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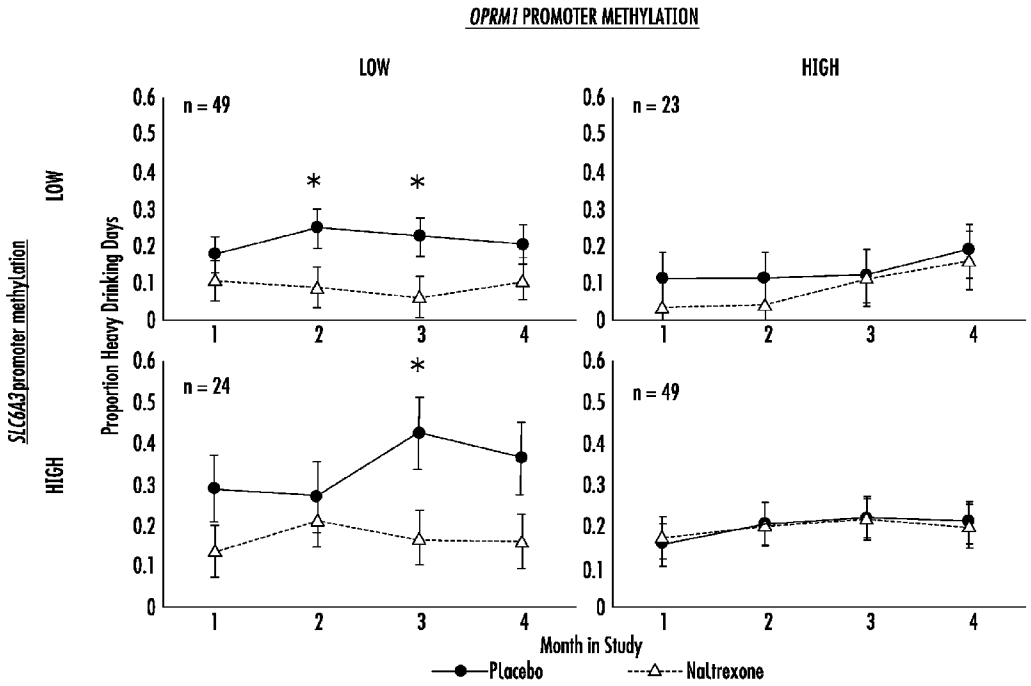

FIG. 1

(57) Abstract: Disclosed are method for predicting naltrexone response in subjects with AUD. In some embodiments, the methods include performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions are subsequences of a gene selected from a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject. Also provided are method for treating patients diagnosed with AUD with the medication naltrexone based on a methylation status of a combination of specific methylation sites located associated with an OPRM1 gene, a COMT gene,

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and/or an SLC6A3 gene in obtained from each AUD patient.

EPIGENETIC MODERATORS OF NALTREXONE EFFICACY IN REDUCING HEAVY DRINKING IN INDIVIDUALS DIAGNOSED WITH ALCOHOL USE DISORDER

CROSS REFERENCE TO RELATED APPLICATION

The presently disclosed subject matter claims the benefit of U.S. Provisional Patent Application Serial No. 63/192,952, filed May 25, 2021, the disclosure of which incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

The content of the electronically submitted sequence listing in ASCII text file (Name: 1586_24_PCT_ST25.txt; Size: 285 kilobytes; and Date of Creation: May 25, 2022) filed with the instant application is incorporated herein by reference in its entirety.

GRANT STATEMENT

This invention was made with government support under Grant Numbers AA017435 and AA017633 awarded by The National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health. The government has certain rights in the invention.

TECHNICAL FIELD

The presently disclosed subject matter relates in some embodiments to methods for predicting naltrexone responses in patients diagnosed with alcohol use disorders. The presently disclosed subject matter also relates in some embodiments to methods for treating subjects with alcohol use disorders with a treatment strategy for the subject that is predicated at least in part on the methylation statuses of subsequences of the OPRM1, COMT, and/or SLC6A3 genomic loci in the subject.

BACKGROUND

The opioid antagonist naltrexone reduces heavy drinking among individuals with Alcohol Use Disorder (AUD; Jonas et al., 2014a), but is not effective for everyone. Genomic factors might account for variability in its efficacy. The most extensively studied genomic factor is the rs1799971 single nucleotide polymorphism (SNP) in the gene encoding the μ-opioid receptor (MOR) OPRM1, which is an A/G SNP that encodes an aspartic acid or an asparagine at amino acid 40 of the human OPRM1 polypeptide in the endorphin binding domain and is associated with increased MOR binding affinity for β-endorphin (Bond et al., 1998). This gain-of-function SNP could also increase MOR affinity for naltrexone (Weerts et al., 2013), increasing naltrexone effects. However, meta-analyses of randomized controlled trials (RCTs) of naltrexone for AUD have found only weak evidence in support of rs1799971 moderation of naltrexone response (Hartwell et al., 2020; Jonas et al., 2014b).

Naltrexone is believed to reduce drinking through opioid-mediated downstream effects on alcohol-induced dopamine release (Benjamin et al., 1993; Gonzales & Weiss, 1998), so genomic

factors associated with dopamine reuptake and inactivation might also moderate its effects. Accordingly, in a human laboratory study of short-term naltrexone dosing among non-treatment-seeking AUD individuals, it was previously reported that variation at OPRM1 rs1799971, and a 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3, the gene encoding the dopamine transporter (DAT), interacted to predict naltrexone effects on alcohol self-administration and alcohol cue-elicited activation of the ventral striatum (Anton et al., 2012; Schacht et al., 2013; see also U.S. Patent Application Publication No. 2018/0371542 A1, each of which is incorporated herein by reference in its entirety). The SLC6A3 VNTR 10-repeat (10R) allele, relative to the 9-repeat (9R) allele, has been associated with relatively greater striatal DAT expression in AUD (Heinz et al., 2000), presumably reducing synaptic dopamine accumulation, and naltrexone, relative to placebo, reduced self-administration and cue-elicited ventral striatal activation most among individuals who carried the rs1799971 G allele and were homozygous for the SLC6A3 10R allele. These findings suggested that the predisposition to greater MOR affinity for naltrexone putatively conferred by the rs1799971 G allele might be beneficial only in the presence of genetically influenced reductions in synaptic dopamine accumulation.

This finding was subsequently replicated and extended in a secondary analysis of a 16-week naltrexone RCT among treatment-seeking AUD patients (Anton et al., 2020; see also U.S. Patent Application Publication No. 2018/0369238, each of which is incorporated herein by reference in its entirety). While OPRM1 rs1799971 genotype alone did not significantly moderate naltrexone effects on heavy drinking, there were epistatic interactions between rs1799971 and both the SLC6A3 VNTR and the rs4680 (val158met) SNP in COMT, the gene encoding the dopamine-inactivating enzyme catechol-O-methyltransferase (COMT; Anton et al., 2020). The rs4680 val allele has been associated with a three- to four-fold increase in COMT efficacy (Chen et al., 2004; Lachman et al., 1996), presumably reducing synaptic dopamine accumulation in a manner similar to the SLC6A3 10-repeat allele (but possibly in different brain areas, since DAT and COMT expression vary across the brain). As disclosed herein, naltrexone, relative to placebo, most effectively reduced heavy drinking among individuals who carried the rs1799971 G allele and were homozygous for either the SLC6A3 10R or the rs4680 val alleles. These data again suggested that a combination of genetically predisposed enhanced MOR function and reduced synaptic dopamine accumulation was associated with superior naltrexone response.

Although somatically derived (inherited/germline) epistatic genetic effects may have utility in predicting naltrexone efficacy, a more universal mechanism by which genomic factors could affect naltrexone response is epigenetic modification (which might either be inherited or more likely acquired after birth: e.g., by excessive alcohol use). DNA methylation at cytosine residues in CpG (cytosine, followed by guanine) dinucleotides, which are disproportionately clustered into islands in gene promoter regions, influences transcription factor binding and recruits histone deacetylase

complexes that compact chromatin, thereby decreasing gene expression (Jones, 2021). Given naltrexone's neurochemical mechanism of action, and our previous observations of the interaction of specific genes variants that influence naltrexone efficacy in AUD, differences in OPRM1, SLC6A3, and/or COMT methylation could also moderate naltrexone response. Methylation of each gene's promoter has been associated with downstream effects on its expression and the function of the protein it encodes. Greater OPRM1 promoter methylation of a neural-derived cell line was associated with decreased MOR expression (Andria & Simon, 1999); greater SLC6A3 promoter methylation in blood was associated with less striatal DAT availability (Wiers et al., 2018); and greater COMT promoter methylation in a human cell line was associated with less COMT expression (Swift-Scanlan et al., 2014). Importantly, SLC6A3 promoter methylation in blood correlated highly with methylation in the substantia nigra (Wiers et al., 2018), and COMT promoter methylation in peripheral leukocytes correlated highly with neural tissue methylation in a variety of brain regions (Murphy et al., 2005), including the prefrontal cortex (PFC; Ursini et al., 2011), where COMT is the primary mechanism of dopamine inactivation (Matsumoto et al., 2003). While most of this work was done in animals and some not replicated in man there is some evidence that peripheral SLC6A3 and COMT methylation could be biomarkers of neural methylation.

OPRM1, SLC6A3, and COMT promoter methylation have previously been associated with AUD and drinking. OPRM1 promoter methylation was greater among AUD individuals, relative to controls (Zhang et al., 2012), and methylation of several individual OPRM1 CpG sites predicted AUD relapse during treatment, although OPRM1 methylation did not independently moderate naltrexone effects on drinking (Lin et al., 2020). Similarly, SLC6A3 promoter methylation was greater among AUD individuals than controls (Hillemacher et al., 2009; Wiers et al., 2015), although some studies found no differences between these groups (Jasiewicz et al., 2015; Nieratschker et al., 2014). COMT promoter hypomethylation was associated with more hazardous drinking, albeit only in rs4680 met-allele carriers (Swift-Scanlan et al., 2014) but has not been previously associated with naltrexone response. So, while there is some evidence that alcohol consumption over a period of time could influence DNA methylation across the whole genome and in specific genes, there is considerable variation/inconsistency amongst individuals. Importantly, there has been no previous indication of any specific CpG methylation pattern influencing/predicting naltrexone or any other specific treatment response.

