

# 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

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**Context:** Naltrexone hydrochloride treatment for alcohol dependence works for some individuals but not for everyone. Asn40Asp, a functional polymorphism of the  $\mu$ -opioid receptor gene (*OPRM1*), might predict naltrexone response.

**Objective:** To evaluate whether individuals with alcoholism who are heterozygous (Asp40/Asn40) or homozygous (Asp40/Asp40) for the *OPRM1* Asp40 allele respond better to naltrexone.

**Design:** Pharmacogenetic analysis conducted between January 1, 2001, and January 31, 2004.

**Setting:** Eleven academic sites in the COMBINE Study.

**Participants:** Recently abstinent volunteers who met all 3 of the following conditions: (1) DSM-IV criteria for primary alcohol dependence; (2) participation in the COMBINE Study; and (3) availability of DNA.

**Interventions:** Alcoholic subjects were treated for 16 weeks with 100 mg of naltrexone hydrochloride (234 Asn40 homozygotes and 67 with at least 1 copy of the Asp40 allele) or placebo (235 Asn40 homozygotes and 68 with at least 1 copy of the Asp40 allele). All participants received medical management (MM) alone or with combined behavioral intervention (CBI).

**Main Outcome Measures:** Time trends in percentage of days abstinent, percentage of heavy drinking days, and rates of good clinical outcome.

**Results:** Alcoholic subjects with an Asp40 allele receiving MM alone (no CBI) had an increased percentage of days abstinent ( $P = .07$ ) and a decreased percentage of heavy drinking days ( $P = .04$ ) if treated with naltrexone vs placebo, while those with the Asn40/Asn40 genotype showed no medication differences. If treated with MM alone and naltrexone, 87.1% of Asp40 carriers had a good clinical outcome, compared with only 54.8% of individuals with the Asn40/Asn40 genotype (odds ratio, 5.75; confidence interval, 1.88-17.54), while, if treated with placebo, 48.6% of Asp40 carriers and 54.0% of individuals with the Asn40/Asn40 genotype had a good clinical outcome (interaction between medication and genotype,  $P = .005$ ). No gene  $\times$  medication interactions were observed in those treated with both MM and CBI.

**Conclusions:** These results confirm and extend the observation that the functionally significant *OPRM1* Asp40 allele predicts naltrexone treatment response in alcoholic individuals. This relationship might be obscured, however, by other efficacious treatments. *OPRM1* genotyping in alcoholic individuals might be useful to assist in selecting treatment options.

**Trial Registration:** clinicaltrials.gov Identifier: NCT00006206

*Arch Gen Psychiatry.* 2008;65(2):135-144

**W**HILE PHARMACOTHERAPY for alcohol dependence is limited, several drugs have received Food and Drug Administration approval.<sup>1,2</sup> Meta-analyses of many single-site and multisite studies of the opioid antagonist medication naltrexone hydrochloride have suggested that the effect size for response over placebo is in the small to moderate range.<sup>3-6</sup> For instance, in the

Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) Study,<sup>7</sup> naltrexone showed its prime efficacy when used in the context of medical management (MM)

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(number needed to treat, 6-7) but not when combined with a specialized alcohol counseling approach. On the basis of this extensive experience, it is clear that

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not all individuals with alcohol dependence respond to naltrexone. Attempts to identify individual factors that would be associated with a positive response have been limited and inconsistent. Therefore, any improvement in the ability to predict naltrexone response would have immense theoretical and clinical value.

It is well known that naltrexone is a specific opioid antagonist targeting endogenous opioid receptors, particularly (but not exclusively)  $\mu$ -receptors.<sup>8,9</sup> It has also been observed that alcohol increases the release of the endogenous opioids  $\beta$ -endorphin and enkephalin in animals<sup>10,11</sup> and humans.<sup>12</sup> Blocking opioid receptors with naltrexone leads to less alcohol-induced pleasure, high, and intoxication<sup>13</sup> and, ultimately, less craving<sup>14</sup> and relapse.<sup>15</sup> Indeed, there is a high concentration of  $\mu$ -opioid receptors in human brain areas, such as the nucleus accumbens, amygdala, cingulate gyri, and basal ganglia,<sup>16</sup> that have been implicated in the reward pathway associated with alcohol and substance abuse. Occupancy of these receptors is related to alcohol craving.<sup>17</sup> It is also known that  $\mu$ -opioid receptors primarily bind  $\beta$ -endorphin and transduce this binding via G-protein signaling that ultimately alters neuronal firing and leads to neuroadaptive changes. Although not a consistent finding,<sup>18</sup> the discovery that a  $\mu$ -opioid receptor (*OPRM1*) missense polymorphism, Asn40Asp,<sup>19</sup> was capable of changing  $\beta$ -endorphin binding, function, and receptor levels<sup>20,21</sup> led to speculation of functional effects in humans and formed the basis for examination of differential naltrexone response.

The asparagine-to-aspartate amino acid substitution at position 40 is caused by an A118G single nucleotide substitution, leading to structural variation in the receptor extracellular domain. This substitution has been reported to increase binding of  $\beta$ -endorphin and increase functional activity *in vitro*.<sup>20</sup> In addition, early reports have suggested that the 15% to 25% of humans<sup>22,23</sup> who carry the Asp40 allele show a greater response to alcohol,<sup>24,25</sup> greater endocrine response to opioid antagonists,<sup>26</sup> and less response to painful stimuli.<sup>27</sup> In addition, a similar polymorphism has been reported to reduce alcohol-induced stimulation and alcohol consumption in non-human primates.<sup>28</sup> However, as reported when this polymorphism was first described,<sup>19</sup> a meta-analysis of human population studies suggested that this *OPRM1* polymorphism is not associated with a higher genetic risk of developing alcohol dependence.<sup>23</sup>

Most germane to this study was a report by Oslin and colleagues<sup>29</sup> that suggested, in an exploratory manner, that treatment-seeking alcoholic-dependent individuals with at least 1 copy of the Asp40 allele responded to naltrexone better than did those without the allele. That report, however, combined 3 disparate studies with limited outcome analysis (bivariate abstinence and relapse to heavy drinking). Also, the analytic plan used a number of covariates (such as age, sex, and marital status) that limited the potential generalizability of the finding. Subsequent to that report, a reanalysis of a Veterans Affairs Naltrexone Cooperative Study<sup>30</sup> found that individuals who had at least 1 copy of the Asp40 allele showed relapse to heavy drinking rates similar to those of Asn40 homozygotes when treated with naltrexone; however, they did not evaluate other drinking outcome variables.<sup>31</sup>

The COMBINE Study was a multisite study designed to address whether naltrexone, acamprosate calcium, or specialized counseling, called *combined behavioral intervention* (CBI), when given in the context of an MM approach, were individually better than placebo or whether combinations of these would be better than any one alone.<sup>32</sup> The results of that trial<sup>7</sup> indicated that naltrexone was better than placebo on a number of drinking outcome variables, but the naltrexone effect was largely observable only when CBI, ie, alcohol-specialty therapy, was not used concomitantly with MM and naltrexone. Acamprosate was not effective in its own right and did not significantly add to or detract from the response of naltrexone alone.

