Published in final edited form as:
Am J Psychiatry. 2013 September 1; 170(9): . doi:10.1176/appi.ajp.2013.12091163.

DETERMINATION OF GENOTYPE COMBINATIONS THAT CAN PREDICT THE OUTCOME OF THE TREATMENT OF ALCOHOL DEPENDENCE USING THE 5-HT$_3$ ANTAGONIST ONDANSETRON

Bankole A. Johnson, DSc, MD[a], **Chamindi Seneviratne, MD**[a], **Xin-Qun Wang, MS**[b], **Nassima Ait-Daoud, MD**[a], and **Ming D. Li, PhD**[a]

[a]Department of Psychiatry and Neurobehavioral Sciences University of Virginia, Charlottesville, Virginia, USA

[b]Department of Public Health Sciences, University of Virginia, Charlottesville, Virginia, USA

Abstract

Objective—Previously, we reported that the 5′-HTTLPR-LL and rs1042173-TT (*SLC6A4*-LL/TT) genotypes in the serotonin transporter gene predicted a significant reduction in the severity of alcohol consumption among alcoholics receiving the 5-HT$_3$ antagonist ondansetron. In this study, we explored additional markers of ondansetron treatment response in alcoholics by examining polymorphisms in the *HTR3A* and *HTR3B* genes, which regulate directly the function and binding of 5-HT$_3$ receptors to ondansetron.

Method—We genotyped 1 rare and 18 common single-nucleotide polymorphisms in *HTR3A* and *HTR3B* in the same sample that we had genotyped for *SLC6A4*-LL/TT in the previous randomized, double-blind, 11-week clinical trial. Participants were 283 European Americans who received oral ondansetron (4 μg/kg twice daily) or placebo along with weekly cognitive behavioral therapy. Associations of individual and combined genotypes with treatment response on drinking outcomes were analyzed.

Results—Individuals carrying one or more of genotypes rs1150226-AG and rs1176713-GG in *HTR3A* and rs17614942-AC in *HTR3B* showed a significant overall mean difference between ondansetron and placebo in drinks per drinking day (−2.50; effect size (ES)=0.867), percentage of heavy drinking days (−20.58%; ES=0.780), and percentage of days abstinent (18.18%; ES=0.683). Combining these *HTR3A/HTR3B* and *SLC6A4*-LL/TT genotypes increased the target cohort from approaching 20% (identified in our previous study) to 34%.

Conclusions—We present initial evidence suggesting that a combined 5-marker genotype panel can be used to predict the outcome of treatment of alcohol dependence with ondansetron. Additional, larger pharmacogenetic studies would help to validate our results.

Corresponding author: Bankole A. Johnson, DSc, MD, Alumni Professor and Chairman, Department of Psychiatry and Neurobehavioral Sciences, University of Virginia, P.O. Box 800623, Charlottesville, VA 22908-0623. Telephone: 434-924-5457; Fax: 434-244-7565; bankolejohnson@virginia.edu.

Disclosures: B. A. Johnson served as a consultant to Johnson & Johnson (Ortho-McNeil Janssen Scientific Affairs, LLC) 5 years ago, Transcept Pharmaceuticals, Inc. 4 years ago, Eli Lilly and Company 3 years ago, and Organon 3 years ago; he currently consults for D&A Pharma, ADial Pharmaceuticals, LLC (with which he also serves as Chairman), and Psychological Education Publishing Company (PEPCo), LLC. M. D. Li has served as a consultant and board member of ADial Pharmaceuticals, LLC. The other authors report no financial relationships with commercial interests.

ClinicalTrials.gov Identifier: NCT00382642 (see http://www.clinicaltrials.gov/ct2/show/NCT00382642?term=ondansetron+AND+alcohol&rank=6)

INTRODUCTION

Alcoholism is a heterogeneous complex disorder with multiple subtypes or endophenotypes (1). Perhaps because of this heterogeneity, the therapeutic effect sizes of the approved medicines for the treatment of alcohol dependence have been relatively small (2). A personalized approach based on a person's genetic makeup is increasingly being investigated for delivering optimum treatment to the “right” patient. Previously, in a randomized double-blind clinical trial of alcohol-dependent individuals of European descent, we showed that treatment of severe drinking with ondansetron, a specific serotonin-3 (5-HT_3) antagonist, was significantly more efficacious in a subpopulation carrying the serotonin-transporter-linked polymorphic region LL (5′-HTTLPR-LL) and rs1042173-TT (*SLC6A4*-LL/TT) genotype combination in the serotonin transporter (5-HTT) gene, *SLC6A4* (3). Briefly, in that study, we found that *SLC6A4*-LL/TT carriers treated with ondansetron had 2.63 fewer standard drinks per drinking day and a 16.99% higher percentage of days abstinent compared with non-carriers of the *SLC6A4*-LL/TT genotypes. In contrast, when subjects were not subgrouped according to their 5′-HTTLPR and rs1042173 genotypes, there was no statistically significant difference between the ondansetron and placebo groups.

Although the *SLC6A4*-LL/TT genotypes predicted ondansetron treatment response, ondansetron cannot bind directly to 5-HTT molecules since it is an antagonist at the 5-HT_{3A} receptor subunit (4–7). Determination of the 5-HTT genotypes that we selected as being predictive of ondansetron treatment response in our previous study (3) was, therefore, indirect and based on previous *in vitro* and *in vivo* experimental evidence indicating that *SLC6A4*-LL/TT is associated with markedly reduced 5-HTT expression levels in alcohol-dependent individuals (8–11). The postsynaptic 5-HT_3 receptors are formed by 5-HT_{3A} homopentamers or 5-HT_{3A} and 5-HT_{3B} heteropentamer (5-HT_{3AB}) complexes that evince even faster ion transport and conduction. The 5-HT_{3B} subunits do not bind with serotonin or ondansetron but are important for stabilizing the receptor complex at the cell surface (12, 13). The 5-HT_3 A and B subunits are encoded by the *HTR3A* and *HTR3B* genes located adjacently on chromosome 11, separated only by an intergenic region of 28 kb. Hodge and colleagues (14), using a transgenic mice model lacking the gene for the 5-HT_{3A} subunit, showed that the reduction of alcohol consumption produced by 5-HT_3 antagonism is dependent on the presence of 5-HT_{3A}-containing receptors. Recently, a genetic association study conducted by Enoch and colleagues (15) that examined African-American male patients with a lifetime diagnosis of alcohol, cocaine, and/or heroin dependencies showed an association between these diseases and the particular genotypes of *HTR3A*, *HTR3B*, and the 5′-HTTLPR. These findings were supported by earlier work from the same group that showed an association between certain *HTR3B* genotypes and alcohol dependence with antisocial behaviors (16).

Taken together, these risk association studies implicate a collective effect of 5-HT_3 and 5′-HTTLPR genotypes on susceptibility for alcoholism. In the present study, we further extended our previously tested hypothesis to include functional variants in the *SLC6A4*, *HTR3A*, and *HTR3B* genes that would interact with each other to mediate ondansetron treatment response. This new extended hypothesis was tested in the same sample that was examined in our previously published phase II pharmacogenetic trial with 283 alcoholics of European descent. If such predictors were found in the *HTR3A* and *HTR3B* genes, they would help identify a larger population of alcoholics who would respond to ondansetron in treating alcohol dependence.

