):60–65. [PubMed: 12627468]

Hoehe MR, Kopke K, Wendel B, Rohde K, Flachmeier C, Kidd KK, Berrettini WH, Church GM. Sequence variability and candidate gene analysis in complex disease: association of mu opioid receptor gene variation with substance dependence. Hum Mol Genet. 2000; 9(19):2895–2908. [PubMed: 11092766]

Ikeda K, Ide S, Han W, Hayashida M, Uhl GR, Sora I. How individual sensitivity to opiates can be predicted by gene analyses. Trends Pharmacol Sci. 2005; 26(6):311–317. [PubMed: 15925706]

Inturrisi CE. Clinical pharmacology of opioids for pain. Clin J Pain. 2002; 18(4 Suppl):S3–S13. [PubMed: 12479250]

Kieffer BL, Gaveriaux-Ruff C. Exploring the opioid system by gene knockout. Prog Neurobiol. 2002; 66(5):285–306. [PubMed: 12015197]

Kraus J, Borner C, Giannini E, Hickfang K, Braun H, Mayer P, Hoehe MR, Ambrosch A, Konig W, Hollt V. Regulation of mu-opioid receptor gene transcription by interleukin-4 and influence of an allelic variation within a STAT6 transcription factor binding site. J Biol Chem. 2001; 276(47): 43901–43908. [PubMed: 11572871]

Krystal JH, Cramer JA, Krol WF, Kirk GF, Rosenheck RA. Naltrexone in the treatment of alcohol dependence. N Engl J Med. 2001; 345(24):1734–1739. [PubMed: 11742047]

Lerman C, Wileyto EP, Patterson F, Rukstalis M, Audrain-McGovern J, Restine S, Shields PG, Kaufmann V, Redden D, Benowitz N, Berrettini WH. The functional mu opioid receptor (OPRM1) Asn40Asp variant predicts short-term response to nicotine replacement therapy in a clinical trial. Pharmacogenomics J. 2004

Livak KJ. Allelic discrimination using fluorogenic probes and the 5' nuclease assay. Genet Anal. 1999; 14(5–6):143–149. [PubMed: 10084106]

Luo X, Kranzler HR, Zhao H, Gelernter J. Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. Am J Med Genet B Neuropsychiatr Genet. 2003; 120(1):97–108. [PubMed: 12815747]

Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP. A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology. 2003; 28(8):1546–1552. [PubMed: 12813472]

Pan L, Xu J, Yu R, Xu MM, Pan YX, Pasternak GW. Identification and characterization of six new alternatively spliced variants of the human mu opioid receptor gene, Oprm. Neuroscience. 2005; 133(1):209–220. [PubMed: 15893644]

Pasternak GW. Multiple opiate receptors: deja vu all over again. Neuropharmacology. 2004; 47 Suppl 1:312–323. [PubMed: 15464147]

Rommelspacher H, Smolka M, Schmidt LG, Samochowiec J, Hoehe MR. Genetic analysis of the mu-opioid receptor in alcohol-dependent individuals. Alcohol. 2001; 24(2):129–135. [PubMed: 11522434]

Shi MM, Myrand SP, Bleavins MR, de la Iglesia FA. High throughput genotyping for the detection of a single nucleotide polymorphism in NAD(P)H quinone oxidoreductase (DT diaphorase) using TaqMan probes. Mol Pathol. 1999; 52(5):295–299. [PubMed: 10748880]

Sobell MB, Schaefer HH, Mills KC. Differences in baseline drinking behavior between alcoholics and normal drinkers. Behav Res Ther. 1972; 10(3):257–267. [PubMed: 5047682]

Sora I, Elmer G, Funada M, Pieper J, Li XF, Hall FS, Uhl GR. Mu opiate receptor gene dose effects on different morphine actions: evidence for differential in vivo mu receptor reserve. Neuropsychopharmacology. 2001; 25(1):41–54. [PubMed: 11377918]

Stephens M, Donnelly P. A comparison of bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet. 2003; 73(5):1162–1169. [PubMed: 14574645]

Town T, Abdullah L, Crawford F, Schinka J, Ordorica PI, Francis E, Hughes P, Duara R, Mullan M. Association of a functional mu-opioid receptor allele (+118A) with alcohol dependency. Am J Med Genet. 1999; 88(5):458–461. [PubMed: 10490697]