SUMMARY

This summary lists several embodiments of the presently disclosed subject matter, and in many cases, lists variations and permutations of these embodiments. This summary is merely exemplary of the numerous and varied embodiments. Mention of one or more representative features of a given embodiment is likewise exemplary. Such an embodiment can typically exist with or without the feature(s) mentioned; likewise, those features can be applied to other embodiments of

the presently disclosed subject matter, whether listed in this summary or not. To avoid excessive repetition, this Summary does not list or suggest all possible combinations of such features.

The presently disclosed subject matter relates in some embodiments to methods for predicting naltrexone response in subject with an alcohol use disorder (AUD). In some embodiments, the methods comprise, consist essentially of, or consist of performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject. In some embodiments, the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the SLC6A3 gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene. In some embodiments, the one or more regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of SEQ ID NOs: 2-10. In some embodiments, the one or more regions of the SLC6A3 gene comprise one or more of SEQ ID NOs: 12-20. In some embodiments, the one or more regions of the COMT gene comprise one or more of SEQ ID NsS: 28-38. In some embodiments, the SLC6A3 VNTR comprises one or more of SEQ ID NOs: 22-25.

In some embodiments of the presently disclosed methods, the methylation status of at least two and optionally all three of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined.

In some embodiments of the presently disclosed methods, the methylation statuses of at least one region of an OPRM1 gene and at least one region of an SLC6A3 gene and/or a COMT gene are determined. In some embodiments, the at least one region of the OPRM1 gene is selected from the group consisting of nucleotide positions 274, 277, 357, and 419 of SEQ ID NO: 1, and further wherein (i) the at least one region of the SLC6A3 is selected from the group consisting of nucleotide position 576 and 1102 of SEQ ID NO: 11, nucleotide position 1102 of SEQ ID NO: 11, and nucleotide position 46 of SEQ ID NO: 21; and/or (ii) the at least one region of the COMT gene is selected from the group consisting of nucleotides 46 and 107 of SEQ ID NO: 27. In some embodiments, a positive response to naltrexone is predicted if the subject has a combination of methylation values that are:

(a) lower than 0.147 with respect to nucleotide position 27 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.651 with respect to nucleotide position 576 of SEQ ID NO: 11 and/or lower than 0.648 with respect to nucleotide position 1102 of SEQ ID NO: 11 and/or lower than 0.089 with respect to nucleotide position 46 of SEQ ID NO: 21; and/or

(b) lower than 0.147 with respect to nucleotide position 27 of SEQ ID NO: 1 and/or lower than 0.157 with respect to nucleotide position 277 of SEQ ID NO: 1 and/or lower than 0.126 with respect to nucleotide position 357 of SEQ ID NO: 1 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.587 with respect to nucleotide position 46 of SEQ ID NO: 27 and/or lower than 0.546 with respect to position 107 of SEQ ID NO: 27.

In some embodiments of the presently disclosed methods, the genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

In some embodiments, the presently disclosed methods further comprise, consist essentially of, or consist of converting the isolated genomic DNA with bisulfite prior to performing or having performed the one or more methylation assays.

In some embodiments, the presently disclosed subject matter also relates to methods for treating subjects with an alcohol use disorder (AUD). In some embodiments, the presently disclosed methods comprise, consist essentially of, or consist of (a) performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject; and either (b1) treating the subject with an effective amount of naltrexone if the methylation status of one or more regions of the isolated genomic DNA is predictive of the subject advantageously responding to the naltrexone; or (b2) treating the subject with an effective amount of a non-naltrexone agent, optionally wherein the non-naltrexone agent is selected from the group consisting of acamprosate, topiramate, fluoxetine, ondansetron, or any combination thereof. In some embodiments, the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the CSLC6A3 gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene. In some embodiments, the one or more

regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of SEQ ID NOs: 2-10. In some embodiments, the one or more regions of the SLC6A3 gene comprise one or more of SEQ ID NOs: 12-20. In some embodiments, the one or more regions of the COMT gene comprise one or more of SEQ ID NOs: 28-38. In some embodiments, the SLC6A3 VNTR comprises one or more of SEQ ID NOs: 22-25. In some embodiments, the methylation status of at least two and optionally all three of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined. In some embodiments, the methylation statuses of at least one region of an OPRM1 gene and at least one region of an SLC6A3 gene and/or a COMT gene are determined. In some embodiments, the at least one region of the OPRM1 gene is selected from the group consisting of nucleotide positions 274, 277, 357, and 419 of SEQ ID NO: 1, and further wherein (i) the at least one region of the SLC6A3 is selected from the group consisting of nucleotide position 576 and 1102 of SEQ ID NO: 11, nucleotide position 1102 of SEQ ID NO: 11, and nucleotide position 46 of SEQ ID NO: 21; and/or (ii) the at least one region of the COMT gene is selected from the group consisting of nucleotides 46 and 107 of SEQ ID NO: 27. In some embodiments, a positive response to naltrexone is predicted if the subject has a combination of methylation values that are

(a) lower than 0.147 with respect to nucleotide position 27 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.651 with respect to nucleotide position 576 of SEQ ID NO: 11 and/or lower than 0.648 with respect to nucleotide position 1102 of SEQ ID NO: 11 and/or lower than 0.089 with respect to nucleotide position 46 of SEQ ID NO: 21; and/or

(b) lower than 0.147 with respect to nucleotide position 27 of SEQ ID NO: 1 and/or lower than 0.157 with respect to nucleotide position 277 of SEQ ID NO: 1 and/or lower than 0.126 with respect to nucleotide position 357 of SEQ ID NO: 1 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.587 with respect to nucleotide position 46 of SEQ ID NO: 27 and/or lower than 0.546 with respect to position 107 of SEQ ID NO: 27. In some embodiments, the genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

In some embodiments, the presently disclosed methods further comprise, consist essentially of, or consist of converting the isolated genomic DNA with bisulfite prior to performing or having performed the one or more methylation assays.

Thus, it is an object of the presently disclosed subject matter to provide methods for predicting naltrexone responses in subjects with alcohol use disorders and/or for treating subjects

with alcohol use disorders with a treatment strategy that is predicted to be appropriate for the subject based at least in part upon the outcome of whether or not the subject would be predicted to respond adequately to naltrexone.

An object of the presently disclosed subject matter having been stated hereinabove, and which is achieved in whole or in part by the compositions and methods disclosed herein, other objects will become evident as the description proceeds when taken in connection with the accompanying Figures as best described herein below.

BRIEF DESCRIPTION OF THE FIGURES

The accompanying drawings, which are incorporated herein by reference and constitute a part of this specification, illustrate several representative embodiments of the presently disclosed subject matter and together with the description illustrate the disclosed compositions and methods.

Figure 1 is a series of graphs showing the effects of naltrexone (open triangles), relative to placebo (closed circles), on percent heavy drinking days (PHDD) during the 16-week trial as a function of SLC6A3 promoter methylation (y-axis) and OPRM1 promoter methylation (x-axis). SLC6A3 and OPRM1 methylation are split into low and high groups (below and above the median methylation levels) for display purposes. Naltrexone, relative to placebo, reduced PHDD more among participants with lower SLC6A3 promoter and OPRM1 promoter methylation. Figures are estimated marginal means (± standard errors) from the linear mixed model where the independent variables were promoter methylation levels (low or high) and naltrexone or placebo treatment, while the dependent variable was percent heavy drinking days over the treatment period (months).

Figure 2 is a series of graphs showing the effects of naltrexone (open triangles), relative to placebo (closed circles), on percent heavy drinking days (PHDD) during the 16-week trial as a function of COMT promoter and OPRM1 promoter methylation. COMT and OPRM1 methylation are split into low and high groups (below and above the median methylation levels) for display purposes. Naltrexone, relative to placebo, reduced PHDD more among participants with lower COMT promoter and OPRM1 promoter methylation. Figures are estimated marginal means (± standard errors) from the linear mixed model where the independent variables were promoter methylation levels (low or high) and naltrexone or placebo treatment, while the dependent variable was percent heavy drinking days over the treatment period (months).

Figure 3 is a series of graphs showing the effects of naltrexone (open triangles), relative to placebo (closed circles), on percent heavy drinking days (PHDD) during the 16-week trial as a function of SLC6A3 VNTR and OPRM1 promoter methylation. SLC6A3 and OPRM1 methylation are split into low and high groups (below and above the median methylation levels) for display purposes. Naltrexone, relative to placebo, reduced PHDD more among participants with lower SLC6A3 VNTR and OPRM1 promoter methylation. Figures are estimated marginal means (± standard errors) from the linear mixed model. where the independent variables were promoter

methylation levels (low or high) and naltrexone or placebo treatment, while the dependent variable was percent heavy drinking days over the treatment period (months).