METHOD

Participants

The study population analyzed here is identical to the sample used in our previous study that tested the genetic effects of the *SLC6A4* 5-–HTTLPR-LL and rs1042173-TT genotypes on ondansetron's efficacy (3). Briefly, all 283 subjects were alcohol-dependent individuals with no comorbid axis I diagnoses as assessed by the Diagnostic and Statistical Manual of Mental Disorders, *4th edition* (17) who scored >8 on the Alcohol Use Disorders Identification Test (18) and were enrolled in an 11-week, randomized, double-blind clinical trial in which they received either oral ondansetron (4 μg/kg twice daily) or placebo along with weekly cognitive behavioral therapy (19). Details of the inclusion and exclusion criteria for the participants have been provided previously (3).

Study Design

We performed an *a priori* stratification based on a subject's 5′-HTTLPR genotype, with additional genotyping of single-nucleotide polymorphism (SNP) rs1042173 in the 3′-untranslated region (3′-UTR) of the *SLC6A4*. Candidate genotyping for polymorphisms in *HTR3A* and *HTR3B* were performed, retrospectively, and were not used as stratification factors (see Figures 1 and 2). We assessed the effect of genotype on three different measures of alcohol consumption—drinks per drinking day (DDD; i.e., the amount of severe drinking), percentage of heavy drinking days (PHDD; i.e., the frequency of severe drinking), and percentage of days abstinent (PDA; i.e., the frequency of not drinking)—for those who received either ondansetron or placebo. Information on daily alcohol consumption was collected for the 90 days prior to enrollment and during the study period using the timeline follow-back method (20). Similar to our previous study, we employed a mixed-effects statistical model to examine genetic associations with drinking patterns throughout the 3-month treatment period, rather than at a single time point.

Genotyping

Genomic DNA was extracted from the blood of each subject at baseline with a Gentra Puregene® kit (QIAGEN Inc., Valencia, CA). For *SLC6A4* polymorphisms, genotyping data for 5′-HTTLPR L/S and rs1042173 polymorphisms were obtained from our previous pharmacogenetic trial (3, 21). For *HTR3A* and *HTR3B* polymorphisms, we genotyped 10 SNPs in *HTR3A* and 9 SNPs in *HTR3B* using a commercially available TaqMan® premade genotyping assay (Applied Biosystems, Foster City, CA) on an ABI 7900 platform. Average densities of their distribution on the two genes were about 1 SNP every 2 kb in *HTR3A* and every 5 kb in *HTR3B*. Candidate SNPs were selected based on their location on the gene, having a minor allele frequency of >5%, and previously reported findings by other researchers.

Because we did not detect a significant association of ondansetron treatment response with rs25531 genotypes alone or in combination with 5′-HTTLPR genotypes in our previous study, we have not examined 5′-HTTLPR tri-allelic effects in the present study.

Genotyping procedures for ancestral informative marker SNPs to assess population stratification using a 24-ancestral marker panel have been described previously (3, 21). Biological and primer/probe sequence information for all of the above-mentioned *SLC6A4*, *HTR3A*, and *HTR3B* polymorphisms is shown in Supplementary Table 1.

Statistical Analysis

Departure from Hardy-Weinberg Equilibrium (HWE) was assessed using Haploview (v. 4.0) software (22). Quality of clinical data was assessed as described previously (3). Additional

to the primary outcome variable, DDD, we tested two other secondary outcome measures—PHDD and PDA. One heavy drinking day is defined as a day on which the subject consumed ≥5 drinks for male or ≥4 drinks for female patients.

To study the effect of treatment and genotypes on DDD, PHDD, and PDA, we used mixed-effects linear regression models, which can accommodate missing data at random. The models included random intercept and slope (for temporal trend) and were adjusted for participants' average drinking levels prior to the study, age, gender, and proportions of genetic ancestry as covariates. Proportions of genetic ancestry were calculated using the Structure program (http://pritch.bsd.uchicago.edu/software/structure2_2.html) as described previously (3).

To minimize type I error, we have used two filtering steps in our study. First, we examined the drinking data for all 19 *HTR3A* and *HTR3B* SNPs (Supplementary Table 1) and restricted further analysis to only those that had a difference between the ondansetron and placebo groups of more than 1.5 DDD and a p-level <0.05 (Figure 1). The 1.5 DDD cutoff was chosen because in our primary analysis, we detected a reduction of about 1.5 standard DDD in ondansetron-treated alcoholics who possessed the 5′-HTTLPR-LL genotype, which was the randomization factor (3). Thus, the 1.5 DDD cutoff was set with the empirical goal of discovering other genetic markers that would be at least as predictive of a response to ondansetron treatment. For all 19 *HTR3A* and *HTR3B* SNPs, we tested statistical models that included 2-way interaction terms between genotypes of a given SNP. Genotype associations with drinking outcome measures for each SNP were tested using dominant (homozygous major allele genotype plus heterozygous genotype vs. homozygous minor allele genotype), recessive (homozygous minor allele genotype plus heterozygous genotype vs. homozygous major allele genotype), or heterozygous (heterozygous genotype vs. homozygous major allele genotype plus homozygous minor allele genotype) genetic models. The differences in means between “genotype × treatment” combinations for all genotypes are presented in Supplementary Table 2. Second, in addition to the above-mentioned screening process, to further minimize type I error due to multiple testing, we calculated false discovery rate (FDR) q-values (23) for all “genotype × treatment” association analyses that survived the screening process, as shown in Supplementary Table 2.

Subsequent to the screening process, the selected SNPs were subjected to a filtering process, based on their associations with the two secondary outcome measures, PHDD and PDA. Figure 1 summarizes the SNP selection process used in the study. Only the SNPs that survived filtering were analyzed for their interactive effects on the primary outcome measure—DDD—and the secondary outcome measures—PHDD and PDA. All interactions were tested at two levels: 1) the effect of treatment on DDD, PHDD, and PDA in those who possessed one, two, or all of the *HTR3A* and *HTR3B* genotypes, regardless of their previously identified 5′-HTTLPR and rs1042173 genotypes, and 2) the combined effect of the *HTR3A* and *HTR3B* genotypes together with the previously identified *SLC6A4*-LL/TT genotypes. The sample sizes included in the combined genotypes × treatment groups are given in Figure 2. Statistical power analyses (24) were performed as described previously (3).

Validation of detected genotype combinations using the generalized multifactor dimensionality reduction (GMDR) method—As shown above and illustrated in Figure 1, our statistical approach in identifying interactive effects of different genotype combinations was based on pooling individually significant variants and analyzing their combined effects on the drinking measures. This approach, however, could potentially fail to identify individual variants having significant epistatic effects but having no significant main genetic effects on the phenotypes of interest. To address this concern and

validate our results from the primary statistical analysis, we performed an independent SNP-by-SNP interaction analysis using the GMDR method (25), one of the commonly used approaches for detecting genetic epistatic effects (26, 27). The GMDR method allowed us to test for the best interaction models consisting of 2 to 5 variants by performing an exhaustive search using all 21 variants genotyped in the present study. As the response variable for GMDR analyses, we used predicted values for the three different drinking outcome measures during the entire treatment period. These predictive values were generated by using the same mixed-effects linear regression models that we used above for detecting significant interactive effects of different genotype combinations on three outcome measures, which included random intercept and slope (for temporal trend) adjusted for age, gender, ethnicity defined by ancestral informative markers, center, and corresponding baseline drinking outcome measures.

RESULTS

Demographic Characteristics

Demographic characteristics of the sample analyzed in the current study are the same as in our previous publication (3). Briefly, the current study included all 283 participants who identified themselves as being of European descent. Within this European-American cohort, 122 Caucasian and 18 Hispanic individuals received ondansetron; 118 Caucasian and 25 Hispanic individuals received the placebo. As discussed previously (3), potential population stratification was assessed with the 24-SNP ancestral informative marker panel using the Structure program. Results from the Structure program revealed no population admixture between those who identified themselves as Hispanic and Caucasian or between ondansetron- and placebo-treated individuals. Therefore, all 283 subjects were included in the final analyses, and the individual ancestral scores were utilized as covariates in place of self-reported ancestry.