The goal of this preplanned pharmacogenetic study within the larger COMBINE Study was to examine the role of Asn40Asp as a predictor of naltrexone treatment response.<sup>33</sup> We hypothesized that this variant would primarily predict response to naltrexone in the MM-only condition, given that this is the condition in which there was evidence that naltrexone was effective in the primary analyses.

## METHODS

### OVERVIEW OF STUDY DESIGN

The parent study rationale, design, and methods have been previously detailed<sup>34</sup> and the results reported.<sup>7</sup> For this pharmacogenetic study, conducted from January 1, 2001, through January 31, 2004, we focused primarily on white subjects receiving naltrexone (with or without acamprosate) or placebo. In brief, after baseline assessment and attainment of 4 days of abstinence, eligible alcohol-dependent individuals were randomized to naltrexone, with or without acamprosate, or to double placebo for 16 weeks of outpatient treatment. All of the groups received MM, a 9-session intervention focused on enhancing medication adherence and abstinence by means of a model that could be adapted to primary care settings. Half of the subjects were randomly assigned to receive a more intensive CBI delivered by alcoholism treatment specialists. An additional group received CBI alone without any pills or MM, but that group's results are not included in this report. Of the 1383 subjects randomized in the main trial, 1013 subjects provided genetic samples; of those, 911 samples (684 whites) were successfully genotyped (**Figure 1**). Those receiving naltrexone or placebo and MM with or without CBI (n=604) are the subjects of this report.

### PARTICIPANT RECRUITMENT, ASSESSMENT, AND RANDOMIZATION

At 11 academic sites, participants were recruited by advertisements and from clinical referrals. All participants signed informed consents (accompanied by a certificate of confidentiality issued by the National Institute on Alcohol Abuse and Alcoholism) approved by each site's institutional review board for the main clinical trial. Participants gave consent separately for the pharmacogenetic substudy. Consenting for this substudy began after commencement of randomization for the main trial; therefore, not all participants were available to provide consent for the substudy. Baseline drinking histories, psychosocial data, health screens (including laboratory general health panels), and specific alcohol biomarkers were obtained. Eligibility criteria included (1) alcohol dependence, determined by *DSM-IV*<sup>35</sup> criteria, using the Structured Clinical Interview for *DSM-IV*<sup>36</sup>; (2) 4 to 21 days of abstinence; and (3) consumption of more than 14 drinks (women) or 21 drinks (men) per week, with at least 2 heavy drinking days (defined as  $\geq 4$  drinks

for women and  $\geq 5$  for men) during a consecutive 30-day period within the 90 days before baseline evaluation. Exclusion criteria included (1) history of substance abuse (other than nicotine or cannabis) by *DSM-IV* criteria in the past 90 days (except 6 months for opioid abuse) or by urine drug screen, (2) psychiatric disorder requiring medication, or (3) unstable medical conditions (eg, serum liver enzyme levels  $> 3$  times normal).

In the initial pharmacogenetic analysis of this report, the study sample was restricted to the 604 white participants because (1) previous reports on the *OPRM1* allele prediction of naltrexone response were restricted to whites only, (2) the Asp40 allele is reported to be present in less than 5% of African Americans but in 15% to 25% of whites, and (3) white subjects were well represented at all treatment sites and made up the majority of the study subjects providing acceptable *OPRM1* allele frequencies for analysis. However, in a secondary analysis, we used all subjects irrespective of reported race/ethnicity (an additional 104 subjects), to check the sensitivity of the findings on the good clinical outcome measure.

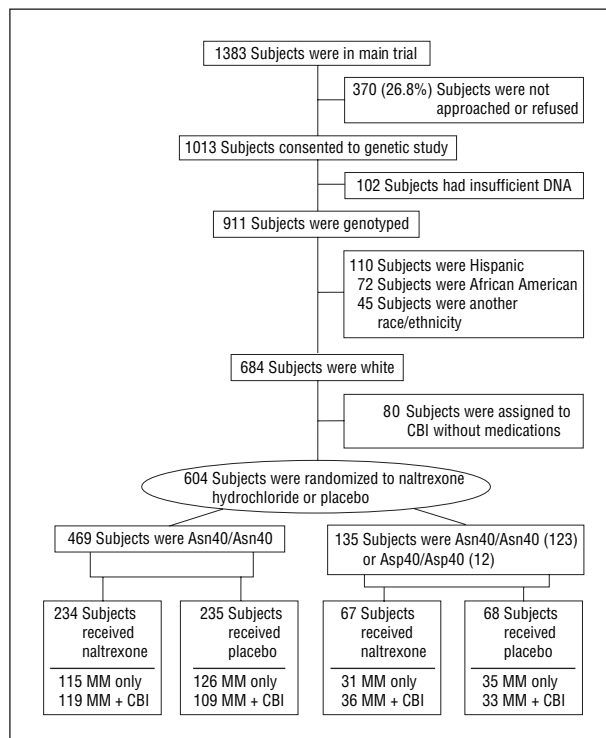
## ASSESSMENT

Drinking measures obtained from structured interviews at baseline<sup>37,38</sup> and during the 16-week treatment period<sup>39</sup> served as the basis for evaluating the genetic contribution to naltrexone response. At the 9 MM visits during treatment, research assistants (neither blinded to nor providing psychosocial treatment) assessed alcohol consumption<sup>39</sup> and craving.<sup>40</sup> At each appointment, adverse medication effects were assessed by a health care professional using the Systematic Assessment of Treatment Emergent Effects interview.<sup>41,42</sup> Complete blood cell count and liver and kidney function tests were conducted at baseline and every 4 weeks. The  $\gamma$ -glutamyltransferase level and percentage of carbohydrate-deficient transferrin<sup>43,44</sup> were measured at baseline and at weeks 8 and 16. Race and ethnicity were self-designated by participants, using an item allowing open-ended responses. All subjects and study site personnel, including investigators, research staff, evaluators, health care (MM) providers, and CBI therapists, were blind to medication assignment and genotype.