Figure 4A is a depiction of the nucleotide sequence of the OPRM1 promoter region (SEQ ID NO: 1). Lower-case letters indicate untranscribed nucleotides and upper-case letters indicate the exonic sequence. The transcription start site (GAT) is highlighted in dark gray (black and white version of Figure) and in red (color version of Figure) in line 3 and the translation start site (ATG) in light gray (black and white version of Figure) and in blue (color version of Figure) in line 6. CpG dinucleotides on the Illumina BeadChip are highlighted in light gray (black and white version of Figure) and in green (color version of Figure), and superscript numbers refer to the specific Illumina ID for each site (see also Figure 4B). Figure 4B is a Table showing Illumina IDs and chromosomal positions from Genome Reference Consortium Human Build 37; GRCh37. Sites 1-10 highlighted in gray (black and white version of Figure) or yellow (color version of Figure) in Figure 4B are ones that are the most significant predictors of naltrexone response in combination with other sites as follows:

Figures 5A and 5B are depictions of the nucleotide sequences of SLC6A3 promoter (Figure 5A; SEQ ID NO: 11) and 3' untranslated region (UTR; Figure 5B; SEQ ID NO: 21) regions analyzed. Lower-case letters indicate untranscribed nucleotides and upper-case letters indicate the exonic sequence. The transcription start site (GAG) is highlighted in dark gray (black and white version of Figure) or in red (color version of Figure). CpG dinucleotides on the Illumina BeadChip are highlighted in light gray (black and white version of Figure) or in green (color version of Figure), and superscript numbers refer to the specific Illumina ID for each site (see also Figure 5C). Figure 5C is a Table showing Illumina IDs and chromosomal positions (Genome Reference Consortium Human Build 37; GRCh37). Sites highlighted in light gray (black and white version of Figure) or in yellow (color version of Figure) in Figure 5C are ones that are the most significant predictors of naltrexone response in combination with other sites listed on site interaction table.

Figures 6A and 6B are depictions of the COMT promoter sequences analyzed. Figure 6A depicts the P2 (membrane-bound COMT) promoter (SEQ ID NO: 26). Figure 6B depicts the P1 (soluble COMT) promoter (SEQ ID NO: 27). Lower-case letters indicate untranscribed nucleotides and upper-case letters indicate the first exon (top) and third (bottom) exons of the gene. The translation start sites (ATG) for the membrane-bound and soluble isoforms are highlighted in light gray and dark gray (black and white version of Figure) or in blue and purple (color version of Figure), respectively, in lines 2 and 4 of Figure 6B. CpG dinucleotides on the Illumina BeadChip are highlighted in light gray (black and white version of Figure) or in green (color version of Figure), and superscript numbers refer to the specific Illumina ID for each site (see also Figure 6C). Figure 6C is a Table showing Illumina IDs and chromosomal positions (from Genome Reference Consortium Human Build 37; GRCh37). Sites highlighted in light gray (black and white version of

Figure) or in yellow (color version of Figure) in Figure 6C are ones that are the most significant predictors of naltrexone response in combination with other sites listed on the site interaction Table above.

DETAILED DESCRIPTION

The current study tested methylation of the OPRM1 promoter and its interactions with methylation of the SLC6A3 and COMT promoters and the SLC6A3 VNTR as moderators of naltrexone effects on drinking. We used genomic DNA extracted from peripheral leukocytes from participants in our 16-week naltrexone randomized control trial (RCT; Schacht et al., 2017), in which we subsequently discovered that SLC6A3 and COMT polymorphisms interacted with the OPRM1 rs1799971 SNP to predict naltrexone response (Anton et al., 2020). It was hypothesized that OPRM1, SLC6A3, and COMT promoter methylation and SLC6A2 VNTR methylation would interact in their effects on naltrexone response in a manner similar to, but different and not attributable to, those polymorphisms, such that naltrexone-treated individuals with lower OPRM1 methylation (and presumably greater MOR expression) and either lower SLC6A3 or COMT methylation (and presumably greater DAT or COMT expression, engendering less synaptic dopamine accumulation) might demonstrate the least heavy drinking when treated with naltrexone.

Numerous clinical trials have tested genomic factors that may moderate naltrexone efficacy in Alcohol Use Disorder (AUD). Epigenetic processes (some of which could be acquired by example by chronic heavy alcohol consumption), such as DNA methylation, which modulates gene expression by inhibiting transcription factor binding in gene promoters and/or at other relevant genomic sites, could also affect naltrexone efficacy. Since naltrexone putatively reduces drinking through direct effects on opioid, and, indirectly, on dopamine signaling, methylation of opioid- and dopamine-related genes might moderate its effects. This study tested methylation of the promoters of the mu opioid receptor (OPRM1), dopamine transporter (SLC6A3), and catechol-O-methyltransferase (COMT) genes as well as the SLC6A3 VNTR as moderators of naltrexone effects on heavy drinking in a 16-week randomized, placebo-controlled trial among 145 treatment-seeking AUD patients. OPRM1 methylation did not independently moderate naltrexone effects, but interacted with both SLC6A3 and COMT methylation to do so ($p<0.05$ and $p<0.01$, respectively), such that naltrexone-treated individuals with lower methylation of the OPRM1 and SLC6A3 or COMT promoters (presumably associated with greater expression of these genes), relative to placebo and to those with higher OPRM1 and SLC6A3 or COMT methylation, had significantly fewer heavy drinking days. This effect was consistent with previous pharmacogenetic data from this sample (Anton et al., 2020), which suggested that individuals with functional alleles at polymorphisms in OPRM1, SLC6A3, and COMT influenced naltrexone response. The methylation effects detailed here persisted even when these genotypes were accounted for in the statistical models, and thus represent new discoveries that were not related to the heritable gene variations

previously described. Taken together, these data suggest epigenetic modification of genes associated with opioid and dopamine signaling are a novel predictor of naltrexone efficacy in AUD.

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that they are not limited to specific synthetic methods or specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

I. Definitions

All technical and scientific terms used herein, unless otherwise defined below, are intended to have the same meaning as commonly understood by one of ordinary skill in the art. References to techniques employed herein are intended to refer to the techniques as commonly understood in the art, including variations on those techniques or substitutions of equivalent techniques that would be apparent to one of skill in the art. While the following terms are believed to be well understood by one of ordinary skill in the art, the following definitions are set forth to facilitate explanation of the presently disclosed subject matter.

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, some embodiments includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms an embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed, then "less than or equal to 10" as well as "greater than or equal to 10" are also disclosed. It is also understood that the throughout the application, data are provided in a number of different formats, and that these data represent in some embodiments endpoints and starting points and in some embodiments ranges for any combination of the data points. For example, if a particular data point "10" and a particular data point "15" are disclosed, it is understood that greater than, greater than or equal to, less than, less than or equal to, and equal to

10 and 15 are considered disclosed as well as between 10 and 15. It is also understood that each unit between two particular units are also disclosed. For example, if 10 and 15 are disclosed, then 11, 12, 13, and 14 are also disclosed.

The term "and/or", when used in the context of a list of entities, refers to the entities being present singly or in combination.

The terms "optional" and "optionally" as used herein indicate that the subsequently described event, circumstance, element, and/or method step may or may not occur and/or be present, and that the description includes instances where said event, circumstance, element, or method step occurs and/or is present as well as instances where it does not.

"Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically, a probe can be made from any combination of nucleotides, nucleotide derivatives, and/or analogs thereof as are available in the art.

"Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art which do not interfere with the enzymatic manipulation.

As used herein, the term "COMT" refers to a catechol-O-methyltransferase (COMT) gene or gene product, such as but not limited to those gene products described in Accession Nos. NM_000754.3 (SEQ ID NO: 40) and NP_000745.1 (SEQ ID NO: 41) in the GENBANK® biosequence database. The human COMT locus is located on chromosome 22 and corresponds to nucleotides 19,929,263-19,957,498 of GENBANK® Accession No. NC_000022.10 (SEQ ID NO: 42).

As used herein, the term "rs4680" refers to an SNP in the COMT gene. The wild-type allele has a G at the nucleotide position corresponding to nucleotide 721 of the human COMT cDNA of SEQ ID NO: 40, which encodes a valine amino acid at amino acid 158 of the human COMT polypeptide of SEQ ID NO: 41. The substitution polymorphism has an A at the nucleotide position corresponding to nucleotide 721 of the human COMT cDNA of SEQ ID NO: 40, which encodes a methionine amino acid at amino acid 158 of the human COMT polypeptide of SEQ ID NO: 41. Hence, this SNP is sometimes also referred to as "Val158Met" or grammatical variants thereof. Similarly, the term "rs4680 genotype" refers to both whether a patient has a G or an A at nucleotide 721 of the human COMT cDNA of SEQ ID NO: 40 as well as whether a patient has a valine or a methionine at amino acid 158 of the human COMT polypeptide of SEQ ID NO: 41.

As used herein, the terms "DAT1", "SLC6A3" and "DAT1/SLC6A3" refers to a solute carrier family 6 member 3 (SLC6A3) gene or gene product, such as but not limited to those gene products described in Accession Nos. NM_001044.4 (SEQ ID NO: 43) and NP_001035.1 (SEQ ID

NO: 44) in the GENBANK® biosequence database. The SLC6A3 gene is also referred to as the dopamine transporter 1 (DAT1) gene. The human SLC6A3 locus is located on chromosome 5 and corresponds to the reverse complement of nucleotides 1,392,905-1,445,483 of GENBANK® Accession No. NC_000005.9 (SEQ ID NO: 45).

As used herein, the term "rs28363170" refers to a polymorphism with respect to a 40 nucleotide variable number tandem repeat (VNTR; ACTGGAGCGTGTACTACCCC AGGACGCATGCAGGGCCCCC; SEQ ID NO: 39) present in the 3' untranslated region (UTR) of the nucleotide sequences of dopamine transporter (SLC6A3/DAT1) gene products. The 10- and 9-repeat alleles are the most common alleles. (Doucette-Stamm et al., 1995). Similarly, the term "rs28363170 genotype" refers to which allele(s) of the VNTR a patient has (e.g., how many copies of SEQ ID NO: 39 the patient has in the SLC6A3/DAT1 3' UTR).

As used herein, the term "OPRM1" refers to an opioid receptor mu 1 (OPRM1) gene or gene product, such as but not limited to those gene products described in Accession Nos. NM_000914.5 (SEQ ID NO: 46) and NP_000905.3 (SEQ ID NO: 47) in the GENBANK® biosequence database. The human OPRM1 locus is located on chromosome 6 and corresponds to nucleotides 154,360,375-154,453,491 of GENBANK® Accession No. NC_000006.11 (SEQ ID NO: 48).