Overall genotyping failure rates for all 21 polymorphisms examined in the present study were less than 3%. For genotyping quality control, we performed HWE tests on all 21 polymorphisms in *HTR3A*, *HTR3B*, and *SLC6A4*, and none of the variants showed significant deviations in either the ondansetron or the placebo group (Supplementary Table 1). Thus, they were all included in the analyses detailed below. Furthermore, there were no statistically significant differences in genotype frequencies between the ondansetron and placebo groups for all genotypes included in the analyses (Supplementary Table 2).

Association of Drinking Outcome Measures with HTR3A *and* HTR3B *Polymorphisms at the Individual Level*

The DDD estimates for all genotype groups examined in the present study are listed in Supplementary Table 2. As shown in that table, genotypes rs17614942-AC in *HTR3B* and rs1150226-AG, rs1176713-GG, and rs1176719-AA in *HTR3A* were significantly associated with DDD reductions of more than 1.5 in response to ondansetron treatment ($p<0.05$). Of the 4 *HTR3A* and *HTR3B* SNPs that survived the screening process, rs1176719-AA did not show a statistically significant association with PHDD (PHDD reduction=15.35%; 95% confidence interval [CI]=−37.55% to 6.86%; p=0.175); furthermore, the association of rs1176719-AA with PDA neither was statistically significant nor reached the 10% threshold (PDA improvement=3.48%; 95% CI=−2.98% to 9.94%; p=0.291). Hence, rs1176719 was excluded (Figure 1) from further analyses performed to test the interactive effects. The rs1176713 genotype GG showed only a trend with PHDD and was not significantly associated with PDA; yet, the magnitudes of PHDD and PDA (Table 1) were similar to those of rs1150226-AG and rs17614942-AC. We, therefore, included the following genotypes in the subsequent multilocus interaction analyses: *SLC6A4*-LL/TT, *HTR3B*-

rs17614942-AC, and *HTR3A*-rs1150226-AG and -rs1176713-GG. The FDR q-values for DDD associations with the *HTR3B*-rs17614942-AC and *HTR3A*-rs1150226-AG and -rs1176713-GG genotypes ranged from 0.156 to 0.311 (Supplementary Table 2).

Combined Interactive Effects of Significantly Associated HTR3A, HTR3B, *and Previously Reported* SLC6A4-*LL/TT Genotypes on Drinking*

We have identified three *HTR3A/HTR3B* (HTR3) genotypes (i.e., *HTR3B*-rs176149-AC, *HTR3A*-rs1150226-AG, and *HTR3A*-rs1176713-GG) in the above single-locus analyses and the *SLC6A4*-LL/TT genotype combination in our previous study; all of these polymorphisms were associated with lowering alcohol consumption from heavy to non-heavy levels, reducing it by at least 2 standard drinks on a given drinking day. Yet, if these five polymorphisms are to be utilized as a marker panel for screening individual DNA samples, it is imperative to know their predictability when they are present alone or in combination with each other. To examine combined interactive effects, we adopted an analytical strategy similar to the collapsing method, which is used widely in rare-variant analyses in genetic association studies (28–32). More specifically, the collapsing method investigates for the presence or absence of several rare alleles in a region of interest and then treats them as a single common variant; similarly, in our analysis, we have investigated for the presence or absence of the *HTR3B*-rs176149-AC, *HTR3A*-rs1150226-AG, *HTR3A*-rs1176713-GG, and SLC6A4-LL/TT genotypes and combined them in the following two ways. First, we assessed the combined effects of HTR3 genotypes identified in the present study, regardless of the presence or absence of *SLC6A4*-LL/TT genotypes. Next, we assessed the combined effects of HTR3 and *SLC6A4*-LL/TT genotypes in both the current and previous studies.

***(1) Combined effects of* HTR3A and HTR3B polymorphisms on drinking outcome measures, regardless of presence or absence of SLC6A4-*LL/TT genotypes*—**Combination-1 indicates the effects of carrying *only one* of the three HTR3 genotypes on ondansetron treatment response. Included subgroups are: “rs1150226-AG & no rs176149-AC or rs1176713-GG”, “rs1176713-GG & no rs1150226-AG or rs176149-AC”, and “rs176149-AC & no rs1150226-AG or rs1176713-GG”. All subgroups were present in less than 5% of the total cohort, and overall, Combination-1 represents 11% of the total study population.

Combination-2 indicates the effects of carrying any *two* of the three HTR3 genotypes on ondansetron treatment response. Included subgroups are: “rs1150226-AG & rs176149-AC & no rs1176713-GG” and “rs176149-AC & rs1176713-GG & no rs1150226-AG”. All but one subject in Combination-2 belonged to the subgroup “rs1150226-AG & rs176149-AC & no rs1176713-GG”. This can be partly explainable by the low linkage disequilibrium (LD) between rs1176713 and the other two SNPs and the high LD between rs17614942 and rs1150226. (r^2 values for linkage are given in the LD plot shown in Figure 3.)

Combination-3 indicates the effects of possessing one or any combination of two or all three HTR3 genotypes. However, rs1176713-GG was found only in two of the rs17614942-AC carriers and was not detected in any rs1150226-AG carrier. This lack of individuals possessing all three HTR3 genotypes in our sample resulted in Combination-3 being more like a pooled group of Combinations-1 and -2 (23% of the total study population) rather than a new group consisting of individuals possessing 1, 2, or all 3 HTR3 genotypes. The included subgroups in Combination-3 are “rs1150226-AG & rs17614942-AC & no rs1176713-GG” (48%), “AG & no AC or rs1176713-GG” (22%), “rs1176713-GG & no rs1150226-AG or rs17614942-AC” (21%), “rs17614942-AC & no rs1150226-AG or rs1176713-GG” (6%), and “rs17614942-AC & rs1176713-GG & no rs1150226-AG” (3%).

As shown in Table 2 and Figure 4A,B,C, Combination-3 was significantly associated with all three outcome measures: DDD, PHDD, and PDA. The effect sizes for DDD, PHDD, and PDA associations with ondansetron treatment outcome in Combination-3 were 0.867, 0.780, and 0.683, respectively.

***(2) Combined effects of* HTR3A, HTR3B, *and previously reported* SLC6A4-*LL/TT on drinking outcome measures*—**Our previously reported 5-HTT gene *SLC6A4*-LL/TT genotype combination had a 2.08 (95% CI=−3.75 to −0.41; p=0.015) DDD reduction between *SLC6A4*-LL/TT carriers who received ondansetron and placebo regardless of their genotypes at the 3 HTR3 SNPs; there were no statistically significant differences for PHDD or PDA between *SLC6A4*-LL/TT carriers who received ondansetron and placebo. However, within the group of subjects who received ondansetron, *SLC6A4*-LL/TT carriers, compared with non-carriers, were significantly more likely to have more abstinent days (12.68%; 95% CI=0.48 to 24.89; p=0.042). Next, to study the combined effects of the HTR3 and *SLC6A4*-LL/TT genotypes on the three outcome variables, we pooled all subjects carrying the above-mentioned Combination-3 and/or *SLC6A4*-LL/TT genotypes (Combination-4) and compared them with the non-carriers of Combination-4.