Uhl GR, Sora I, Wang Z. The mu opiate receptor as a candidate gene for pain: polymorphisms, variations in expression, nociception, and opiate responses. Proc Natl Acad Sci U S A. 1999; 96(14):7752–7755. [PubMed: 10393893]

Volpicelli JR, Alterman AI, Hayashida M, O'Brien CP. Naltrexone in the treatment of alcohol dependence. Arch Gen Psychiatry. 1992; 49(11):876–880. [PubMed: 1345133]

Wand GS, McCaul M, Yang X, Reynolds J, Gotjen D, Lee S, Ali A. The mu-opioid receptor gene polymorphism (A118G) alters HPA axis activation induced by opioid receptor blockade. Neuropsychopharmacology. 2002; 26(1):106–114. [PubMed: 11751037]

Xuei X, Flury-Wetherill L, Bierut L, Dick D, Nurnberger J Jr, Foroud T, Edenberg HJ. The opioid system in alcohol and drug dependence: Family-based association study. Am J Med Genet B Neuropsychiatr Genet. 2007

Zhang H, Luo X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvartau E, Gelernter J. Association between two {micro}-opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. Hum Mol Genet. 2006a; 15(6):807–819. [PubMed: 16476706]

Zhang L, Kendler KS, Chen X. The mu-opioid receptor gene and smoking initiation and nicotine dependence. Behav Brain Funct. 2006b; 2:28. [PubMed: 16887046]

Zhang Y, Wang D, Johnson AD, Papp AC, Sadee W. Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. J Biol Chem. 2005; 280(38):32618–32624. [PubMed: 16046395]

Zhou Z, Zhu G, Hariri AR, Enoch M-A, Scott D, Sinha R, Virkkunen M, D.C M, Lipsky RH, Hu X, Hodgkinson C, Xu K, Buzas B, Yuan Q, Shen P, Ferrel RE, Manuck SB, Brown SM, Hauger RL, Stohler CS, Zubieta J-K, Goldman D. Variation in expression of Neuropeptide Y has convergent effects on human emotion and stress response. (submitted).

Zweben A, Cisler RA. Clinical and methodological utility of a composite outcome measure for alcohol treatment research. Alcohol Clin Exp Res. 2003; 27(10):1680–1685. [PubMed: 14574241]

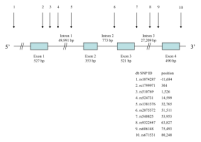

Figure 1.
Schematic (non-scaled) structure of the human μ opioid receptor gene (*OPRM1*) based on the MOR-1 transcript variant (NM_000914.2). Positions of 10 SNPs genotyped and their dbSNP IDs are also shown. Nucleotide +1 is the A of the ATG translation initiation codon, the nucleotide 5’ to +1 is numbered −1.

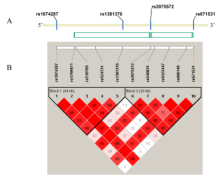

Figure 2.

(A) Scaled schematic structure of *OPRM1 (on line version is in color)*. The yellow (top) bar represents the contig sequence spanning 20kb upstream and 10kb downstream of the 5' and 3' ends of the gene indicated by the green (lower) rectangle. The green vertical bars in the rectangle indicate exons. (B) Linkage disequilibrium (LD) plot of 10 *OPRM1* SNPs based on 685 Caucasians in the COMBINE Study. The D' value of each SNP pair is shown in the squares. The numbers in the squares are D'x 100. Empty squares indicate D' = 1. Squares are colored bright red (dark grey) if the D' value is high and the confidence in the value of D' is high as well. The first and the last markers in each block are also displayed on the structure of *OPRM1* to help the comparison between the two parts of the figure.