## TREATMENT CONDITIONS

### Medications

Each participant took up to 8 pills of active medication or placebo daily for 16 weeks. All naltrexone and placebo pills and all acamprosate and placebo pills were identical in appearance. Subjects in each group took the same number of pills per day. Naltrexone or its placebo was given as 2 pills once a day as follows: 1 placebo and 1 containing 25 mg of naltrexone hydrochloride or placebo on days 1 through 4, 1 placebo and 1 containing 50 mg of naltrexone hydrochloride or placebo on days 5 through 7, and two 50-mg pills (100 mg daily) of naltrexone hydrochloride or placebo on days 8 through 112. Acamprosate or its placebo was administered as 2 pills (500 mg each of acamprosate calcium or placebo) 3 times per day (3 g daily). Naltrexone and its placebo differed in appearance from acamprosate and its placebo. On the basis of tolerability, the MM clinician could reduce the acamprosate pills and then reduce the naltrexone pills. Attempts were made to reestablish the full dose. Doses were chosen on the basis of preliminary evidence that doses higher than commonly prescribed could be more efficacious and provide better coverage for missed doses<sup>45,46</sup> and were well tolerated.<sup>32,47</sup> For this report, we grouped patients according to whether they received active naltrexone or placebo, naltrexone, regardless of whether they received acamprosate or placebo acamprosate.



**Figure 1.** Distribution of subjects in the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) Study who had blood collected for genetic testing (*OPRM1* genotypes). CBI indicates combined behavioral intervention; MM, medical management.

### MM Regimen

The MM<sup>48,49</sup> was delivered by a licensed health care professional over 9 sessions (weeks 0, 1, 2, 4, 6, 8, 10, 12, and 16) during which pills were dispensed. The initial visit averaged 45 minutes and began with a review of the alcohol-dependence diagnosis and the negative consequences of drinking. The provider recommended abstinence, provided education about the medications, and developed a medication adherence plan in collaboration with the patient. Attendance at support groups (eg, Alcoholics Anonymous) was encouraged. Subsequent sessions, averaging 20 minutes, included review of drinking, overall functioning, medication adherence, and adverse effects. Patients who resumed drinking were given advice and encouraged to attend support groups. Problems with medication adherence were addressed. Participants who discontinued medication because of intolerance continued in MM sessions to support abstinence.

### CBI Regimen

The CBI<sup>50,51</sup> was delivered by licensed behavioral health specialists in up to 20 flexible participant need-adjusted 50-minute sessions (an average of 9 sessions were attended). It integrated aspects of cognitive behavioral therapy,<sup>52</sup> 12-step facilitation,<sup>53</sup> motivational interviewing,<sup>54</sup> and support system involvement external to the study.<sup>55,56</sup>

### 5' Nuclease Genotyping

Genomic DNA was extracted from peripheral blood mononuclear cells. The 5' nuclease genotyping assay (*TaqMan*; Applied Biosystems, Foster City, California) is a rapid and accurate method for high-throughput genotyping of single nucleotide

**Table 1. Demographics of White Participants Who Provided Genetic Samples by Treatment Condition<sup>a</sup>**

Characteristic	Medical Management (No CBI)						CBI + Medical Management			
	Total Sample		Placebo		Naltrexone Hydrochloride		Placebo		Naltrexone Hydrochloride	
	Asn40 (n=469)	Asp40 (n=135)	Asn40 (n=126)	Asp40 (n=35)	Asn40 (n=115)	Asp40 (n=31)	Asn40 (n=109)	Asp40 (n=33)	Asn40 (n=119)	Asp40 (n=36)
Demographics										
Age, y, mean (SD)	45.6 (10.49)	44.8 (10.51)	45.2 (10.09)	46.1 (12.08)	45.7 (11.17)	42.5 (10.01)	45.8 (10.05)	45.6 (9.53)	45.7 (10.73)	44.8 (10.28)
Sex, No. (%) M	332 (70.8)	91 (67.4)	91 (72.2)	23 (65.7)	77 (67.0)	21 (67.7)	81 (74.3)	24 (72.7)	83 (69.7)	23 (63.9)
Married, No. (%)	214 (45.6)	52 (38.5)	50 (39.7)	10 (28.6)	51 (44.3)	11 (35.5)	61 (56.0)	15 (45.5)	52 (43.7)	16 (44.4)
Employed, No. (%)	344 (73.3)	110 (81.5)	91 (72.2)	31 (88.6)	88 (76.5)	20 (64.5)	82 (75.2)	26 (78.8)	83 (69.7)	33 (91.7)
Education ≤ high school, No. (%)	124 (26.4)	35 (25.9)	36 (28.6)	11 (31.4)	30 (26.1)	8 (25.8)	25 (22.9)	11 (33.3)	33 (27.7)	5 (13.9)
Current smoker, No. (%)	206 (43.9)	58 (43.0)	51 (40.5)	13 (37.1)	54 (47.0)	13 (41.9)	39 (35.8)	16 (48.5)	62 (52.1)	16 (44.4)
Alcohol use severity indicators, mean (SD)										
% of days abstinent <sup>b</sup>	23.9 (24.58)	21.9 (23.91)	24.5 (25.37)	19.9 (23.99)	23.9 (24.38)	26.9 (27.73)	19.8 (22.44)	21.6 (23.38)	27.1 (25.55)	19.6 (20.99)
Drinks per drinking day <sup>b</sup>	12.6 (7.54)	11.8 (7.14)	12.4 (6.69)	12.3 (8.37)	12.6 (8.37)	13.4 (9.14)	12.4 (7.36)	11.5 (5.19)	12.8 (7.81)	10.2 (5.10)
Overall drinks per day <sup>b</sup>	9.3 (6.18)	9.2 (6.78)	9.2 (6.17)	9.5 (7.38)	9.2 (6.14)	10.5 (9.48)	9.7 (5.97)	8.6 (4.15)	9.0 (6.47)	8.3 (5.27)
Heavy drinking days <sup>b</sup>	20.0 (8.36)	20.6 (8.10)	20.1 (8.40)	20.4 (7.98)	19.9 (8.49)	19.8 (9.10)	21.1 (7.89)	21.2 (7.90)	19.1 (8.58)	20.9 (7.79)
DSM-IV symptoms	5.5 (1.26)	5.5 (1.23)	5.6 (1.29)	5.4 (1.14)	5.5 (1.27)	5.6 (1.36)	5.6 (1.21)	5.6 (1.19)	5.5 (1.27)	5.3 (1.26)
Alcohol dependence score <sup>c</sup>	16.6 (7.26)	16.9 (7.93)	17.2 (7.30)	16.6 (7.45)	16.2 (7.43)	18.1 (10.36)	16.5 (6.99)	17.7 (7.18)	16.6 (7.36)	15.3 (6.58)
OCDS score	26.0 (7.11)	26.5 (7.24)	26.7 (7.50)	24.6 (6.53)	25.2 (7.33)	25.8 (7.91)	26.8 (5.96)	28.2 (7.95)	25.4 (7.37)	27.2 (6.38)
Drinking consequences score <sup>d</sup>	47.8 (19.91)	46.5 (20.52)	49.6 (20.06)	46.8 (21.31)	46.2 (20.05)	45.2 (21.52)	48.1 (19.78)	47.0 (20.61)	47.2 (19.81)	47.1 (19.58)
GGT, IU/L	68.8 (112.4)	61.8 (82.49)	81.0 (168.7)	59.3 (63.46)	62.4 (74.90)	63.3 (80.15)	60.3 (53.88)	64.6 (99.93)	69.7 (107.4)	60.5 (86.51)
% CDT	3.5 (2.06)	3.2 (1.84)	3.7 (2.55)	3.3 (2.27)	3.3 (1.84)	3.1 (1.59)	3.5 (1.95)	3.5 (2.12)	3.5 (1.77)	2.9 (1.14)
No. (%)										
GGT > 63 IU/L	137 (29.2)	33 (24.4)	36 (28.6)	9 (25.7)	32 (27.8)	8 (25.8)	34 (31.2)	10 (30.3)	35 (29.4)	6 (16.7)
CDT > 2.5%	227 (48.4)	58 (43.0)	63 (50.0)	16 (45.7)	50 (43.5)	12 (38.7)	57 (52.3)	17 (51.5)	57 (47.9)	13 (36.1)