Combination-4 indicates the effects of possessing the following genotype subgroups: “LL/TT & no HTR3 genotypes” (32%), “any two HTR3 genotypes & no LL/TT” (26%), “any one HTR3 genotype & no LL/TT” (25%), “LL/TT combined with two HTR3 genotypes” (8.5%), and “LL/TT combined with only one HTR3 genotype” (8.5%). As shown in Table 2, the DDD reductions in Combination-4 were close to two standard drinks in ondansetron-treated individuals, compared with the placebo group (p=0.004) (Figure 4D). In addition, carriers of Combination-4 who were treated with ondansetron also had significantly improved PHDD (Figure 4E) and PDA (Figure 4F). The effect sizes for DDD, PHDD, and PDA associations with ondansetron treatment outcome in Combination-4 carriers were 0.593, 0.416, and 0.428, respectively.

Notably, in all of the above combinations, the ondansetron group not carrying the targeted genotype group (i.e., groups designated as “All Others” in Figure 4), compared with their placebo counterparts, had a trend toward worse outcomes on our three measures of alcohol consumption (i.e., DDD, PHDD, and PDA).

The Combination-4 group included four infrequent (<5% of the total study population) subgroups carrying *SLC6A4*-LL/TT and one or two of the HTR3 genotypes. Associations of these infrequent genotype groups with the primary outcome measure, DDD, are given in Supplementary Table 3. As seen in Supplementary Table 3, DDD reductions in these groups of individuals with markers for both HTR3 and *SLC6A4* were around 4 standard drinks, which was greater than the DDD reductions detected for Combinations 1–4 in Table 2. The greatest reduction in DDD was seen in the combination of *SLC6A4*-LL/TT and rs1176713-GG (Supplementary Table 3; Combination-1).

Independent Determination of SNP-by-SNP Interactive Effects Using the GMDR Method

To validate the significant interactive effects of various genotype combinations detected by the above-mentioned statistical approach, we conducted an independent SNP-by-SNP interaction analysis on all SNPs of the three genes using the GMDR method. As shown in Table 3, the detected significant 5-variant genotype combination for the three outcome measures, determined by the primary statistical approach above, appeared also to be significant based on the parameters of test accuracy, cross-validation consistency, and permutated p-value used commonly in the evaluation of GMDR results (25, 33, 34). This provides an independent determination of the best genotype combinations that can be used to predict treatment outcomes for the three alcohol dependence measures.

As reported previously, except for fatigue (p=0.019), no other adverse events (including QT-interval prolongations) occurred more frequently in the ondansetron-treated group than in the placebo group (3); nor did they occur more frequently among carriers of the above-mentioned genotype combinations than among non-carriers (data not shown). There were no life-threatening events, and the five most commonly reported adverse events were (ondansetron vs. placebo): insomnia (20.5% vs. 22.3%), headache (20.9% vs. 19.4%), appetite disturbance (18.0% vs. 20.1%), fatigue (18.0% vs. 11.7%), and diarrhea (13.1% vs. 15.2%).

DISCUSSION

We identified three genotypes in the *HTR3A* and *HTR3B* (collectively designated as HTR3) genes that were significantly associated with efficacy of ondansetron treatment for alcohol dependence in individuals with European ancestry. We showed that possession of at least one of the *HTR3A*-rs1150226-AG, *HTR3A*-rs1176713-GG, and/or *HTR3B*-rs17614942-AC genotypes, along with the previously identified *SLC6A4*-LL/TT genotypes (i.e., Combination-4), was predictive of ondansetron treatment response; approximately 34% of the cohort carried these genotypes.

A unique strength of our findings was that the 5-genotype Combination-4 (Table 2) was associated not only with a reduction in the amount of severe drinking (DDD) but also with two other measures of treatment response—the frequency of heavy drinking and of abstinence (PHDD and PDA, respectively). Additional to these widely used outcome measures, we also performed an exploratory analysis for the genotype associations with an extreme dichotomous responder vs. non-responder endpoint efficacy variable, percentage of subjects with no heavy drinking days (PSNHDD), which may be used by regulatory agencies as an important outcome measure for larger phase III trials (35). When only 1 heavy drinking day during the final 4 weeks was allowed, we detected at least a 2-fold increase in PSNHDD in the ondansetron-treated population carrying one or more of the Combination-4 genotypes, compared with the placebo group or the ondansetron-treated non-carriers. Notably, however, carriers of Combination-3 that constituted a subset of Combination-4 (approximately 25% of the cohort) had more than a 5-fold increase in PSNHDD when the ondansetron and placebo groups were compared, and they might define a group of “super-responders”.

Furthermore, the greatest DDD reductions were seen in small subpopulations possessing both *SLC6A4*-LL/TT genotypes and any one or more of the HTR3 genotypes. This finding supports our hypothesis that the combined effects of genetic variants for the presynaptic 5-HTT and postsynaptic 5-HT_3 would predict a greater response than genetic variations within each individual gene, as they represent the state of both synaptic availability of serotonin and its postsynaptic receptor levels. Another factor supporting this hypothesis is the greater effect of *SLC6A4* + HTR3 than the combined effect of the *HTR3A* and *HTR3B* genotypes. However, results for the combined effect of *SLC6A4* and HTR3 should be interpreted cautiously as they were limited by the small sample size within each placebo and ondansetron “treatment × genotype” group (Supplementary Table 3), thereby reducing the statistical power to draw definitive conclusions. Nevertheless, these initial statistical trends that generally support a molecular basis for the use of ondansetron as a treatment for a particular cohort of alcohol-dependent individuals warrant further corroboration with studies of larger sample size. The second-best response for DDD was seen in the group that pooled individuals carrying any one or a combination of the three HTR3 genotypes, regardless of LL/TT carrier status (Combination-3 in Table 2). From a biological perspective, this is quite explainable as 5-HT_{3AB} receptors are the primary target of ondansetron, which in fact led us to select *HTR3A* and *HTR3B* as candidate genes for the present study. Even though

ondansetron's therapeutic effect on the selected *SLC6A4* genotypes might appear to be smaller than that on the target HTR3 genotypes, presumably because the biological effect of the 5-HTT on ondansetron pharmacodynamics is an indirect one, their interaction is most intriguing in showing a more robust response across different outcome measures of alcohol consumption.

Notably, the significant associations that we detected with SNPs rs1150226 and rs17614942 were through their heterozygous genotypes; i.e., the heterozygotes showed significantly improved response to ondansetron compared with both homozygous genotype carriers. Although it is argued that such a comparison utilizing the heterozygous genetic model may not reflect underlying biological mechanism(s), a dominant heterozygous effect over both homozygotes, which also is referred to as molecular heterosis, is an increasingly recognized phenomenon in many human gene-disease association studies including addictions (36–40). Another possible explanation for detecting heterozygous associations could be the small sample size in the homozygous minor allele genotype groups, rather than the effects of molecular heterosis.

An additional caveat of our work is that the preclinical studies on the functionality of rs1150226 and rs1176713 of *HTR3A* and rs17614942 of *HTR3B* have not been reported in the literature, and, therefore, the exact molecular mechanism for their interactive effects remains to be determined. However, given their location within the genes, it is possible that all three of these polymorphisms may alter mRNA expression levels. The rs1150226 is located in the promoter region of *HTR3A* within 1 kb of putative binding sites for several transcription factors that have been shown to be modulated by ethanol (41, 42). Furthermore, genetic association analyses have shown an association of rs1150226 with alcoholism comorbid with antisocial personality disorder (16), altered temporal lobe activity (43), and clozapine treatment outcome for schizophrenia (44). Both rs1176713, which is a synonymous SNP located on exon 8 (Leu459Leu) of *HTR3A*, and rs17614942, located in intron 8 of *HTR3B*, could alter expression levels of functional 5-HT_{3AB} subunits through altered splicing of primary mRNA to form nonfunctional secondary mRNA. Interestingly, rs17614942 is located in different regions in different mRNA isoforms (Figure 3). In primary mRNA isoforms AM293589 and NM.006028.3 (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?c=geneid&org=9606&l=9177), the two longer *HTR3B* mRNA transcripts that are found to be present in the brain, rs17614942 is located in intron 8. In rodents, intron 8 consists of a splice acceptor site that results in the long isoform (45); even though this site is not known to be present in humans, it underlines the importance of the intron 8 region in the formation of various mRNA splice variants. In the shorter primary mRNA isoform BF942819 (http://www.ncbi.nlm.nih.gov/IEB/Research/Acembly/av.cgi?c=geneid&org=9606&l=9177), which has not yet been reported to be present in the brain, rs17614942 is located in the 3′-UTR, which also can lead to functional 5-HT_{3B} subunit expression level changes through different molecular mechanisms such as altered mRNA polyadenylation, miRNA binding, and decay. *In vivo* experiments would be required to characterize accurately the functional effects of the variants in Combination-4, both individually and collectively. We, therefore, propose our theorization as a framework upon which to launch further molecular investigations (3).