As used herein, the term "rs1799971" refers to an SNP in the OPRM1 gene. The wild-type allele has an A at the nucleotide position corresponding to nucleotide 423 of the human OPRM1 cDNA of SEQ ID NO: 46, which encodes an asparagine amino acid at amino acid 40 of the human OPRM1 polypeptide of SEQ ID NO: 47. The substitution polymorphism has a G at the nucleotide position corresponding to nucleotide 423 of the human OPRM1 cDNA of SEQ ID NO: 46, which encodes an aspartic acid amino acid at amino acid 40 of the human OPRM1 polypeptide of SEQ ID NO: 47. Hence, this SNP is sometimes also referred to as "Asn40Asp" or grammatical variants thereof. Similarly, the term "rs1799971genotype" refers to both whether a patient has an A or a G at nucleotide 423 of the human OPRM1 cDNA of SEQ ID NO: 46 as well as whether a patient has an asparagine or an aspartic acid at amino acid 40 of the human OPRM1 cDNA of SEQ ID NO: 47.

As is known in the art, in some embodiments multiple gene products can be generated from a particular genetic locus, for example by alternative transcriptional initiation sites, alternative splicing, etc. It is understood that the GENBANK® Accession Nos. presented herein are meant to be exemplary only, and other gene products for which the nucleotide and/or amino acid sequences are not explicitly disclosed herein are also intended to be encompassed by the names of the corresponding genes. Thus, for example, transcript variants of the sequences in the Sequence Listing are also included with the definitions of the genes described herein, as are the amino acid variants encoded thereby.

II. Methods for Predicting Naltrexone Response in Subjects with Alcohol Use Disorder (AUD)

Alcohol use disorder (AUD) is a chronic relapsing brain disease characterized by compulsive alcohol use, loss of control over alcohol intake, and a negative emotional state when not using. An estimated 16 million people have been diagnosed as having AUD in the United States alone. To be diagnosed with AUD, individuals must meet at least two of the criteria outlined in the Diagnostic and Statistical Manual of Mental Disorders (DSM) including amount or duration of consumption, inability to reduce or stop drinking, time spent drinking or recovering, craving, interference of drinking on work, school, or family, maintaining consumption despite problems resulting from consumption, reducing activities to place more emphasis on consumption, increased risk behavior while consuming or intoxicated, continued consumption despite feelings of depression or anxiety, increased average consumption over the past year, and presence of withdrawal symptoms.

Treatment for AUD can comprise counseling, behavioral modification, and pharmacological intervention. Currently, three drugs, Naltrexone, Acamprosate, and Disulfiram, are approved for treating alcohol use disorder.

Both endogenous opiate and dopamine (DA) signaling regulate many aspects of AUD. Alcohol cues and intravenous alcohol self-administration both increase DA release in animals and human brain imaging suggests the same mechanism in the human ventral striatum (VS). This dopamine release and its effects are blocked by exogenously administered naltrexone in animals and man.

Naltrexone has proven efficacy in the treatment of AUD and has been approved by the FDA for this purpose. However, naltrexone does not work for all individuals with AUD and in fact works well for the minority of individuals. This has led to the speculation that genetic differences might underlie naltrexone's effect. Given that naltrexone specifically binds to a brain mu opiate receptor and that binding is linked to effects on the brain dopamine system (in the ventral striatum and elsewhere) it could be hypothesized that genetic variability (either inherited or acquired) could influence naltrexone's efficacy. As stated previously, it had been reported that a single nucleotide variant (SNP) in the coding region of the mu opiate receptor gene (OPRM1) at the 118 position (A118G) was predictive of naltrexone efficacy. However, this was not universally confirmed. Our past work suggested that this OPRM1 SNP, however, was influenced by and/or interacted with several genetic variants in the dopamine systems, a VNTR in the dopamine transporters (DAT1) gene, and/or a SNP in the catechol-O-methyl transferase (COMT) gene. These functional variants suggest that other mechanisms that affect these dopamine system genes might interact with the OPRM1 gene to also affect naltrexone efficacy. One such mechanism is epigenetic (likely acquired, not inherited) methylation of certain CpG sites in the gene promoter area and elsewhere of the DAT1 and COMT genes.

The DA transporter (DAT) is the primary mechanism for striatal DA clearance. A 40-base-pair variable number tandem repeat (VNTR) polymorphism (rs28363170; ACTGGAGCGTGTACTACCCCAGGACGCATGCAGGGCCCCC; SEQ ID NO: 39) in the 3' untranslated region of the DAT1 gene (DAT1/SLC6A3), for which the most common allelic variants are nine (9) and ten (10) repeats, can affect DAT1 function. Relative to the 10-repeat (10R) allele, the 9-repeat (9R) allele has been associated with reduced DAT1 expression and lower striatal DAT1 availability among AUD individuals, potentially leading to relatively increased extrasynaptic DA tone. Consistent with these findings, individuals who carry the 9R allele, relative to 10R homozygotes, display greater VS activation during the anticipation and receipt of monetary reward. Further, nicotine-dependent 9R carriers display greater smoking cue-elicited VS activation and greater VS DA release after smoking.

Thus, in some embodiments the presently disclosed subject matter relates to methods for predicting naltrexone response in subjects with an alcohol use disorder (AUD). In some embodiments, the method comprises, consists essentially of, or consists of performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject.

In some embodiments, the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene.

In some embodiments, the one or more regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of the following subsequences:

SEQ ID NO:	Sequence
2	ATGTGTTTGCACAGAAGAGTGCCCAGTGAAGAGACCTACTCCTTGGATCG[1]
3	TGCACAGAAGAGTGCCCAGTGAAGAGACCTACTCCTTGGATCG[1]CTTTGCG[2]

4	CTAAGGTGGGAGGGGGCTATA**CG**[3]CAGAGGAGAATGTCAGATGCTC AGCTC
5	CGCAGAGGAGAATGTCAGATGCTCAGCTCGGTCCCCTC**CG**[4]CCTGA**C G**[5]CTC
6	GTCTCAGCCAGGACTGGTTTCTGTAAGAAACAGCAGGAGCTGTGGC AG**CG**[6]
7	AGGACTGGTTTCTGTAAGAAACAGCAGGAGCTGTGGCAG**CG**[6]G**CG**[7]A AAGG
8	**CG**[8]TCAGTACC***ATG***GACAGCAGCGCTGCCCCCACGAACGCCAGCAAT TGCA
9	**CG**[9]TACTCAAGTTGCTCCCCAGCACCCAGCCCCGGTTCCTGGGTCAA CTTG
10	**CG**[10]ATCATGGCCCTCTACTCCATCGTGTGCGTGGTGGGGCTCTTCGG AAAC

With reference to the above sequences, nucleotides underlined correspond to exemplary CpG sites for the OPRM1 promoter as identified and numbered in Figures 4A and 4B. The initiator codon ATG is indicated in bold italics. In some embodiments, a methylation status for one or more of CG^{1-10} in SEQ ID NOs: 2-10 is determined.

In some embodiments, the one or more regions of the SLC6A3 gene comprise one or more of the following subsequences:

SEQ ID NO:	Sequence
12	**CG**[1]TGGGGAGATACAGGGAGAGAACTGTCTGCAACCCCGAAGCGGC CCTCA
13	**CG**[2]GCCACTCACCTCGGTGCCTTCTAAGGACCTGGACATCCTGGGCC TTGG
14	TGGTTTTCTTAGGCGAGTGCGAGGCGGGCCCCTCGGTTCCGATGCAG G**CG**[3]
15	**CG**[4]GACCCTGTCTACTGGATAAGAGCC**CG**[5]AGGC**CG**[6]AGGCTGAGAC **CG**[7]CCCA

16	TCGGCGGGAGGGCGGGGCGGGG**CG**[4]GACCCTGTCTACTGGATAAGA GCC**CG**[5]
17	GGAGGGCGGGGCGGGG**CG**[4]GACCCTGTCTACTGGATAAGAGCC**CG**[5] AGGC**CG**[6]
18	GGGG**CG**[4]GACCCTGTCTACTGGATAAGAGCC**CG**[5]AGGC**CG**[6]AGGCT***G AG***AC**CG**[7]
19	**CG**[5]AGGC**CG**[6]AGGCTGAGAC**CG**[7]CCCAG**CG**[8]CTGCGGAGCGGGAGGG GAGGC
20	CCGGGCACAGTCTGGGGTCCCCG**CG**[9]TCCCGCAGACCGCGCCGTCTC CAAA

With reference to the above sequences, nucleotides underlined correspond to exemplary CpG sites for the SLC6A3 promoter as identified and numbered in Figures 5A-5C. The transcription start site GAG is indicated in bold italics.

In some embodiments, the SLC6A3 VNTR comprises one or more of the following subsequences:

SEQ ID NO:	Sequence
22	CCTATCCCCGGACGCATGCAGGGCCCCCACAGGAG**CG**[10]TGTCCTAT CCCCG
23	**CG**[10]TGTCCTATCCCCGGACGCATGCAGGGCCCCCACAGGAGCATGT CCTAT
24	GCATGCAGGGCCCCCACAGGAG**CG**[11]TGTACTACCCCAGGACGCATG CAGG
25	CGCATGCAGGGCCCCCACAGGAG**CG**[11]TGTCCTATCCCCGGACCGGA CGCAT

With reference to the above sequences, nucleotides underlined correspond to exemplary CpG sites for the SLC6A3 VNTR as identified and numbered in Figures 5B and 5C. The transcription start site GAG is indicated in bold italics. In some embodiments, a methylation status for one or more of CG^{1-11} in SEQ ID NOs: 12-20 and 22-25 is determined.