Abbreviations: CBI, combined behavioral intervention; CDT, carbohydrate-deficient transferrin; GGT,  $\gamma$ -glutamyltransferase; OCDS, obsessive compulsive drinking scale.

SI conversion factor: To convert GGT to microkatal per liter, multiply by 0.01667.

<sup>a</sup>Differences across treatment and genotypes were not significant. For the total sample and within each treatment combination, Asn40 (Asn40/Asn40 homozygote) individuals were compared with Asp40 carriers (Asn40Asp or Asp40Asp). For those receiving CBI plus medical management and naltrexone, "employed" had  $P = .004$ . All other  $P$  values were greater than .05.

<sup>b</sup>In the 30 days before randomization.

<sup>c</sup>Assessed using the Alcohol Dependence Scale.<sup>57</sup>

<sup>d</sup>Assessed using the Drinker Inventory of Consequences scale.<sup>58</sup>

polymorphisms, combining polymerase chain reaction amplification and sequence variant detection into a single step. Locus-specific primers and fluorogenic allele-specific probes were designed and manufactured (Applied Biosystems Assays-on-Demand, identification No. C\_8950074).

The 5- $\mu$ L reaction mixture consisted of 2.5  $\mu$ L of a master mix, 0.125  $\mu$ L of 20X assay mix, 8  $\mu$ M detection probe for each allele, 36  $\mu$ M forward and reverse primer each (all reagents supplied as TaqMan, Applied Biosystems), and 10 ng of genomic DNA diluted in 2.375  $\mu$ L of Tris EDTA, pH 8.0 (extracted from serum samples by Quality Biological Inc, Gaithersburg, Maryland). Amplification was performed by PCR (Gene Amp PCR System 9700; Applied Biosystems) using 384-well plates and the following amplification profile: 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds and 60°C for 1 minute. After amplification, end-point fluorescence intensity was measured directly in the reaction plates (7900 Sequence Detector; Applied Biosystems). Genotypes were determined using proprietary software (Sequence Detection System Software Version 2.0; Applied Biosystems). Four genotyping signal clusters were identified, representing Asn40 and Asp40 homozygotes, Asn40/Asp40 heterozygotes, and no-DNA-template controls. Genotyping accuracy was determined by replicate genotyping of 203 DNA samples. The genotyping error rate was 0%. Genotyping completion rate was 99.6%. No significant deviation from Hardy-Weinberg equilibrium was found ( $P = .43$  for the full sample and .49 for whites only). All geno-

typing was done at the National Institute on Alcohol Abuse and Alcoholism Laboratory of Neurogenetics (D.G., director), and results were reported blind to treatment assignment and outcome data, which were held separately at the study coordinating center at the University of North Carolina.

## OUTCOME MEASURES

The drinking variables evaluated for the gene  $\times$  medication interactions in each counseling group were time trends in percentage of days abstinent, percentage of heavy drinking days, and end-of-study categorical measures of response: the number (and percentage) of subjects with a good clinical outcome. Good clinical outcome was as defined in the main trial, ie, 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 fewer alcohol-related problems endorsed on the Drinker Inventory of Consequences scale<sup>57</sup> during the last 8 weeks of treatment. A subject with missing values for this variable was deemed to have a poor (not good) clinical outcome.

## STATISTICAL METHODS

Baseline characteristics for continuous variables are presented as means and standard deviations. Analysis of variance was used

to test equality of means across categories. For each categorical variable, frequencies and percentages are reported, along with the  $P$  value for a  $\chi^2$  test of equality of proportions across the categories.

For the drinking outcomes, percentage of days abstinent and percentage of heavy drinking days, percentages were calculated for each of the four 4-week periods in the 16-week treatment phase of the study. Four separate 4-week percentages were used rather than a single percentage over the 16-week period to allow for examination of trends, to reduce the effect of missing data, and to provide consistency with the analyses in the main trial.<sup>7</sup> These variables were analyzed by means of a general linear model, adjusting for clinical center and the relevant baseline drinking variable. An unstructured covariance matrix was used to account for the repeated measures on each individual. Trends over time were investigated by means of a linear term in the 4 periods. A contrast was constructed to test for interactions between time and treatment-genotype combinations without adjustment for multiple tests.

Good clinical outcome, an a priori but arbitrarily defined categorical variable that allows group comparisons of clinical response, was modeled as a dichotomous variable and analyzed as in the main trial report.<sup>7</sup> These dichotomous outcomes were modeled using logistic regression, adjusting for clinical center and baseline percentage of days abstinent.