Another caveat of our findings is the relatively small sample size to perform statistical comparisons for all different genotype combinations constituted by the 5 variants included in Combination-4. It is, therefore, not possible to dissect fully the relative contribution of each individual genotype with treatment response. However, such an analysis that tested for all 16 different combinations of the 5 variants was well beyond the scope of this phase II clinical trial and would require a multicenter clinical trial that enrolled several thousand treatment-seeking, alcohol-dependent individuals.

Another consideration was that we used a descriptive filtering process to control for type I error, by minimizing the number of inferential contrasts, to understand the relationship between ondansetron treatment response and genotype. Thus, an additional replication study is needed in a much larger sample to validate our results. Furthermore, as the frequency of the identified alleles may vary in different ethnic populations, additional studies are needed to test the applicability of the current findings in other ethnic populations. Nevertheless, our findings were bolstered by the fact that the Combination-4 variants also were included in the detected significant genetic epistatic models by GMDR independent epistatic analyses, which were performed to validate the findings from the primary analysis.

Interestingly, a previous association study conducted by Enoch and colleagues (15) had suggested that rs1176744 polymorphisms in *HTR3B* and *SLC6A4* (L_AL_A) were associated with the development of alcohol dependence and, speculatively, ondansetron treatment response. Confirming these findings, an independent association analysis conducted by our team (46) also showed a highly significant association between alcohol dependence and a genotype combination comprising *HTR3A*-rs10160548, *HTR3B*-rs1176744 and -rs3782025, *SLC6A4*-rs1042173, and 5′-HTTLPR polymorphisms.

In conclusion, we have shown that polymorphisms in the *SLC6A4, HTR3A*, and *HTR3B* genes are predictors of reduced drinking in response to ondansetron. Based on these findings, we hypothesize that the Combination-4 genotypes can be utilized for screening individuals who would respond to ondansetron. Furthermore, the effect sizes of Combination-4 across different measures of alcohol consumption appear to be medium to high. Clinical trials randomized based on Combination-4 genotypes are thus needed to test the replicability of our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank the National Institute on Alcohol Abuse and Alcoholism for its generous support through grants 5 R01 AA010522-17 and 1 R01 AA021163-01 to B. A. Johnson and grant 5 R01 AA019720-02 to N. Ait-Daoud; the National Institute on Drug Abuse for its support of M. D. Li through grant 5 R01 DA012844-12, and Robert H. Cormier, Jr., BA, for his assistance with manuscript preparation.

REFERENCES

1. Enoch M-A, Schuckit MA, Johnson BA, Goldman D. Genetics of alcoholism using intermediate phenotypes. Alcohol Clin Exp Res. 2003; 27:169–176. [PubMed: 12605066]
2. Johnson BA. Update on neuropharmacological treatments for alcoholism: scientific basis and clinical findings. Biochem Pharmacol. 2008; 75:34–56. [PubMed: 17880925]
3. Johnson BA, Ait-Daoud N, Seneviratne C, Roache JD, Javors MA, Wang X-Q, Liu L, Penberthy JK, DiClemente CC, Li MD. Pharmacogenetic approach at the serotonin transporter gene as a method of reducing the severity of alcohol drinking. Am J Psychiatry. 2011; 168:265–275. [PubMed: 21247998]
4. McBride WJ, Lovinger DM, Machu T, Thielen RJ, Rodd ZA, Murphy JM, Roache JD, Johnson BA. Serotonin-3 receptors in the actions of alcohol, alcohol reinforcement, and alcoholism. Alcohol Clin Exp Res. 2004; 28:257–267. [PubMed: 15112933]
5. Niesler B, Walstab J, Combrink S, Möller D, Kapeller J, Rietdorf J, Bönisch H, Göthert M, Rappold G, Brüss M. Characterization of the novel human serotonin receptor subunits 5-HT3C, 5-HT3D, and 5-HT3E. Mol Pharmacol. 2007; 72:8–17. [PubMed: 17392525]

6. Papke RL, Porter Papke JK, Rose GM. Activity of alpha7-selective agonists at nicotinic and serotonin 5HT3 receptors expressed in Xenopus oocytes. Bioorg Med Chem Lett. 2004; 14:1849–1853. [PubMed: 15050614]
7. Boyd GW, Low P, Dunlop JI, Robertson LA, Vardy A, Lambert JJ, Peters JA, Connolly CN. Assembly and cell surface expression of homomeric and heteromeric 5-HT3 receptors: the role of oligomerization and chaperone proteins. Mol Cell Neurosci. 2002; 21:38–50. [PubMed: 12359150]
8. Johnson BA, Javors MA, Roache JD, Seneviratne C, Bergeson SE, Ait-Daoud N, Dawes MA, Ma JZ. Can serotonin transporter genotype predict serotonergic function, chronicity, and severity of drinking? Prog Neuropsychopharmacol Biol Psychiatry. 2008; 32:209–216. [PubMed: 17950969]
9. Barr CS, Newman TK, Becker ML, Champoux M, Lesch KP, Suomi SJ, Goldman D, Higley JD. Serotonin transporter gene variation is associated with alcohol sensitivity in rhesus macaques exposed to early-life stress. Alcohol Clin Exp Res. 2003; 27:812–817. [PubMed: 12766626]
10. Preuss UW, Soyka M, Bahlmann M, Wenzel K, Behrens S, de Jonge S, Krüger M, Bondy B. Serotonin transporter gene regulatory region polymorphism (5-HTTLPR), [3H]paroxetine binding in healthy control subjects and alcohol-dependent patients and their relationships to impulsivity. Psychiatry Res. 2000; 96:51–61. [PubMed: 10980326]
11. Heinz A, Jones DW, Mazzanti C, Goldman D, Ragan P, Hommer D, Linnoila M, Weinberger DR. A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity. Biol Psychiatry. 2000; 47:643–649. [PubMed: 10745057]
12. Dubin AE, Huvar R, D'Andrea MR, Pyati J, Zhu JY, Joy KC, Wilson SJ, Galindo JE, Glass CA, Luo L, Jackson MR, Lovenberg TW, Erlander MG. The pharmacological and functional characteristics of the serotonin 5-HT(3A) receptor are specifically modified by a 5-HT(3B) receptor subunit. J Biol Chem. 1999; 274:30799–30810. [PubMed: 10521471]
13. Krzywkowski K, Davies PA, Feinberg-Zadek PL, Bräuner-Osborne H, Jensen AA. High-frequency HTR3B variant associated with major depression dramatically augments the signaling of the human 5-HT3AB receptor. Proc Natl Acad Sci U S A. 2008; 105:722–727. [PubMed: 18184810]
14. Hodge CW, Kelley SP, Bratt AM, Iller K, Schroeder JP, Besheer J. 5-HT(3A) receptor subunit is required for 5-HT3 antagonist-induced reductions in alcohol drinking. Neuropsychopharmacology. 2004; 29:1807–1813. [PubMed: 15162158]
15. Enoch M, Gorodetsky E, Hodgkinson C, Roy A, Goldman D. Functional genetic variants that increase synaptic serotonin and 5-HT3 receptor sensitivity predict alcohol and drug dependence. Mol Psychiatry. 2011; 16:1139–1146. [PubMed: 20838391]
16. Ducci F, Enoch MA, Yuan Q, Shen PH, White KV, Hodgkinson C, Albaugh B, Virkkunen M, Goldman D. HTR3B is associated with alcoholism with antisocial behavior and alpha EEG power—an intermediate phenotype for alcoholism and comorbid behaviors. Alcohol. 2009; 43:73–84. [PubMed: 19185213]
17. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 4th ed.. American Psychiatric Association; Washington, DC: 1994.
18. Bohn MJ, Babor TF, Kranzler HR. The Alcohol Use Disorders Identification Test (AUDIT): validation of a screening instrument for use in medical settings. J Stud Alcohol. 1995; 56:423–432. [PubMed: 7674678]
19. U.S. Department of Health and Human Services. Cognitive-Behavioral Coping Skills Therapy Manual. USDHHS; Washington, DC: 1992. NIH Pub. No. 92-1895
20. Sobell, LC.; Sobell, MB. Timeline follow-back: a technique for assessing self-reported alcohol consumption. In: Litten, RZ.; Allen, JP., editors. Measuring Alcohol Consumption: Psychosocial and Biochemical Methods. Humana Press; Totowa, NJ: 1992. p. 41-72.
21. Seneviratne C, Huang W, Ait-Daoud N, Li MD, Johnson BA. Characterization of a functional polymorphism in the 3′ UTR of SLC6A4 and its association with drinking intensity. Alcohol Clin Exp Res. 2009; 33:332–339. [PubMed: 19032574]
22. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21:263–265. [PubMed: 15297300]
23. Benjamini Y, Drai D, Elmer G, Kafkafi N, Golani I. Controlling the false discovery rate in behavior genetics research. Behav Brain Res. 2001; 125:279–284. [PubMed: 11682119]