In some embodiments, the one or more regions of the COMT gene comprise one or more of the following subsequences:

SEQ ID NO:	Sequence
28	**CG**[1]GACTCCTGAGCAAGACTAGACCAAGAGGC**CG**[2]GTATGTGGACAC CCC**CG**[3]
29	TGTGGCTAGAAGCAGCC**CG**[1]GACTCCTGAGCAAGACTAGACCAAGA GGC**CG**[2]
30	**CG**[4]CGGACACCTACCGCGGGGA**CG**[5]CCCCGACCCCATCCTACCTGCT GCGCC
31	**CG**[5]CCCCGACCCCATCCTACCTGCTGCGCCCCGCGCCGCGCCCCGCA CCC**CG**[6]
32	**CG**[6]CCCGCCACGGCCTGCGTC**CG**[7]CCACCGGAAGCGCCCTCCTAATC CCCGC
33	**CG**[7]CCACCGGAAGCGCCCTCCTAATCCCCGCAG**CG**[8]CCACCGCCATT GCCGC
34	CGCCCTCCTAATCCCCGCAG**CG**[8]CCACCGCCATTGCCGCCATCGTCG TGGG
35	AAGGCTGGCATTTCTGAACCTTGCCCCTCTGCAAACACAAGGGGG**C G**[9]
36	CCAAGCAAAGGGGCGTGTGGGTGCTGCAGGAGGAGCACAGAGCAC TGG**CG**[10]
37	**CG**[11]CCCTGCAGATGCCGGAGGCCCCGCCTCTGCTGTTGGCAGCTGTG TTGC
38	**CG**[12]AGTTCATCCTGCAGCCCATCCACAACCTGCTCATGGGTGACACC AAGG

With reference to the above sequences, nucleotides underlined correspond to exemplary CpG sites for the COMT P2 and P1 promoters as identified and numbered in Figures 6A-6C. In some embodiments, a methylation status for one or more of CG^{1-12} in SEQ ID NOs: 12-20 and 22-25 is determined.

In some embodiments, the methylation status of at least one and optionally all four of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the SCLC6A3, and

the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined.

Genomic DNA can be isolated from any biological sample isolated from a subject that includes nucleated cells. Exemplary biological samples include easily obtainable cells including but not limited to blood cells. In some embodiments, genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

As disclosed herein, the presently disclosed methods for predicting naltrexone response comprise, consist essentially of, or consist of performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA. Methods for determining methylation statuses of genomic DNA samples are known and include but are not limited to sequencing, methylation-specific PCR (MS-PCR), melting curve methylation-specific PCR (McMS-PCR), MLPA with or without bisulfite treatment, QAMA (Zeschnigk et al., 2004), MSRE-PCR (Melnikov et al., 2005), MethyLight (Eads et al., 2000), ConLight-MSP (Rand et al., 2002), bisulfite conversion-specific methylation-specific PCR (BS-MSP; Sasaki et al., 2003), COBRA (which relies upon use of restriction enzymes to reveal methylation dependent sequence differences in PCR products of sodium bisulfite-treated DNA), methylation-sensitive single-nucleotide primer extension conformation (MS-SNuPE), methylation-sensitive single-strand conformation analysis (MS-SSCA), Melting curve combined bisulfite restriction analysis (McCOBRA; Akey et al., 2002, PyroMethA, HeavyMethyl (Cottrell et al., 2004), MALDI-TOF, MassARRAY, Quantitative analysis of methylated alleles (QAMA), enzymatic regional methylation assay (ERMA), QBSUPT, MethylQuant, Quantitative PCR sequencing and oligonucleotide-based microarray systems, Pyrosequencing, Meth-DOP-PCR, etc. A review of some useful techniques for DNA methylation analysis is provided in Rein et al., 1998; Laird, 2003; and Auerkari, 2006; each of which is incorporated herein in its entirety. See also U.S. Patent No. 7,425,415.

Techniques for assessing methylation status are based on distinct approaches. Some include use of endonucleases. Such endonucleases may either preferentially cleave methylated recognition sites relative to non-methylated recognition sites or preferentially cleave non-methylated relative to methylated recognition sites. Some examples of the former are Acc III, Ban I, BstN I, Msp I, and Xma I. Examples of the latter are Acc II, Ava I, BssH II, BstU I, Hpa II, and Not I. Differences in cleavage pattern are indicative for the presence or absence of a methylated CpG dinucleotide. Cleavage patterns can be detected directly, or after a further reaction which creates products which are easily distinguishable. Means which detect altered size and/or charge can be used to detect

modified products, including but not limited to electrophoresis, chromatography, and mass spectrometry.

Alternatively, the identification of methylated CpG dinucleotides may utilize the ability of the methyl binding domain (MBD) of the MeCP2 protein to selectively bind to methylated DNA sequences (Cross et al., 1994; Shiraishi et al., 1999). The MBD may also be obtained from MBP, MBP2, MBP4, poly-MBD (Jorgensen et al., 2006) or from reagents such as antibodies binding to methylated nucleic acid. The MBD may be immobilized to a solid matrix and used for preparative column chromatography to isolate highly methylated DNA sequences. Variant forms such as expressed His-tagged methyl-CpG binding domain may be used to selectively bind to methylated DNA sequences. Eventually, restriction endonuclease digested genomic DNA is contacted with expressed His-tagged methyl-CpG binding domain. Other methods are well known in the art and include amongst others methylated-CpG island recovery assay (MIRA). Another method, MB-PCR, uses a recombinant, bivalent methyl-CpG-binding polypeptide immobilized on the walls of a PCR vessel to capture methylated DNA and the subsequent detection of bound methylated DNA by PCR.

Further approaches for detecting methylated CpG dinucleotide motifs use chemical reagents that selectively modify either the methylated or non-methylated form of CpG dinucleotide motifs. Suitable chemical reagents include hydrazine and bisulfite ions. The methods of the invention preferably use bisulfite ions. The bisulfite conversion relies on treatment of DNA samples with sodium bisulfite which converts unmethylated cytosine to uracil, while methylated cytosines are maintained (Furuichi et al., (1970). This conversion finally results in a change in the sequence of the original DNA. It is general knowledge that the resulting uracil has the base pairing behavior of thymidine which differs from cytosine base pairing behavior. This makes the discrimination between methylated and non-methylated cytosines possible. Useful conventional techniques of molecular biology and nucleic acid chemistry for assessing sequence differences are well known in the art and explained in the literature. See for example, Sambrook et al., 2001; Gait, 1984; Hames & Higgins, 1985; and the series, Methods in Enzymology, Academic Press, Inc.

Some techniques use primers for assessing the methylation status at CpG dinucleotides. Two approaches to primer design are possible. Firstly, primers may be designed that themselves do not cover any potential sites of DNA methylation. Sequence variations at sites of differential methylation are located between the two primers and visualization of the sequence variation requires further assay steps. Such primers are used in bisulfite genomic sequencing, COBRA, Ms-SnuPE and several other techniques. Secondly, primers may be designed that hybridize specifically with either the methylated or unmethylated version of the initial treated sequence. After hybridization, an amplification reaction can be performed and amplification products assayed using any detection system known in the art. The presence of an amplification product indicates that a sample hybridized to the primer. The specificity of the primer indicates whether the DNA had been modified or not,

which in turn indicates whether the DNA had been methylated or not. If there is a sufficient region of complementarity, e.g., 12, 15, 18, or 20 nucleotides, to the target, then the primer may also contain additional nucleotide residues that do not interfere with hybridization but may be useful for other manipulations. Examples of such other residues may be sites for restriction endonuclease cleavage, for ligand binding or for factor binding or linkers or repeats. The oligonucleotide primers may or may not be such that they are specific for modified methylated residues.

A further way to distinguish between modified and unmodified nucleic acid is to use oligonucleotide probes. Such probes may hybridize directly to modified nucleic acid or to further products of modified nucleic acid, such as products obtained by amplification. Probe-based assays exploit the oligonucleotide hybridization to specific sequences and subsequent detection of the hybrid. There may also be further purification steps before the amplification product is detected e.g. a precipitation step. Oligonucleotide probes may be labelled using any detection system known in the art. These include but are not limited to fluorescent moieties, radioisotope labelled moieties, bioluminescent moieties, luminescent moieties, chemiluminescent moieties, enzymes, substrates, receptors, or ligands.

In some embodiments, the methylation status of at least a subsequence of a genetic locus selected from OPMR1, DAT1/SLC6A3, and COMT (or portions thereof, in some embodiments the CpG islands) is determined using methylation specific PCR (MSP), or an equivalent amplification technique. In the MSP approach, DNA may be amplified using primer pairs designed to distinguish methylated from unmethylated DNA by taking advantage of sequence differences as a result of sodium-bisulfite treatment (Herman et al., 1996; and PCT International Patent Application Publication No. WO 97/46705). For example, bisulfite ions modify non-methylated cytosine bases, changing them to uracil bases. Uracil bases hybridize to adenine bases under hybridization conditions. Thus an oligonucleotide primer which comprises adenine bases in place of guanine bases would hybridize to the bisulfite-modified DNA, whereas an oligonucleotide primer containing the guanine bases would hybridize to the non-modified (methylated) cytosine residues in the DNA. Amplification using a DNA polymerase and a second primer yield amplification products which can be readily observed, which in turn indicates whether the DNA had been methylated or not. Whereas PCR is a preferred amplification method, variants on this basic technique such as nested PCR and multiplex PCR are also included within the scope of the invention.

Bisulfite sequencing offers another alternative to determine the methylation status of at least one gene selected from OPMR1, SLC6A3, and COMT. Primers may be designed for use in sequencing through the important CpG islands of the concerned gene. Thus, primers may be designed in both the sense and antisense orientation to direct sequencing across the region of interest of the selected gene.

As mentioned earlier, an exemplary technique for assessing the methylation status of the relevant gene requires amplification to yield amplification products. The presence of amplification products may be assessed directly using methods well known in the art. They simply may be visualized on a suitable gel, such as an agarose or polyacrylamide gel. Detection may involve the binding of specific dyes, such as ethidium bromide, which intercalate into double-stranded DNA and visualization of the DNA bands under a UV illuminator for example. Another means for detecting amplification products comprises hybridization with oligonucleotide probes. Alternatively, fluorescence or energy transfer can be measured to determine the presence of the methylated DNA.