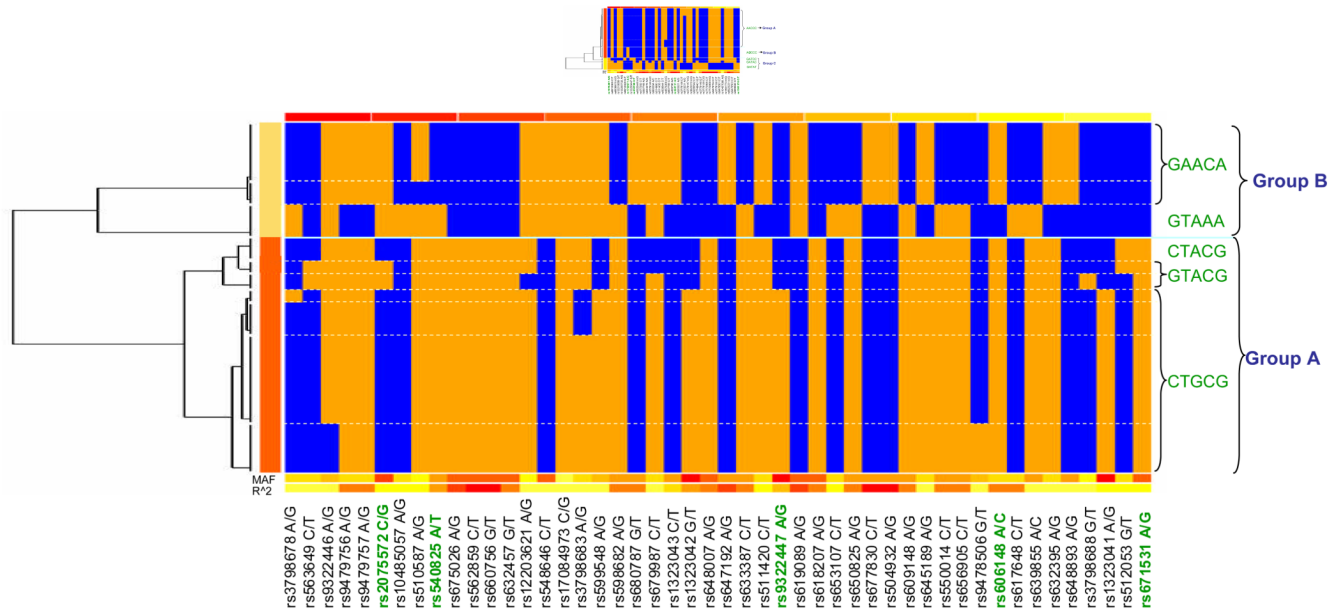

Figure 3.

Close correspondence of haplotypes in the COMBINE Study and HapMap and haplotype grouping (*on line version is in color*). Alleles of markers (SNPs) genotyped in both datasets are highlighted in green (bold). The first allele and second allele in the haplotypes are depicted in blue (dark grey) and brown (light grey), respectively. The height of each haplotype is proportional to the CEU haplotype frequency in HapMap. Haplotypes with a frequency ≥ 3% are shown in both datasets. a). Block 1: The SNP (rs1799971) for Asn40Asp(A118G) is highlighted in bold italics b). Block 2.

Table 1

Demographics and pre-study drinking data of Caucasian participants who did not receive Combined Behavioral Intervention and who had haplotype data for either the *OPRM1* block 1 or block 2 haplotype

	Placebo (n=160)	Naltrexone (n=146)
Categorical variables, No. (%)		
Male	114 (71.3)	98 (67.1)
Married	60 (37.5)	62 (42.5)
Employed	121 (75.6)	108 (74.0)
Years of education ≤ 12	46 (28.8)	38 (26.0)
Current smoker	64 (40.0)	67 (45.9)
GGT above normal limit	45 (28.1)	40 (27.4)
CDT above normal limit	79 (49.4)	62 (42.5)
Continuous variables, mean (SD)		
Age, years	45.3 (10.51)	45.0 (10.98)
Percent days abstinent (PDA)[1]	23.7 (25.09)	24.6 (25.06)
Drinks per drinking day[1]	12.4 (7.08)	12.7 (8.51)
Overall drinks per day[1]	9.3 (6.45)	9.4 (6.96)
Heavy drinking days[1]	20.1 (8.31)	19.9 (8.59)
Alcohol dependence score (ADS)	17.1 (7.33)	16.6 (8.14)
OCDS score	26.2 (7.35)	25.3 (7.43)
Drinking Consequences (DRINC)	49 (20.37)	46 (20.30)
% CDT	3.6 (2.49)	3.2 (1.78)
GGT IU/L	76.5 (152.7)	62.6 (75.76)
Number of alcohol dependent symptoms from SCID	5.6 (1.26)	5.5 (1.28)