24. Cohen, J. Statistical Power Analysis for the Behavioral Sciences. 2nd ed.. Lawrence Erlbaum Associates; Hillsdale, NJ: 1988.
25. Lou XY, Chen GB, Yan L, Ma JZ, Zhu J, Elston RC, Li MD. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. Am J Hum Genet. 2007; 80:1125–1137. [PubMed: 17503330]
26. Cordell HJ. Detecting gene-gene interactions that underlie human diseases. Nat Rev Genet. 2009; 10:392–404. [PubMed: 19434077]
27. Moore JH, Williams SM. Epistasis and its implications for personal genetics. Am J Hum Genet. 2009; 85:309–320. [PubMed: 19733727]
28. Dering C, Hemmelmann C, Pugh E, Ziegler A. Statistical analysis of rare sequence variants: an overview of collapsing methods. Genet Epidemiol. 2011; 35(Suppl 1):S12–S17. [PubMed: 22128052]
29. Li B, Leal SM. Methods for detecting associations with rare variants for common diseases: application to analysis of sequence data. Am J Hum Genet. 2008; 83:311–321. [PubMed: 18691683]
30. Asimit J, Zeggini E. Rare variant association analysis methods for complex traits. Annu Rev Genet. 2010; 44:293–308. [PubMed: 21047260]
31. Bansal V, Libiger O, Torkamani A, Schork NJ. Statistical analysis strategies for association studies involving rare variants. Nature Rev Genet. 2010; 11:773–785. [PubMed: 20940738]
32. Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic. PLoS Genet. 2009; 5(2):e1000384. [PubMed: 19214210]
33. Li MD, Lou XY, Chen G, Ma JZ, Elston RC. Gene-gene interactions among CHRNA4, CHRNB2, BDNF, and NTRK2 in nicotine dependence. Biol Psychiatry. 2008; 64:951–957. [PubMed: 18534558]
34. Li MD, Mangold JE, Seneviratne C, Chen GB, Ma JZ, Lou XY, Payne TJ. Association and interaction analyses of GABBR1 and GABBR2 with nicotine dependence in European- and African-American populations. PLoS One. 2009; 4:e7055. [PubMed: 19763258]
35. Falk D, Wang XQ, Liu L, Fertig J, Mattson M, Ryan M, Johnson B, Stout R, Litten RZ. Percentage of subjects with no heavy drinking days: evaluation as an efficacy endpoint for alcohol clinical trials. Alcohol Clin Exp Res. 2010; 34:2022–2034. [PubMed: 20659066]
36. Gratacos M, Gonzalez JR, Mercader JM, de Cid R, Urretavizcaya M, Estivill X. Brain-derived neurotrophic factor Val66Met and psychiatric disorders: meta-analysis of case-control studies confirm association to substance-related disorders, eating disorders, and schizophrenia. Biol Psychiatry. 2007; 61:911–922. [PubMed: 17217930]
37. Pooley EC, Fairburn CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ. A 5-HT2C receptor promoter polymorphism (HTR2C - 759C/T) is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Med Genet B Neuropsychiatr Genet. 2004; 126B: 124–127. [PubMed: 15048662]
38. Lee HS, Kim SH, Lee HJ, Kim L, Lee SK, Jang DW, Lee MS, Son BG, Suh KY, Kim S. Gender-specific molecular heterosis of dopamine D2 receptor gene (DRD2) for smoking in schizophrenia. Am J Med Genet. 2002; 114:593–597. [PubMed: 12210271]
39. Retz W, Rosler M, Supprian T, Retz-Junginger P, Thome J. Dopamine D3 receptor gene polymorphism and violent behavior: relation to impulsiveness and ADHD-related psychopathology. J Neural Transm. 2003; 110:561–572. [PubMed: 12721816]
40. Gosso MF, de Geus EJ, Polderman TJ, Boomsma DI, Heutink P, Posthuma D. Catechol O-methyl transferase and dopamine D2 receptor gene polymorphisms: evidence of positive heterosis and gene-gene interaction on working memory functioning. Eur J Hum Genet. 2008; 16:1075–1082. [PubMed: 18382477]
41. Szabo G, Mandrekar P. Ethanol-mediated regulation of transcription factors in immunocompetent cells. Front Biosci. 2002; 7:a80–a89. [PubMed: 11991856]
42. Wang H, Zhou H, Chervenak R, Moscatello KM, Brunson LE, Chervenak DC, Wolcott RM. Ethanol exhibits specificity in its effects on differentiation of hematopoietic progenitors. Cell Immunol. 2009; 255:1–7. [PubMed: 18834972]

43. Windemuth A, Calhoun VD, Pearlson GD, Kocherla M, Jagannathan K, Ruaño G. Physiogenomic analysis of localized FMRI brain activity in schizophrenia. Ann Biomed Eng. 2008; 36:877–888. [PubMed: 18330705]

44. Souza RP, de Luca V, Meltzer HY, Lieberman JA, Kennedy JL. Influence of serotonin 3A and 3B receptor genes on clozapine treatment response in schizophrenia. Pharmacogenet Genomics. 2010; 20:274–276. [PubMed: 20168265]

45. Bruss M, Eucker T, Gothert M, Bonisch H. Exon-intron organization of the human 5-HT3A receptor gene. Neuropharmacology. 2000; 39:308–315. [PubMed: 10670426]

46. Seneviratne C, Franklin J, Beckett K, Ma JZ, Ait-Daoud N, Payne TJ, Johnson BA, Li MD. Association, interaction, and replication analysis of genes encoding serotonin transporter and 5-HT3 receptor subunits A and B in alcohol dependence. Hum Genet. in press.