A specific example of the MSP technique is designated real-time quantitative MSP (QMSP), and permits reliable quantification of methylated DNA in real time or at end point. Real-time methods are generally based on the continuous optical monitoring of an amplification procedure and utilize fluorescently labelled reagents whose incorporation in a product can be quantified and whose quantification is indicative of copy number of that sequence in the template. One such reagent is a fluorescent dye, called SYBR Green I that preferentially binds double-stranded DNA and whose fluorescence is greatly enhanced by binding of double-stranded DNA. Alternatively, labeled primers and/or labeled probes can be used for quantification. They represent a specific application of the well known and commercially available real-time amplification techniques such as TAQMAN®, MOLECULAR BEACONS®, AMPLIFLUOR® and SCORPION® DZYNA®, PLEXOR™ etc.

In some embodiments, an Infinium MethylationEPIC BeadChip (available from Illumina, San Diego, California) is employed for determining the methylation status of one or more of the OPMR1, SLC6A3, and COMT genes, or portions thereof, in some embodiments the CpG islands.

III. Methods for Predicting Naltrexone Response and for Treating Subjects with AUD

In some embodiments, the presently disclosed subject matter also relates to methods for treating subjects with alcohol use disorders. In some embodiments, the methods comprise, consist essentially of, or consist of performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject, followed by a decision with respect to an appropriate treatment to follow. In some embodiments, the subject is treated with an effective amount of naltrexone if the methylation status of one or more regions of the isolated genomic DNA is predictive of the subject advantageously responding to the naltrexone. If the methylation status of one or more regions of the isolated genomic DNA is not predictive of the subject advantageously

responding to the naltrexone an alternative treatment is selected. In some embodiments, such a subject is treated with an effective amount of an active agent other than naltrexone (referred to herein as a "non-naltrexone agent"). Various non-naltrexone agents can be employed, including but not limited to acamprosate, topiramate, fluoxetine, ondansetron, or any combination thereof.

For the mu opiate gene (OPRM1) the following CpG (cg) sites with a value lower than the mean listed must be present in combination with one of the DAT (SCL6A3) gene CpG (cg) sites with a value lower than the mean listed for naltrexone to be most effective. It would be expected that if the above criteria are met, the patient would have a highly significant chance ($p<0.05$) of responding to naltrexone compared to the situation where the above criteria are not met. That is, after testing and receiving specific methylation levels/frequencies for these specific CpG sites, a clinician/prescriber could/would choose to recommend/prescribe naltrexone if the above criteria were met.

OPRM1

Illumina ID	**Chromosomal position**	**Nucleotide Position in SEQ ID NO: 1**	**Mean Methylation Values**
cg05215925	154360587	274	0.147
cg22719623	154360732	419	0.488

DAT1 (SCL6A3)

Illumina ID	**Chromosomal position**	**Nucleotide Position in SEQ ID NO: 11/21***	**Mean Methylation Values**
cg16180821	1446969	576	0.651
cg15600751	1394054	1102	0.648
cg12882697	1445549	46	0.089

* cg16180821 and cg15600751 are present in SEQ ID NO: 11 and cg12882697 is present in SEQ ID NO: 21.

For the mu opiate gene (OPRM1) the following CpG (cg) sites with a value lower than the mean listed must be present in combination with one of the COMT gene CpG (cg) sites with a value lower than the mean listed for naltrexone to be most effective. It would be expected that if the above criteria are met, the patient would have a highly significant chance ($p < 0.05$) of responding to naltrexone compared to the situation where the above criteria are not met. That is, after testing and receiving specific methylation levels/frequencies for these specific CpG sites the clinician/prescriber could/would choose to recommend/prescribe naltrexone if the above criteria were met.

OPRM1

Illumina ID	**Chromosomal position**	**Nucleotide Position in SEQ ID NO: 1**	**Mean Methylation Values**
cg05215925	154360587	274	0.147

cg14348757	154360590	277	0.157
cg12838303	154360670	357	0.126
cg22719623	154360732	419	0.488

COMT

Illumina ID	Chromosomal position	Nucleotide Position in SEQ ID NO: 27	Mean Methylation Values
cg06346307	19949965	46	0.587
cg22546130	19950026	107	0.546

Thus, in some embodiments the presently disclosed subject matter also relates to methods for treating subjects with an alcohol use disorder (AUD). In some embodiments, the presently disclosed methods comprise, consist essentially of, or consist of (a) performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject; and either (b1) treating the subject with an effective amount of naltrexone if the methylation status of one or more regions of the isolated genomic DNA is predictive of the subject advantageously responding to the naltrexone; or (b2) treating the subject with an effective amount of a non-naltrexone agent, optionally wherein the non-naltrexone agent is selected from the group consisting of acamprosate, topiramate, fluoxetine, ondansetron, or any combination thereof. In some embodiments, the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the CSLC6A3 gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene. In some embodiments, the one or more regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of SEQ ID NOs: 2-10. In some embodiments, the one or more regions of the SLC6A3 gene comprise one or more of SEQ ID NOs: 12-20. In some embodiments, the one or more regions of the COMT gene comprise one or more of SEQ ID NOs: 28-38. In some embodiments, the SLC6A3 VNTR comprises one or more of SEQ ID NOs: 22-25. In some embodiments, the methylation status of at least two and optionally all three of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined. In some embodiments, the methylation statuses of at least one region of an OPRM1 gene and at least one region of an SLC6A3 gene and/or a COMT gene are determined.

In some embodiments, the at least one region of the OPRM1 gene is selected from the group consisting of nucleotide positions 274, 277, 357, and 419 of SEQ ID NO: 1, and further wherein (i) the at least one region of the SLC6A3 is selected from the group consisting of nucleotide position 576 and 1102 of SEQ ID NO: 11, nucleotide position 1102 of SEQ ID NO: 11, and nucleotide position 46 of SEQ ID NO: 21; and/or (ii) the at least one region of the COMT gene is selected from the group consisting of nucleotides 46 and 107 of SEQ ID NO: 27. In some embodiments, a positive response to naltrexone is predicted if the subject has a combination of methylation values that are

(a) lower than 0.147 with respect to nucleotide position 27 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.651 with respect to nucleotide position 576 of SEQ ID NO: 11 and/or lower than 0.648 with respect to nucleotide position 1102 of SEQ ID NO: 11 and/or lower than 0.089 with respect to nucleotide position 46 of SEQ ID NO: 21; and/or

(b) lower than 0.147 with respect to nucleotide position 27 of SEQ ID NO: 1 and/or lower than 0.157 with respect to nucleotide position 277 of SEQ ID NO: 1 and/or lower than 0.126 with respect to nucleotide position 357 of SEQ ID NO: 1 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.587 with respect to nucleotide position 46 of SEQ ID NO: 27 and/or lower than 0.546 with respect to position 107 of SEQ ID NO: 27. In some embodiments, the genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

In some embodiments, the presently disclosed methods further comprise, consist essentially of, or consist of converting the isolated genomic DNA with bisulfite prior to performing or having performed the one or more methylation assays.

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how the compounds, compositions, articles, devices and/or methods claimed herein are made and evaluated, and are intended to be purely exemplary and are not intended to limit the disclosure. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

Materials and Methods for the EXAMPLES

Overview. Detailed methods for the parent RCT, including a CONSORT diagram, are described in Anton et al, 2020 and Schacht et al, 2017. The Medical University of South Carolina Institutional Review Board approved all procedures, and all participants provided informed consent

before participation. The study consisted of an initial assessment session, a baseline visit, and 9 follow-up visits over the course of a 16-week treatment period. Briefly, participants seeking AUD treatment were recruited from the community with media advertisements, assessed for inclusion/exclusion criteria, and genotyped for rs1799971. One of the aims of the parent RCT was to test the effect of rs1799971 genotype on naltrexone efficacy, so participants who carried the minor (G) allele were over-selected, such that these individuals ultimately comprised ~50% of the 146 participants randomized to medication.

Participants. Participants were required to be ages 18-70; report heavy drinking (at least five/four standard drinks per day for men/women) on at least 50% of the days in the 90 days before assessment; and meet DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, revised 4th edition) diagnostic criteria for Alcohol Dependence, as assessed by the Structured Clinical Interview for DSM-IV (First et al, 2002). Participants were also required to self-identify as Caucasian or Asian, secondary to low rs1799971 G-allele frequency among individuals of African descent; we previously reported that analysis of population allele frequencies for 50 SNPs included on the methylation assay used here indicated a high degree of correspondence between self-reported and SNP-identified ancestry. Participants who reported cocaine or marijuana use in the 90 days before assessment were included, as long as they did not meet DSM-IV criteria for dependence on either substance or any other except nicotine and had a negative urine drug screen upon medication randomization. Exclusion criteria were: current psychotropic medication use other than antidepressants (for which a stable dose for at least one month was required); current DSM-IV Axis I diagnosis or suicidal/homicidal ideation; history of significant medical illness; liver enzyme (ALT or AST) levels > three times normal; and past-month naltrexone, disulfiram, or acamprosate use. Female participants could not be pregnant or nursing. The Table below lists demographic characteristics for the sample. Medication, randomization, and assessment. Participants were required to maintain abstinence for at least four days before medication randomization, and were then urn randomized (Stout et al, 1994) to receive naltrexone (25 mg for two days, then 50 mg thereafter) or placebo for 16 weeks. Randomization was stratified by rs1799971 genotype, with sex, smoking status (non-smoker vs. smoker, defined as $\geq$ 10 cigarettes per day), cocaine use, antidepressant use, and AUD family history balanced across medication groups. Study medications were identically over-encapsulated with 100 mg of riboflavin (see (Schacht et al, 2017) for data on adherence, which was high and did not vary between medication groups) and distributed in labeled blister packs. Participants and investigators were blind to genotype and medication assignment. After randomization, participants returned at weeks 1, 2, 3, 4, 6, 8, 10, 12, and 16 for medical management sessions, during which daily drinking since the last visit was assessed with the calendar-based Timeline Follow-back interview (Sobell and Sobell, 1992). Participants who dropped out after randomization were compensated to return at week 16 to provide missing drinking

data. Forty participants ultimately dropped out, at similar rates across medication groups, but full drinking data were available on 89% of participants.