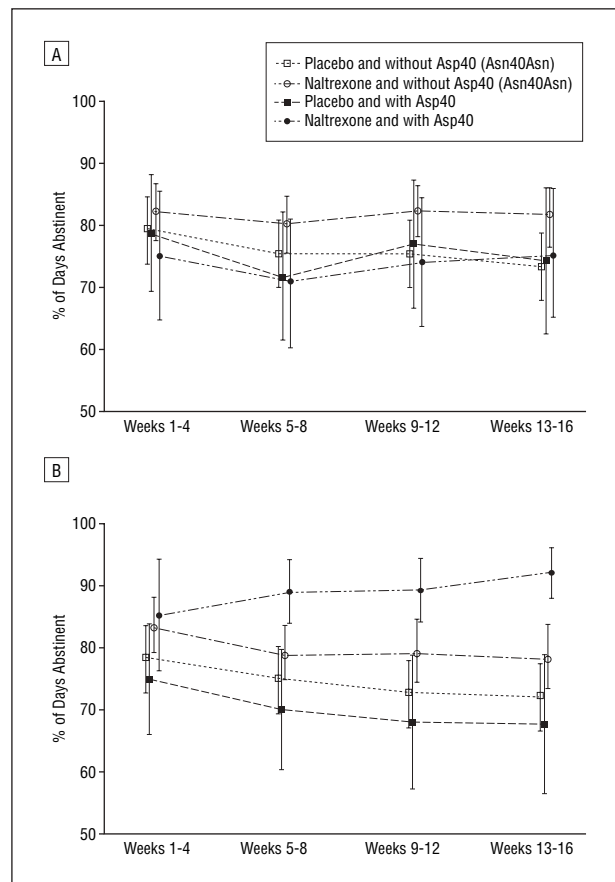
Whether the association of the Asp40 allele to naltrexone response might be exerted through chance association to other nonspecific clinical trial and subject variables was evaluated by examining differences in treatment withdrawal, research completion (full 16-week drinking data reported), medication adherence (number who took at least 80% of prescribed medication), and main adverse events caused by naltrexone (nausea, fatigue, and headache) by means of logistic regression with allele status and medication group as independent and interacting factors.

## RESULTS

The demographic characteristics of the pharmacogenetic study sample (whites who received naltrexone or placebo) in **Table 1** are broken down by treatment group and by presence or absence of the Asp40 allele. All of the pertinent baseline demographic, drinking, and alcohol severity measures were similar across treatment and genotype groups, with no statistically significant differences. In addition, there were minimal differences in these variables between individuals who did and did not have DNA available.

### PERCENTAGE OF DAYS ABSTINENT

There were no significant main effects of genotype or medication, and no gene  $\times$  medication  $\times$  time interactions, in patients who received CBI in addition to MM (**Figure 2A**). However, in patients who received MM alone (no CBI), there was an interaction between treatment and genotype for the time trend of percentage of days abstinent ( $P = .07$ ) among the 4 groups (Figure 2B). In pairwise comparisons, the time trends for naltrexone-treated carriers of Asp40 differed significantly from all other groups ( $P = .01-.03$ ). Alcoholic patients who had at least 1 copy of the Asp40 allele and who were treated with naltrexone showed an increasing trend in abstinent days over time, while those without the Asp40 allele (Asn40/Asn40 genotype) responded similarly to naltrexone as to

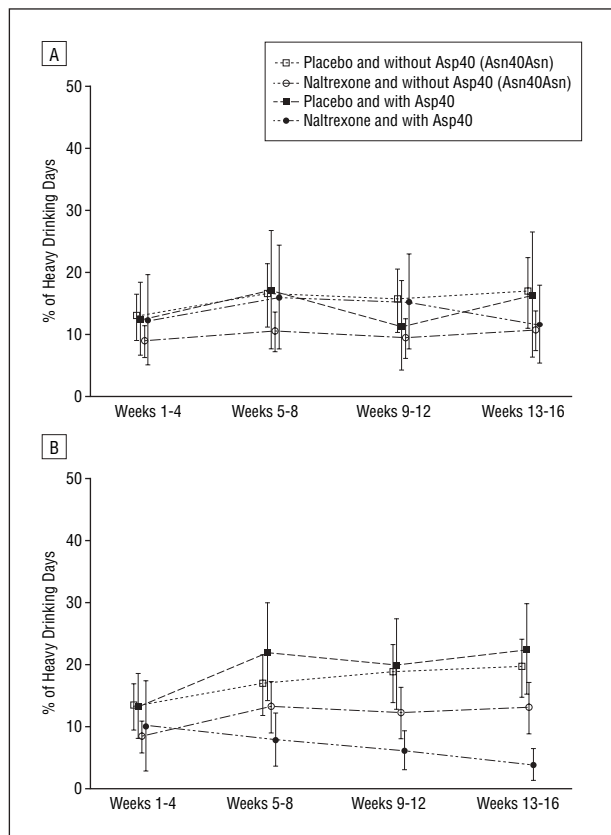


**Figure 2.** Medication effects on percentage of days abstinent (mean and 95% confidence interval) by *OPRM1* genotype. A, Groups assigned to medical management and combined behavioral intervention (CBI) (test of group  $\times$  time interaction,  $P = .35$ ). B, Groups assigned to medical management alone (test of group  $\times$  time interaction,  $P = .07$ ; pairwise comparison of other groups with naltrexone hydrochloride and with Asp40 group,  $P = .01-.03$ ).

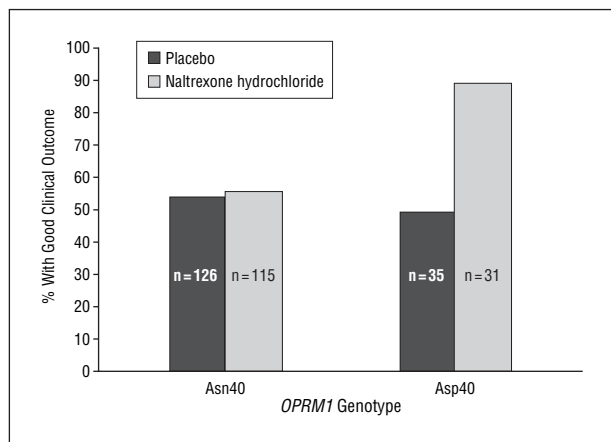
placebo with fewer abstinent days over time. Carriers and noncarriers of the Asp40 allele who received placebo both showed a decreasing trend in abstinence over time.