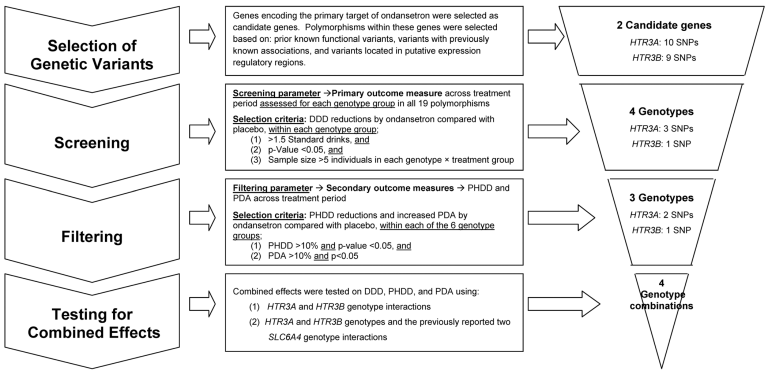

Figure 1.

SNP selection process and statistical analyses workflow.

Footnote: SNP=single-nucleotide polymorphism; DDD=drinks per drinking day; PHDD=percentage of heavy drinking days; PDA=percentage of days abstinent.

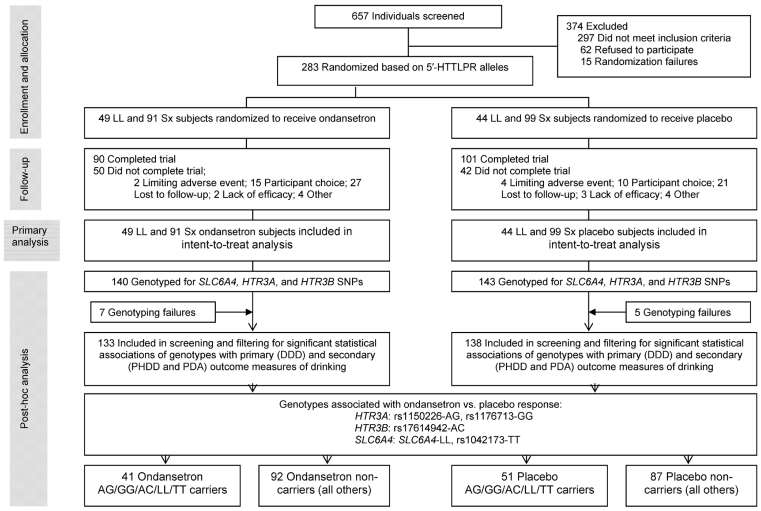

Figure 2.

CONSORT diagram of alcohol-dependent participants in the post-hoc analysis.

Footnote: 5′-HTTLPR=serotonin-transporter-linked polymorphic region; SNP=single-nucleotide polymorphism; DDD=drinks per drinking day; PHDD=percentage of heavy drinking days; PDA=percentage of days abstinent.

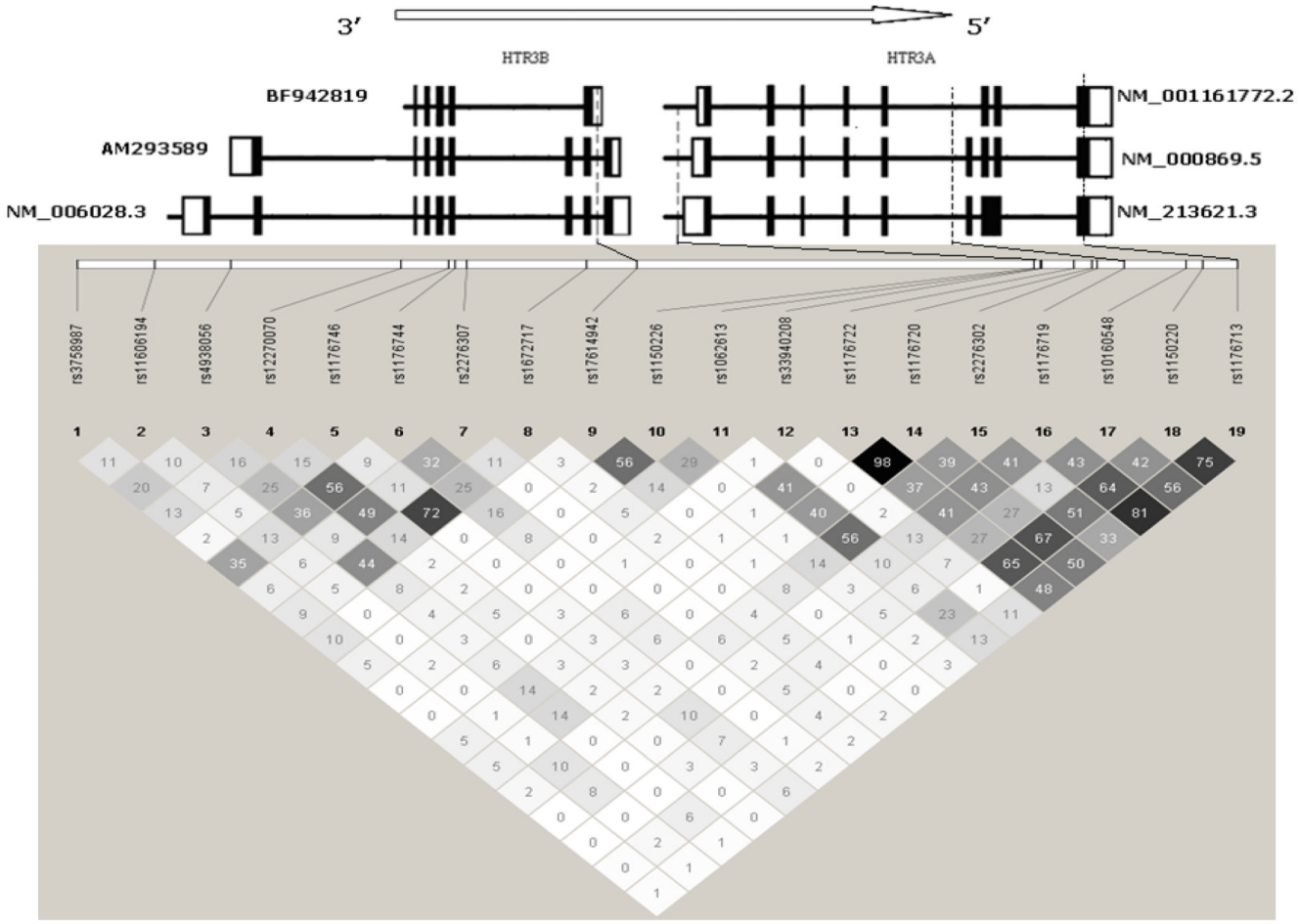

Figure 3.

Linkage disequilibrium analysis of all $HTR3_{AB}$ single-nucleotide polymorphisms (SNPs) in the total cohort and the location of significantly associated SNPs.

Footnote: Values in squares of the linkage disequilibrium plot are r^2 numbers: The dotted lines point toward the location of significantly associated SNPs in reference to various splice variants of *HTR3A* and *HTR3B* primary mRNA. The numbers next to each splice variant indicate their accession numbers.