Demographic Characteristics and Baseline Alcohol Use

	Naltrexone (n = 73)		Placebo (n = 72)		
	No.	**%**	**No.**	**%**	**Test for difference***
Demographics					
Sex, M	51	69.9	49	68.1	$p = 0.81$
Employed	56	76.7	57	79.2	$p = 0.72$
Education ≤ 12 years	12	16.4	11	15.3	$p = 0.85$
Current nicotine user	32	43.8	26	36.1	$p = 0.34$
Recent cocaine use	8	11.0	11	15.3	$p = 0.44$
Current antidepressant use	21	28.8	27	37.5	$p = 0.26$
	Mean	**SD**	**Mean**	**SD**	
Demographics					
Age, years	50.7	9.3	48.1	10.8	$p = 0.13$
Alcohol use					
Drinks per drinking day	11.9	5.2	10.5	4.3	$p = 0.11$
Drinks per day	10.3	5.3	9.0	4.5	$p = 0.08$
Heavy drinking days (%)	79.7	21.5	80.2	22.9	$p = 0.89$
Days from last drink to randomization	6.6	4.1	7.3	4.7	$p = 0.33$

* p values indicate significance of χ^2 and t tests for differences between naltrexone and placebo groups.

DNA collection and genotyping. Genomic DNA was extracted (Gentra Puragene Blood Kit; Qiagen Inc., Valencia, CA) from peripheral blood mononuclear cells collected at the initial assessment session, stored at -80°C, and used to genotype the rs1799971 and rs4680 SNPs and the SLC6A3 VNTR. Details of these assays were previously described (Anton et al, 2020).

Methylation assay. Genomic DNA was quantitated using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, MA). An Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA), which assays methylation at 866,895 cytosine residues, 99.7% of which are CpG sites, was used to generate a comprehensive genome-wide profile of DNA methylation. For each subject, 500 ng genomic DNA was bisulfite converted, denatured, and amplified, fragmented, resuspended, and hybridized to the BeadChips (eight samples per chip). Each group of eight samples was balanced by medication group, age, and nicotine use, such that each chip included four samples from naltrexone-treated participants and four from placebo-treated participants, with age (median split) and nicotine

use (defined as ≥ 10 cigarettes per day) evenly distributed within each group of four samples. Age and nicotine use were chosen because these characteristics are known to affect global DNA methylation (Horvath et al, 2012; Joehanes et al, 2016). During hybridization, the amplified and fragmented DNA annealed to fluorophore-linked probes specific to each CpG site (one for methylated and one for unmethylated sites). Processed BeadChips were then scanned on the Illumina iScan System, which used a laser to excite the fluorophores from each probe and record their fluorescence. Summaries of the probe interrogations yielded average signals for the proportion of alleles that were methylated, vs. unmethylated, at each CpG site.

Quality control. The RnBeads R package (Assenov et al, 2014) was used to examine box plots of the quality of the staining, hybridization, extension, target removal, and bisulfite conversion of the genomic DNA. The distributions and medians of negative control box plots were also examined for each sample. Based on these metrics, one participant's data were judged to be of low quality and were excluded from analysis, leaving 145 participants for analysis. Of the initial 866,895 probes, the RnBeads quality control pipeline removed 17,371 probes from the data because they overlapped with SNPs; 6,105 probes using the Greedycut algorithm, which iteratively removes the probes of greatest impurity; and 2,899 probes because they were located in specific contexts (non-CpG positions), leaving 840,520 CpG sites for analysis. Data were normalized using the method from (Pidsley et al, 2013).

To further assess data quality and to examine convergent validity, participants' age and sex were estimated from the methylation data, and methylation differences between smokers (n = 57) and non-smokers (n = 88) were tested. First, Horvath's DNA Methylation Age Calculator (Horvath, 2013), which estimates age from the methylation of 30,084 CpG sites, was used to predict participants' ages; these predictions correlated highly with participants' self-reported ages ($r = 0.882$, $p<0.001$). Second, the Horvath algorithm and RnBeads also predict sex based on sex chromosome methylation; both predictions exactly matched participants' self-reported sex. Finally, differences in whole-genome methylation between smokers and non-smokers were tested in RnBeads, and the five CpG sites that were most significantly differentially methylated between these groups were compared with the five CpG sites that most strongly discriminated smokers from non-smokers in a previous paper (Joehanes et al, 2016). Four of the top five sites in our data (Illumina probe IDs cg05575921, cg21161138, cg21566642, and cg01940273, none of which were associated with OPRM1, SLC6A3, or COMT) were among the top five most differentially methylated sites in the Joehanes analysis.

Regions analyzed. Figures 4-6 show the CpG sites on the BeadChip located in each gene's promoter. Although 840,520 CpG sites were available for analysis, not every site in each promoter was represented on the BeadChip. All of the available sites within each promoter were included. For OPRM1, the promoter was defined, consistent with a recent study of OPRM1 methylation effects

on naltrexone response (Lin et al, 2020), as the region within 130 nucleotides upstream and 600 nucleotides downstream of the transcription start site (TSS); this comprised 10 CpG sites (Illumina probe IDs cg22370006, cg14262937, cg06649410, cg23143142, cg23706388, cg05215925, cg14348757, cg12838303, cg22719623, cg15085086). For SLC6A3, NCBI AceView lists three possible promoters, one located upstream of the TSS and two in intronic regions. In keeping with prior studies' definitions, the seven CpG sites on the BeadChip in the AceView 5' upstream region (cg16180821, cg13202751, cg14502484, cg05030481, cg27037018, cg04210284, cg12882697) were used. COMT has isoforms that encode both soluble and membrane-bound COMT, each of which has its own promoter (P1 and P2, respectively) (Tenhunen et al, 1994). The four CpG sites in the P1 promoter (cg06346307, cg22546130, cg23601416, cg01335087) and eight in the P2 promoter (cg17810098, cg23268677, cg15834517, cg24899205, cg07019740, cg11032634, cg03205258, cg12175949) on the BeadChip were included. Finally, on an exploratory basis, CpG sites in the SLC6A3 VNTR region were also analyzed. The BeadChip includes three CpG sites in this region (cg15600751, cg1632193, and cg10838500), but one (cg10838500) occurs in the type "E" repeat that is present only in the 10R allele (Fuke et al, 2001). Thus, methylation at the cg15600751 and cg1632193 sites was averaged for this analysis.

Statistical analysis. Interactions between methylation levels (averaged across the proportion of methylated alleles at each site in each region) and medication were tested with linear mixed models (SPSS v. 25 MIXED) in which methylation and medication group (naltrexone vs. placebo) were between-subjects factors and time in study (month 1 to 4) was a repeated within-subjects factor. The dependent variable was percent heavy drinking days (PHDD; i.e., the proportion of study days on which women/men drank 4/5 or more standard drinks), as in our previous analyses (Anton et al, 2020; Schacht et al, 2017). Significant methylation by medication interactions indicated that naltrexone effects on PHDD across all study months differed as a function of methylation; significant methylation by medication by time interactions indicated that these effects differed as a function of both methylation and time in study. For each model, the highest-level significant interaction was interpreted by median-splitting methylation levels for each gene and testing, post hoc, the simple effect of medication within each combination of methylation level (e.g., high vs. low OPRM1, SLC6A3, and COMT methylation). Effect sizes (Cohen's d) were calculated for groups in which this simple effect was significant.

Three primary models were tested: one included OPRM1 promoter methylation, medication, time, and all interactions of these factors, and the second and third added either SLC6A3 promoter methylation or COMT promoter methylation, allowing it to interact with OPRM1 promoter methylation, medication, and time. Alpha for the three primary models was set at a Bonferroni-corrected threshold of $p = 0.0167$ (i.e., 0.05/3). Alpha for post hoc tests was left at $p = 0.05$, as these tests were conducted only to interpret higher-level interactions. A fourth exploratory model

included SLC6A3 VNTR (non-promoter) methylation, OPRM1 promoter methylation, medication, time, and all interactions. Each model used an unstructured covariance matrix and, to control for age-related changes in methylation and assay batch effects, also included terms for age and the specific BeadChip on which the sample was run. To test whether methylation effects could be accounted for by the previously reported epistatic interactions (Anton et al, 2020), additional statistical models were performed in which the germ-line single nucleotide, or VNTR polymorphisms, in OPRM1 rs1799971, SLC6A3 VNTR, and COMT rs4680 genotypes (dichotomized as G-allele carriers vs. A-allele homozygotes, 9R-allele carriers vs. 10R-allele homozygotes, and met-allele carriers vs. val-allele homozygotes, respectively) and their interactions with medication and time were to evaluate the novel/independent effects of the methylation levels on naltrexone response.

EXAMPLE 1

SLC6A3 Promoter and OPRM1 Promoter

The highest-level significant interaction was between SLC6A3 promoter methylation, OPRM1 promoter methylation, medication group, and time. When SLC6A3 and OPRM1 methylation were median-split (Figure 1; medians were SLC6A3 = 0.150, OPRM1 = 0.170), naltrexone, relative to placebo, significantly reduced PHDD at months 2 ($F(1, 217.46) = 4.87$, mean difference between naltrexone and placebo = 16.2% HDD (95% CI = 1.7-30.7%), $d = 0.61$, $p = 0.028$) and 3 ($F(1, 232.53) = 4.79$, mean difference between naltrexone and placebo = 16.5% HDD (95% CI = 1.7-31.4%), $d = 0.62$, $p = 0.030$) among individuals with lower methylation of both promoters. The simple effect of medication was not significant at any time point among individuals with any other combination of SLC6A3 and OPRM1 methylation except those with high SLC6A3 and low OPRM1 methylation, among whom this effect was significant at month 3 ($F(1, 241.14) = 5.35$, mean difference between naltrexone and placebo = 25.7% HDD (95% CI = 3.8-47.7%), $d = 0.98$, $p = 0.022$)), as a function of increased PHDD in the placebo group at that time point. The SLC6A3 by OPRM1 by medication by time interaction remained significant even when rs28363170 (SLC6A3 VNTR variants) genotype, the rs1799971 (OPRM1 A118G SNP) genotype, and their interactions with each other and with medication and time were included in the model, suggesting that the methylation by medication interaction was novel/independent of these other effects.