### PERCENTAGE OF HEAVY DRINKING DAYS

Alcoholic patients who received CBI plus MM did not show significant genotype, medication, or genotype  $\times$  medication  $\times$  time differences in the percentage of heavy drinking days (**Figure 3A**). However, in alcoholic patients receiving MM alone (no CBI), there was a significant ( $P = .04$ ) difference in the time trend for percentage of heavy drinking days across the 4 genotype  $\times$  medication groups (Figure 3B), again attributable to the combined effect of naltrexone and the Asp40 allele. In pairwise comparisons, alcoholic patients who were treated with naltrexone and who had at least 1 copy of Asp40 differed in time trend of heavy drinking days from all other groups, the pairwise  $P$  values ranging from .01 to .03. As Figure 3B shows, alcoholic patients with at least 1 copy of the Asp40 allele and who were treated with naltrexone had fewer heavy drinking days over time than did Asp40 carriers who were treated with placebo and Asp40 noncarriers (Asn40 homozygotes) who were treated with either naltrexone or placebo.



**Figure 3.** Medication effects on percentage of heavy drinking days (mean and 95% confidence interval) by *OPRM1* genotype. A, Groups assigned to medical management and combined behavioral intervention (CBI) (test of group  $\times$  time interaction,  $P = .35$ ). B, Groups assigned to medical management alone (test of group  $\times$  time interaction,  $P = .04$ ; pairwise comparison of other groups with naltrexone hydrochloride and with Asp40 group,  $P = .01-.03$ ).



**Figure 4.** Good clinical outcome based on *OPRM1* and medication group in those receiving medical management alone (no combined behavioral intervention) (test of genotype  $\times$  medication interaction,  $P = .005$ ). All subjects with missing values were considered not to have a good response. Asn40 includes subjects who were Asn40/Asn40 homozygotes. Asp40 includes those with either Asn40/Asp40 or Asp40/Asp40 genotypes.

### CLINICAL OUTCOME

**Figure 4** shows a significant genotype  $\times$  medication interaction ( $P = .005$ ) in those treated with MM only (without CBI). Naltrexone-treated patients who carried the

Asp40 allele had the best outcome (87.1% good outcome), while patients with the Asp40 allele but receiving placebo did worse (48.6% good outcome). Noncarriers of the Asp40 allele (Asn40 homozygotes) had similar rates of good clinical outcome regardless of whether they received naltrexone (54.8% good outcome) or placebo (54.0% good outcome), and both were lower than the rate of Asp40 carriers receiving naltrexone. Comparing Asp40 carriers with noncarriers, the odds ratio of having a good clinical outcome after naltrexone treatment was 5.75 (95% confidence interval, 1.88-17.54). This analysis was also repeated to include subjects of all races (Asp40 allele frequencies: African American, 7%; Hispanic, 34%; other, 35%), leading to essentially the same result, with a significant medication  $\times$  allele interaction ( $P = .03$ ) and a genotype odds ratio of 3.33 (95% confidence interval, 1.49-7.47). In addition, adding sex as a covariate to the white-only analysis did not materially change the findings.

### EFFECT OF GENOTYPE AND MEDICATION GROUP ON STUDY COMPLETION, MEDICATION ADHERENCE, AND ADVERSE EVENTS

There were no significant gene  $\times$  treatment group differences in study completion rates, drinking data obtained, medication adherence, or reported adverse events (**Table 2**). The lack of influence of these salient intervening variables supports the specificity of the naltrexone  $\times$  *OPRM1* genotype treatment interaction.

### COMMENT

These data, from the large and well-controlled multisite COMBINE Study, support and extend evidence that the reportedly functional *OPRM1* Asn40Asp substitution, altering  $\beta$ -endorphin binding to the receptor, influences treatment response to naltrexone among alcoholic patients. In the main COMBINE Study trial,<sup>7</sup> naltrexone showed efficacy compared with placebo only in patients not receiving CBI therapy. Therefore, we had hypothesized that the MM-alone (no CBI) group would most likely show a gene  $\times$  medication interaction. In the COMBINE Study, CBI, a more intensive and specific alcohol intervention, may have compensated for the placebo effect, thereby suppressing the chances of observing a main effect of naltrexone or a genetic interaction. The data presented herein are consistent with this thinking. A gene  $\times$  medication interaction may be observable only in patients who can show obvious benefit from the medication over placebo. External influences, such as alcoholism severity, psychosocial instability, other illnesses, and the use of an effective adjunctive treatment (in this case, CBI), might all obscure meaningful biological effects of genes on which a specific medication can act. In our case, the MM-alone (no CBI) condition enabled enough interindividual variability in clinical response for both the naltrexone<sup>7</sup> and the naltrexone  $\times$  gene interaction to emerge.

Oslin and colleagues<sup>29</sup> previously reported in a retrospective analysis of several disparate studies that nal-

naltrexone reduced relapse drinking after controlling for therapy, age (> 55 years), sex, and marital status, and including only patients who received at least 5 weeks of treatment. The analysis presented in that report suggested a naltrexone × gene interaction on 1 outcome variable (relapse to a day of heavy drinking) in 141 subjects (42 with an Asp40 allele). Nevertheless, using somewhat different and more complex drinking and outcome variables, we have replicated and extended their initial observations. More recently, Gelernter and colleagues,<sup>31</sup> in a reanalysis of the Veterans Affairs Naltrexone Cooperative Study,<sup>30</sup> did not find a significant gene × naltrexone interaction on relapse drinking. Interestingly, they did report a main effect of naltrexone, which was not observed in the initial intent-to-treat analysis. The reasons for these discrepancies are not obvious, but the authors speculate that individuals who provided genetic samples may have been more motivated, more adherent to the medication regimen, and, perhaps, more socially stable than those who did not, leading to a more favorable naltrexone response in those subjects. The lack of an observable naltrexone × gene interaction in their hands is difficult to reconcile with the favorable data of Oslin et al<sup>29</sup> and the data reported herein. However, the COMBINE Study used a naltrexone hydrochloride dosage of 100 mg/d, as did 2 of the 3 studies in the report by Oslin et al,<sup>29</sup> while the Veterans Affairs Naltrexone Cooperative Study<sup>30</sup> used a standard 50-mg/d dosage. It is unknown whether this could alter the treatment interaction with the Asp40 allele. It is also important that we observed that the gene × naltrexone interaction emerges over time, consistent with the antireinforcement effects of naltrexone. This is particularly evident in a reduction in heavy drinking days over time, consistent with previous observations on naltrexone's action.<sup>59</sup> Therefore, various outcome variables and time effects may need to be considered to completely appreciate gene × medication interactions in the treatment of alcohol dependence.