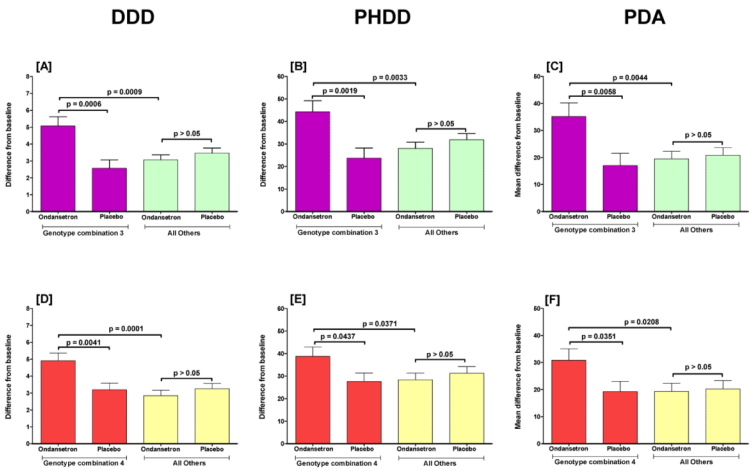

Figure 4.

Comparison of means for the three drinking outcome measures: drinks per drinking day (DDD) [A, D], percentage of heavy drinking days (PHDD) [B, E], and percentage of days abstinent (PDA) [C, F].

Footnote: Graphs in the first row are for ondansetron- or placebo-treated subjects carrying any one or a combination of the *HTR3A*-rs1150226-AG, *HTR3A*-rs1176713-GG, and/or *HTR3B*-rs17614942-AC genotypes (genotype Combination-3, represented by the purple bars) who showed the best treatment response and for those not carrying the above-mentioned genotypes ("All Others", represented by the green bars). Graphs in the second row are for ondansetron- or placebo-treated subjects carrying any one or a combination of the *SLC6A4*-LL/TT, *HTR3A*-rs1150226-AG, *HTR3A*-rs1176713-GG, and/or *HTR3B*-rs17614942-AC genotypes (genotype Combination-4, represented by the red bars) and for those not possessing any of the above-mentioned genotypes ("All Others", represented by the yellow bars).

Table 1

Significant Individual *HTR3A* and *HTR3B* SNP Associations with Three Drinking Measures[a]

Gene	SNP	Genotype		Population Carrying the Genotype Combination (% in Total Cohort)	Outcome Measure: Estimated Mean Difference (95% CI) and p-Value		
		Reference	Associated		DDD	PHDD	PDA
HTR3B	rs17614942	AA/CC	AC	O=17/135 P=19/139 (13%)	−2.73 (−4.59 to −0.87) 0.004	−20.45% (−37.58% to −3.32%) 0.019	17.95% (0.95% to 34.95%) 0.039
HTR3A	rs1150226	AA/GG	AG	O=20/135 P=24/139 (16%)	−1.81 (−3.51 to −0.12) 0.036	−20.65% (−36.17% to −5.12%) 0.009	19.75% (4.30% to 35.19%) 0.012
	rs1176713	AA/AG	GG	O=6/134 P=9/139 (5%)	−3 87 (−6.95 to −0.78) 0.014	−23 98% (−51.83% to 3.87%) 0.091	18.20% (−9.31% to 45.70%) 0.195

SNP=single-nucleotide polymorphism; CI=confidence interval; O=ondansetron; P=placebo; DDD=drinks per drinking day; PHDD=percentage of heavy drinking days; PDA=percentage of days abstinent.

[a]All comparisons are between the ondansetron and placebo groups.

Table 2

Association of Frequent Genotype Combinations (>5%) with Primary and Secondary Outcome Measures of Ondansetron Treatment Response[a]

Combination	Genotype Combination	Population Carrying the Genotype Combination (% in Total Cohort)	Estimated Mean Difference (95% CI) and p-Value		
			DDD	PHDD	PDA
1	*Possessing only one of the three identified HTR3 genotypes:* (*HTR3A*-rs1150226-AG alone or *HTR3B*-rs17614942-AC alone or *HTR3A*-rs1176713-GG alone)	O=15/133 P=16/138 (11%)	−2 35 (−4.41 to −0.29) 0.025	−19.05% (−37.73% to −0.36%) 0.046	16.12% (−2.49% to 34.74%) (0.090)
2	*Possessing a combination of any two of the three identified HTR3 genotypes:* (*HTR3A*-rs1150226-AG & *HTR3B*-rs17614942-AC or *HTR3A*-rs1176713-GG & *HTR3B*-rs17614942-AC)	O=14/133 P=18/138 (12%)	−2 65 (−4.65 to −0.66) 0.009	−22.43% (−40.77% to −4.09%) 0.017	20.23% (2.06% to 38.40%) 0.029
3	*Possessing any one, two, or all three of the identified HTR3 genotypes:* (i.e., *HTR3A*-rs1150226-AG &/or *HTR3A*-rs1176713-GG &/or *HTR3B*-rs17614942-AC)	O=29/133 P=34/138 (23%)	−2.50 (−3.92 to −1.08) 0.0006	−20.58% (−33.53% to −7.62%) 0.002	18.18% (5.26% to 31.10%) 0.006
4	*Possessing any one, two, three, or all four of the identified HTR3 and* SLC6A4 *genotypes:* (*SLC6A4*-LL/TT &/or *HTR3A*-rs1150226-AG &/or *HTR3A*-rs1176713-GG &/or *HTR3B*-rs17614942-AC)	O=41/133 P=51/138 (34%)	−1.71 (−2.88 to −0.54) 0.004	−11.13% (−21.94% to −0.31%) 0.044	11.57% (0.81% to 22.33%) 0.035

CI=confidence interval; O=ondansetron; P=placebo; DDD=drinks per drinking day; PHDD=percentage of heavy drinking days; PDA=percentage of days abstinent.

[a]All comparisons are between the ondansetron and placebo groups and are listed in descending order of magnitude of their DDD reductions; mean differences for DDD are in standard drinks; negative values indicate improvement from baseline.

Table 3

SNP-by-SNP Interaction Models Detected to Be Significant Using the GMDR Method, with the Closest Resemblance to Combinations 1–4 from the Primary Analysis[a]

Outcome Measure	Interaction Model	Test Accuracy	Cross-Validation Consistency	Permutated p-Value[b]
DDD	*HTR3A*: rs1176713–*SLC6A4*: 5′-HTTLPR	0.674	10/10	0.0001
	HTR3A: rs1176713–*SLC6A4*: 5′-HTTLPR, rs1042173	0.595	10/10	0.039
	HTR3A: rs1150226,rs1176713–*HTR3B*:rs17614942–*SLC6A4*: 5′-HTTLPR, rs1042173	0.483	10/10	0.576
PHDD	*HTR3A*: rs1176713–*SLC6A4*: 5′-HTTLPR	0.612	7/10	0.003
	HTR3A: rs1176713–*SLC6A4*: 5′-HTTLPR, rs1042173	0.594	10/10	0.031
	HTR3A: rs1150226,rs1176713–*HTR3B*:rs17614942–*SLC6A4*: 5′-HTTLPR, rs1042173	0.565	10/10	0.094
PDA	*HTR3A*: rs1176713–*SLC6A4*: 5′-HTTLPR	0.593	8/10	0.033
	HTR3A: rs1176713–*SLC6A4*: 5′-HTTLPR, rs1042173	0.628	10/10	0.003
	HTR3A: rs1150226-rs1176713–*HTR3B*:rs17614942–*SLC6A4*: 5′-HTTLPR, rs1042173	0.598	10/10	0.029

[a] SNP=single-nucleotide polymorphism; GMDR=generalized multifactor dimensionality reduction; DDD=drinks per drinking day; PHDD=percentage of heavy drinking days; PDA=percentage of days abstinent.

[b] p-Values were generated with 10^6 permutations.