[1] In the 30 days prior to randomization. Abbreviations: GGT, gamma-glutamyl transpeptidase; CDT, carbohydrate-deficient transferrin; OCD, obsessive-compulsive drinking scale; SCID, structured clinical interview for DSM disorders

Table 2

List of SNPs, their positions and genotyping data based on the whole cohort (915 subjects)

NCBI db SNP ID	Chr position[1]	SNP	SNP gene location	Duplicates	Discrepancy rate[2]	Completion rate[3]
1. rs1074287	154,440,923	A>G	upstream	261 (28.90%)	0%	98.69%
2. rs1799971	154,452,911	A>G	Exon 1 (Asn40Asp)	203 (22.28%)	0%	99.56%
3. rs510769	154,454,133	C>T	intron 1	204 (22.90%)	0%	97.38%
4. rs524731	154,467,206	C>A	intron 1	204 (22.90%)	0%	97.38%
5. rs1381376	154,485,372	C>T	intron 1	201 (22.31%)	0%	98.47%
6. rs2075572	154,504,118	C>G	intron 2	201 (22.46%)	0%	97.81%
7. rs540825	154,506,560	T>A	intron 3	203 (22.61%)	0%	98.14%
8. rs9322447	154,516,434	G>A	intron 3	194 (22.15%)	0.52%	95.74%
9. rs606148	154,528,100	C>A	intron 3	186 (20.81%)	0.54%	97.70%
10. rs671531	154,532,855	G>A	downstream	234 (26.47%)	0.43%	96.61%

[1] chromosome positions are based on NCBI Human Genome Assembly Build 35

[2] ratio of the number of discordant genotypes to the number of duplicates

[3] ratio of the number of valid genotypes to the number of subjects genotyped (915) at each locus

Table 3

List of SNPs, their minor allele frequencies and HWE P values for distribution of genotypes at each locus based on the whole cohort (915 subjects) and Caucasians (687 subjects)

	Whole cohort		Caucasians	
NCBI dbSNP ID	**MAF[1]**	**HWE[2] P value**	**MAF**	**HWE P value**
rs1074287	0.278	0.427	0.254	0.442
rs1799971	0.125	0.434	0.126	0.485
rs510769	0.258	0.469	0.253	0.447
rs524731	0.198	0.085	0.196	0.158
rs1381376	0.156	0.625	0.159	0.536
rs2075572	0.436	0.254	0.427	0.471
rs540825	0.211	0.688	0.237	0.957
rs9322447	0.493	0.178	0.47	0.399
rs606148	0.082	0.635	0.086	0.612
rs671531	0.332	0.699	0.335	0.923

[1] minor allele frequency

[2] Hardy-Weinberg equilibrium

Table 4

Haplotypes in *OPRM1* block 1 and their frequencies in COMBINE and HapMap. Haplotypes with a frequency ≥ 3% are shown

Haplotype	Frequency in COMBINE	Frequency in HapMap
AACCC	0.607	0.583
GATAT	0.139	0.108
AG[1]CCC	0.125	0.158
GATCC	0.05	0.042
GATAC	0.046	0.033

[1] Asp40

Table 5

Haplotypes in *OPRM1* block 2 and their frequencies in COMBINE and HapMap. Haplotypes with a frequency ≥ 3% are shown

Haplotype	Frequency in COMBINE	Frequency in HapMap
CTGCG	0.518	0.449
GAACA	0.226	0.208
GTACG	0.091	0.075
GTAAA	0.088	0.100
CTACG	0.046	0.058

Table 6

Grouping of haplotypes for haplotype-based analyses based on relatedness and function in *OPRM1* block 1 and block 2

Block 1	**Block 2**
Group A: AACCC	Group A: CTGCG, GTACG, CTACG
Group B: A**G**CCC (single haplotype carrying Asp40)	Group B: GAACA, GTAAA
Group C: GATCC, GATAC, GATAT	
Group O: all the remaining haplotypes in block 1 (frequency: < 0.03)	Group O: all the remaining haplotypes in block 2 (frequency < 0.03)