Despite several reports suggesting that Asp40 carriers may respond differently to alcohol, prestudy drinking and alcoholism severity in our study did not differ between alcoholic subjects with and without Asp40. This is in contrast to a Korean study in which homozygous Asp40 carriers had higher levels of pretreatment drinking.<sup>60</sup> Finally, although we did not perform a case-control study, the Asp40 and Asn40 allele frequencies observed in our alcoholic subjects are similar to allele frequencies in population studies, suggesting no major effect of the Asp40 allele on the development of alcoholism or its severity.

Our data speak most clearly to the interaction of the *OPRM1* genotype and naltrexone. While the strength of this interaction leading to differential treatment efficacy might be surprising in view of the clinical diversity, different neurobiologic origins of vulnerability, and genetic heterogeneity of alcoholism, naltrexone is a unique medication engineered for specificity of action at the μ-opioid receptor, although it also binds to δ- and κ-opioid receptors. Therefore, either a direct effect on μ-opioid receptor function or a shift in balance between μ-opioid and δ- and κ-opioid receptor function caused by the *OPRM1* genetic variant might form the basis of nal-

**Table 2. Retention, Medication Adherence, and Adverse Events by Genotype and Medication Group for Those Receiving Medical Management Alone (No Combined Behavioral Intervention)<sup>a</sup>**

	No. (%)				P Value
	Placebo		Naltrexone Hydrochloride		
	Asn40 (n=126)	Asp40 (n=35)	Asn40 (n=115)	Asp40 (n=31)	
Withdrew	16 (12.7)	7 (20.0)	20 (17.4)	4 (12.9)	.62
Full drinking data obtained	119 (94.4)	34 (97.1)	111 (96.5)	30 (96.8)	.92
80% of pills taken	113 (89.7)	30 (85.7)	96 (83.5)	25 (80.6)	.37
Adverse events					
Headache	34 (27.0)	7 (20.0)	43 (37.4)	11 (35.5)	.14
Fatigue	46 (36.5)	10 (28.6)	37 (32.2)	12 (38.7)	.73
Nausea	67 (53.2)	16 (45.7)	50 (43.5)	12 (38.7)	.34

<sup>a</sup>Asn40 includes subjects who were Asn40/Asn40 homozygotes. Asp40 includes those with either Asn40/Asp40 or Asp40/Asp40 genotype.

trexone's therapeutic action. This may not be as clear for medications that lack such specificity of action.

The increased naltrexone response in Asp40 carriers could have immense clinical importance, especially because, in our population, noncarriers (Asn40 homozygotes) responded no better than if given placebo. In our hands, and in the context of MM, the odds of a good clinical outcome with naltrexone were 5 times better in Asp40 carriers than noncarriers. Because almost 25% of the treatment-seeking population carries the Asp40 allele, genetic testing of individuals before naltrexone treatment might be worth the cost and effort, especially if structured behavioral treatment were not being considered. Given that alternative treatments such as CBI,<sup>7</sup> acamprosate,<sup>1,2,6</sup> and topiramate<sup>61</sup> can be offered, one could make the case that naltrexone should be used first, or primarily, in *OPRM1* Asp40 allele carriers. Naltrexone is relatively easy to administer and free of serious adverse effects and, as we observed in the Asp40 carriers we studied, it appears to be highly effective.

Future reports from this study will evaluate whether other more extensive genotyping (other single-nucleotide polymorphisms and haplotypes) of the *OPRM1* gene might lead to improved prediction of naltrexone response, adverse events, or other salient clinical information.

There are a number of limitations of this study. Although genetic samples were obtained to examine this and other treatment-related issues, subjects were not randomized by *OPRM1* genotype. It is possible that consent for the substudy differed by genotype because some subjects (about 27%) either completed or dropped out of the study before genetic consent and an additional 10% of the remaining had nonusable DNA. Although key variables seem to be equally distributed between patients with and without the Asp40 allele, it is possible that some other genetic/nongenetic influence biased responding. Also, the numbers of Asp40 carriers were somewhat limited, especially in the MM-only cells. However, confluence of effect across drinking variables, along with the consis-

tency of the observation of naltrexone response in the subgroup that also showed a naltrexone effect in the main trial, argues against a spurious result. Nevertheless, the foregoing caveats suggest that these results be replicated in a prospective trial where individuals are initially randomized by *OPRM1* Asp40 status.

In addition, because the numbers of different racial/ethnic minorities were relatively low, we could not directly assess the effect of the allele differences in these subgroups. However, adding individuals of African American and Hispanic origin into the analysis did not change the directionality of the main finding that naltrexone improved clinical global outcome primarily in Asp40 carriers. Future studies might want to consider this issue more thoroughly. In addition, we did not separately explore the effects of acamprosate. However, acamprosate did not demonstrate efficacy or added benefits to naltrexone in the main COMBINE Study trial.<sup>7</sup> Also, acamprosate is thought to be a glutamate modulator and would not be expected to show differential efficacy based on changes in the  $\mu$ -opioid receptor.

In summary, in alcoholic individuals who received naltrexone in the context of MM (the most purely pharmacologic condition studied), there was a greater response and improvement if a person had at least 1 copy of the *OPRM1* Asp40 allele. This finding could have considerable theoretical importance for drug development (such as studying only Asp40 carriers during opioid antagonist development) and also for selectively targeting  $\mu$ -opioid antagonist medications and other treatments for alcohol-dependent individuals.

**Submitted for Publication:** May 10, 2007; final revision received July 27, 2007; accepted August 28, 2007.

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**Author Contributions:** Dr Couper takes responsibility for the integrity of the data and the accuracy of the data analysis. With the exception of Dr Goldman, all authors had full access to the results of all analyses. (Because Dr Goldman's laboratory is likely to continue to do more genotyping of COMBINE Study samples, it is desirable for personnel at the laboratory to remain blinded to outcome data.)

**Financial Disclosure:** Dr Anton has reported receiving consultation fees and honoraria from Forest Laborato-

ries and Alkermes Inc (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/Ortho-McNeil; consultation fees and grant funding from Pfizer Inc; and consultation fees from AstraZeneca, Axis Shield, Cephalon, Drug Abuse Sciences, and Sanofi-Aventis. In the near future, he anticipates receiving consultation 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 Inc, DuPont, GlaxoSmithKline, Forest Laboratories, Liplha Pharmaceuticals, Ortho-McNeil, Bristol-Myers Squibb, Pfizer Inc, Sanofi-Aventis, and Mallinckrodt; serving as a consultant to Alkermes Inc, Forest Laboratories, GlaxoSmithKline, Ortho-McNeil, Pfizer Inc, and Johnson & Johnson; and receiving travel reimbursement from Alkermes Inc; she is an inventor on patents held by Yale University entitled "Smoking Cessation Treatments Using Naltrexone and Related Compounds." Dr Swift has reported receiving grant funding from Ortho-McNeil and Pfizer Inc; and serving as a consultant to or on the advisory board or speakers bureau of Alkermes Inc, Cephalon, Ortho-McNeil, Pfizer Inc, Transoral Pharmaceuticals, and Forest Laboratories. Dr Pettinati has reported receiving research support from Alkermes Inc, AstraZeneca, Bristol-Myers Squibb, Cephalon, Forest Laboratories, Liplha Pharmaceuticals and Merck KGaA, and Ortho-McNeil, and serving as a consultant to or on the advisory board or speakers bureau of Alkermes Inc, AstraZeneca, Cephalon, and Forest Laboratories.

**Funding/Support:** This study was supported by National Institute on Alcohol Abuse and Alcoholism cooperative agreements U10AA11715, 11716, 11721, 11727, 11756, 11768, 11773, 11776, 11777, 11783, 11787, 11799, and 11773 and the National Institute on Alcohol Abuse and Alcoholism Intramural Research Program (Dr Goldman). Liplha Pharmaceuticals supplied the medication for this study.

**Previous Presentation:** This study was presented in part at the American College of Neuropsychopharmacology Meeting; December 5, 2006; Fort Lauderdale, Florida.

**Additional Contributions:** We acknowledge the work of all of the COMBINE Study investigators and staff whose names are listed on the COMBINE Study Web site (<http://www.csc.unc.edu/combine>) and Barbara Bozarth, MS, who provided editorial and manuscript preparation assistance. We dedicate this report to the memory of James D. Hosking, PhD, who, before his untimely death, served as principal investigator of the COMBINE Study Coordinating Center (University of North Carolina).

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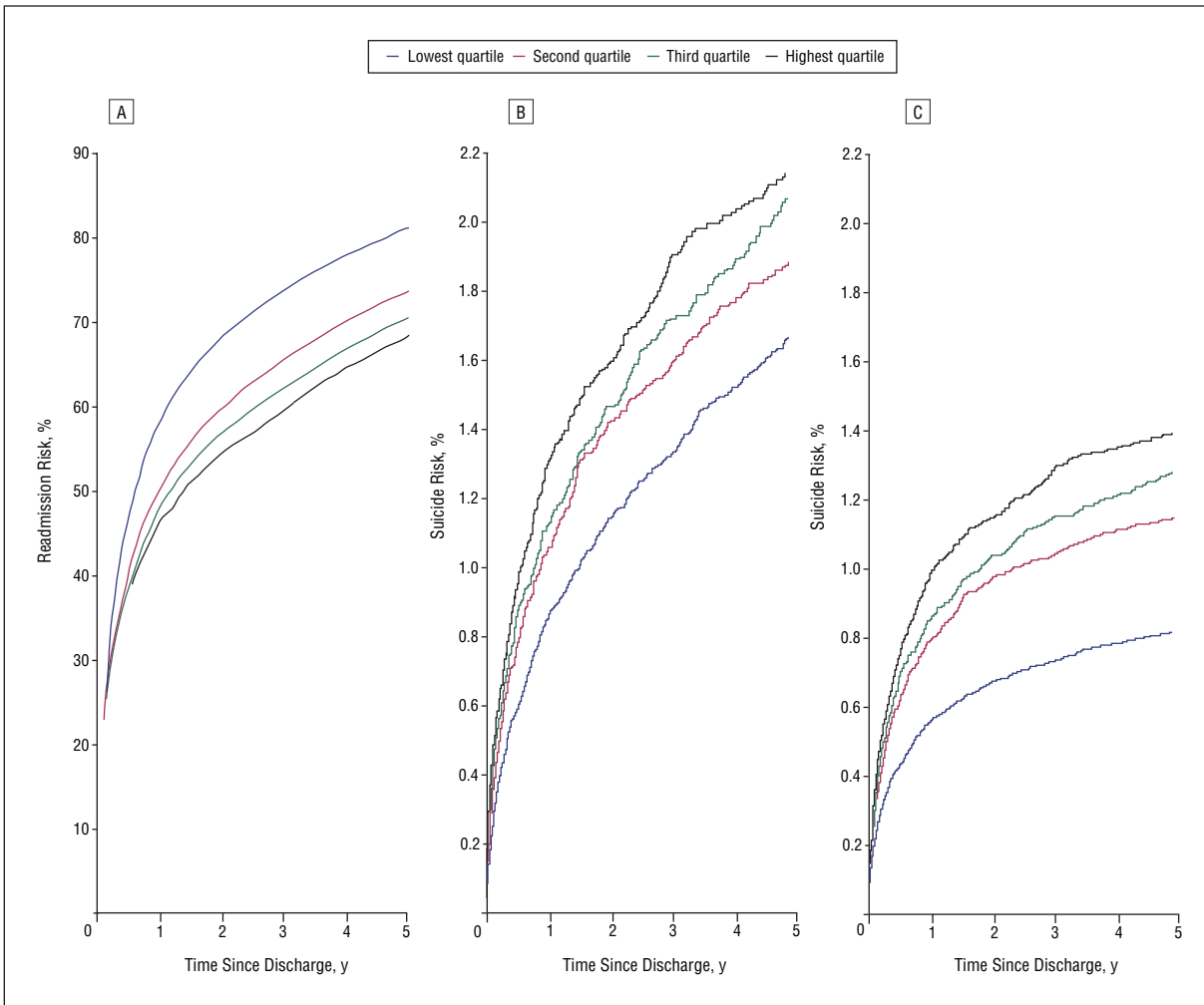
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### Correction

**Error in Figure.** In the Original Article by Esben Agerbo, MS, titled "High Income, Employment, Postgraduate Education, and Marriage: A Suicidal Cocktail Among Psychiatric Patients," published in the December issue of the *Archives* (2007; 64[12]:1377-1384), an error occurred in **Figure 1A** on page 1380. In that figure, the colors applied to the graph in part A were incorrect, resulting in misidentification of the curves. The corrected figure is reproduced here with its legend.



**Figure 1.** Kaplan-Meier plots of the risk of readmission (A) and suicide (B) and an Aalen-Johansen plot of the risk of suicide (C) in the 4 population-based income quartiles.