Table 7

Percent of patients with Good Clinical Outcome (GCO) based on *OPRM1* block 1 diplotypes and medication. N is the number of subjects in each group

	Placebo			Naltrexone			
Combinations of haplotypes	**N**	**GCO (%)**	**P value[1]**	**N**	**GCO (%)**	**P value[1]**	**Interaction P value[2]**
AA	54	57.4	0.61	54	53.7	**0.01**	**0.03**
AB	25	48.0		20	**90.0**		
AC	53	49.1		43	58.1		

A = AACCC (haplotype carrying Asn40 in position 2)

B = AGCCC (haplotype carrying Asp40 in position 2)

C = GATCC, GATAC, GATAT (other haplotypes carrying Asn40 in position 2)

[1]The p-values within each medication category (naltrexone or placebo) are from a chi-squared test of equality of proportions of those with GCO across the diplotypes.

[2]The interaction p-values are from logistic regression models with dependent variable being “having a good clinical outcome (yes or no)” and the independent variables naltrexone status (active or placebo), diplotype, and their interaction, with baseline percent days abstinent used as a covariate.

Table 8

Interaction of (genotypes at) each of the five SNPs in *OPRM1* block 1 with medication reflected by percent of patients with Good Clinical Outcome (GCO)

Position[1] of SNP	Genotype groups	Placebo[2]	GCO (%)	Naltrexone[2]	Genotype by medication interaction (P value)[4]
1	AA vs.	54.8		62.5	0.99
	AG or GG	51.4		60.0	
2	AA vs.	54.1		54.4	**0.006**
	AG or GG[3]	50.0		**87.1**	
3	CC vs.	56.0		65.0	0.83
	CT or TT	50.0		56.9	
4	CC vs.	54.3		62.8	0.98
	CA or AA	51.0		59.3	
5	CC vs.	53.9		62.2	0.99
	CT or TT	51.2		59.6	

[1] with reference to Table 2.

[2] each group had at least 31 subjects

[3] Asn40/Asp40 or Asp40/Asp40

[4] The interaction p-values are from logistic regression models with the dependent variable being “having a good clinical outcome (yes or no)” and the independent variables naltrexone status (active or placebo), genotype, and their interaction, with baseline percent days abstinent used as a covariate.

Table 9

Interaction of (genotypes at) each of the four SNPs in *OPRM1* block 1 with medication reflected by percent of patients with Good Clinical Outcome (GCO) when position 2 is held constant AA (Asn40/Asn40)

Position of SNP[1]	Genotype groups	Position 2[1]	Placebo[2]	GCO (%)	Naltrexone[2]	Genotype by medication interaction (P value)[3]
1	AA vs.	AA	57.1		52.6	0.49
	AG or GG		51.5		56.1	
3	CC vs.	AA	58.9		56.1	0.68
	CT or TT		50.0		52.6	
4	CC vs.	AA	57.3		52.5	0.34
	CA or AA		48.9		56.6	
5	CC vs.	AA	55.4		53.4	0.62
	CT or TT		51.3		56.1	

[1] with reference to Table 2.

[2] each group had at least 39 subjects

[3] The interaction p-values are from logistic regression models with the dependent variable being “having a good clinical outcome (yes or no)” and the independent variables naltrexone status (active or placebo), genotype, and their interaction, with baseline percent days abstinent used as a covariate.

Table 10

Percent of patients with Good Clinical Outcome (GCO) based on *OPRM1* block 2 diplotypes and medication. N is the number of subjects in each group

	Placebo			Naltrexone			
Combinations of haplotypes	**N**	**GCO (%)**	**P value[1]**	**N**	**GCO (%)**	**P value[1]**	**Interaction P value[2]**
AA	75	53.3	0.93	64	71.9	0.01	0.07
AB	64	51.6		60	51.7		
BB	14	57.1		10	30.0		

A = CTGCG, GTACG, CTACG

B = GAACA, GTAAA

[1]The p-values within each medication category (naltrexone or placebo) are from a chi-squared test of equality of proportions of those with GCO across the diplotypes.

[2]The interaction p-values are from logistic regression models with dependent variable being “having a good clinical outcome (yes or no)” and the independent variables naltrexone status (active or placebo), diplotype, and their interaction, with baseline percent days abstinent used as a covariate.