EXAMPLE 2

COMT Promoter and OPRM1 Promoter

The highest-level significant interaction was between COMT promoter methylation, OPRM1 promoter methylation, and medication group. Across all study months, naltrexone, relative to placebo, reduced PHDD more among individuals with lower COMT methylation and lower OPRM1 methylation. When COMT and OPRM1 methylation were median-split (Figure 2; medians were COMT = 0.341, OPRM1 = 0.170), naltrexone, relative to placebo, significantly reduced PHDD

across all study months only among individuals with lower methylation of both promoters (F(1, 154.12) = 5.41, mean difference between naltrexone and placebo = 19.7% HDD (95% CI = 3.0-36.5%), d = 0.85, p = 0.021). This interaction remained significant even when rs4680 (COMT val158met SNP) genotype, rs1799971 (OPRM1 A118G SNP) genotype, and their interactions with each other and with medication and time were included in the statistical model, suggesting that the methylation by medication interaction was novel/independent of these other effects.

EXAMPLE 3

SLC6A3 VNTR and OPRM1 promoter

The highest-level significant interaction was between SLC6A3 VNTR methylation, OPRM1 promoter methylation, and medication group. Across all study months, naltrexone, relative to placebo, reduced PHDD more among individuals with lower SLC6A3 VNTR methylation and lower OPRM1 methylation. When SLC6A3 VNTR and OPRM1 promoter methylation were median-split (Figure 3; medians were SLC6A3 = 0.618, OPRM1 = 0.170), naltrexone, relative to placebo, significantly reduced PHDD across all study months only among individuals with lower methylation of both regions (F(1, 146.43) = 9.93, mean difference between naltrexone and placebo = 28.5% HDD (95% CI = 10.6-46.3%), d = 1.25, p = 0.002). This interaction remained significant even when rs28363170 genotype, rs1799971 genotype, and their interactions with each other and with medication and time were included in the model, again suggesting that the methylation by medication interaction was independent of these other effects.

Discussion of the EXAMPLES

Taken together, these data suggest that methylation differences in genes underlying opioid signaling and dopamine reuptake and inactivation interact to predict naltrexone treatment effects on heavy drinking among AUD outpatients. Specifically, methylation of the promoter regions of SLC6A3 and COMT interacted with OPRM1 promoter methylation to influence naltrexone efficacy, as did, in an exploratory analysis, methylation of the SLC6A3 3' UTR VNTR region. Effect sizes for naltrexone, relative to placebo, on heavy drinking in the subgroups in which it was most effective were in the medium to large range (0.53-1.04), greater than the overall small effect of naltrexone on heavy drinking across all AUD individuals (Maisel et al, 2013). These findings suggested a novel epigenetic predictor of naltrexone response.

The finding that OPRM1 methylation did not independently moderate naltrexone response is consistent with a recent secondary analysis of another AUD naltrexone RCT (Lin et al, 2020), as well as with a recent meta-analysis that concluded that OPRM1 rs1799971 genotype does not consistently moderate naltrexone effects (Hartwell et al, 2020). This meta-analysis included the primary analysis of the data used in the current study (Schacht et al, 2017), which also did not support this OPRM1 alone pharmacogenetic effect. Collectively, these findings suggest that, despite the fact

that naltrexone directly antagonizes the MOR, the effects of genetic or epigenetic alteration of OPRM1 alone are likely not sufficiently large to consistently affect naltrexone response.

In contrast, the current data suggest that epigenetic changes in genes that may underlie naltrexone's downstream effects on dopamine signaling interact with OPRM1 methylation to predict its effects on drinking. Alcohol acutely elicits striatal dopamine release (Boileau et al, 2003) and naltrexone blocks this phenomenon (Benjamin et al, 1993; Gonzales et al, 1998). DAT and COMT are the primary methods of dopamine inactivation in the striatum (Ciliax et al, 1999) and PFC (Matsumoto et al, 2003), respectively. SLC6A3 VNTR and OPRM1 rs1799971 variation have previously been reported to interact in their effects on acute response to alcohol, such that individuals carrying the gain-of-function alleles of each polymorphism displayed lower hedonic response (Weerts et al, 2017). Since lower OPRM1, SLC6A3, and COMT promoter methylation has been associated with relatively increased expression of these genes (Andria et al, 1999; Murphy et al, 2005; Wiers et al, 2018), naltrexone might more effectively reduce heavy drinking among individuals with lower methylation of these regions because this pattern of methylation predisposes greater MOR availability and more effective synaptic dopamine clearance after alcohol-induced dopamine release. With respect to methylation of the SLC6A3 3' UTR, CpG methylation outside of gene promoters can also regulate gene expression (Maunakea et al, 2010), and 3' UTRs contain regulatory regions that can influence a variety of posttranscriptional modifications that affect gene expression (Barrett et al, 2012). Thus, greater methylation of this region could modulate posttranscriptional functions that affect SLC6A3 expression. We previously reported epistatic genetic effects on naltrexone efficacy in this sample, but the significance of the methylation effects persisted when these genetic effects were included in the models, suggesting that, even after accounting for variance attributable to epistatic effects, interactions between OPRM1 methylation and SLC6A3 and COMT methylation independently predicted naltrexone effects.

Thus, disclosed herein is the discovery that AUD individuals with less methylation of the OPRM1 and SLC6A3 or COMT promoters, as well as the SLC6A3 3'UTR VNTR, were more likely to benefit from naltrexone, relative to placebo, than individuals with other combinations of methylation in these regions.

While there have been published studies regarding how "inherited" genes and their germ-line mutations might be associated with naltrexone response, these genetic differences cannot/do not pick up genetic modifications that might occur due to "environmental exposure", such as heavy alcohol consumption. Therefore, the use of epigenetic methylation patterns is a fundamentally different, novel, and biologically meaningful way of understanding biological predictors of medication response. And while there has been some discovery that heavy alcohol consumption can modify the epigenetic methylation patterns of many genes overall, there has been few attempts to relate these changes to medication treatment response in Alcohol Use Disorder. It should also be

recognized that there are many CpG sites in various genes that may, or may not, be more, or less, methylated due to alcohol exposure, and the discovery of which CpG sites that have relevance to disease and treatment response, is not an obvious or trivial issue. This is partly because methylation of multiple sites (especially in areas of gene transcription promoters) are likely needed for biological variation. As such, it is the pattern of these methylation sites that is important. For instance, several papers have examined the methylation of the OPRM1 gene have found various CpG sites to be hypermethylated in heavy drinkers compared to non-heavy drinkers (Zhang et al., 2012) and that a few CpG site methylation levels were associated with "relapse drinking" (Lin et al., 2020) but specifically NOT associated with naltrexone response. Therefore, the presently disclosed subject matter provides (1) a focus on various combinations of CpG methylation sites; and even more importantly, (2) at the next level, how methylation sites in several other important genes (DAT (SLC6A3 and COMT) might be additive to the methylation pattern of a single gene such as the OPRM1 promoter CpG sites. It is the direction of the methylation amounts/frequencies (lower or higher) at specific CpG sites, as well as the discovery of non-obvious combinations of CpG sites across several genes that predicts naltrexone response that is not obvious and therefore novel. This discovery will allow clinicians/prescribers specific new knowledge to choose which patient with Alcohol Use Disorder to treat with naltrexone, improving clinical care and reducing patient burden.

Summarily, disclosed herein is the identification that several out of many OPRM1 gene CpG methylation sites when combined with several other out of many COMT and DAT gene CpG methylation sites that are associated with naltrexone response for Alcohol Use Disorder individuals. So in essence, it is not the discovery of these sites or even if they are different between heavy drinkers (Alcohol Use Disorder) and non-heavy drinkers that is important for the presently disclosed subject matter, but how to apply these differences for improved treatment outcome to a specific medication.

REFERENCES

All references listed below, as well as all references cited in the instant disclosure, including but not limited to all patents, patent applications and publications thereof, scientific journal articles, and database entries (e.g., GENBANK® database entries and all annotations available therein) are incorporated herein by reference in their entireties to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

Akey et al. (2002) Assaying DNA methylation based on high-throughput melting curve approaches. Genomics 80(4):376-384.

Andria & Simon (1999) Localization of promoter elements in the human mu-opioid receptor gene and regulation by DNA methylation. Brain Res Mol Brain Res 70(1):54-65.

Anton et al. (2020) Opioid and Dopamine Genes Interact to Predict Naltrexone Response in a Randomized Alcohol Use Disorder Clinical Trial. Alcohol Clin Exp Res 44(10):2084-2096.

Anton et al. (2012) Naltrexone modification of drinking effects in a subacute treatment and bar-lab paradigm: influence of OPRM1 and dopamine transporter (SLC6A3) genes. Alcohol Clin Exp Res 36(11):2000-2007.

Assenov et al. (2014) Comprehensive analysis of DNA methylation data with RnBeads. Nat Methods 11(11):1138-1140.

Auerkari (2006) Methylation of tumor suppressor genes p16(INK4a), p27(Kip1) and E-cadherin in carcinogenesis. Oral Oncology 42:5-13.

Barrett et al. (2012) Regulation of eukaryotic gene expression by the untranslated gene regions and other non-coding elements. Cell Mol Life Sci 69(21):3613-3634.

Benjamin et al. (1993) Naltrexone reverses ethanol-induced dopamine release in the nucleus accumbens in awake, freely moving rats. Brain Research 621(1):137-140.

Boileau et al. (2003) Alcohol promotes dopamine release in the human nucleus accumbens. Synapse (New York, NY) 49(4):226-231.

Bond et al. (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci U S A 95(16):9608-9613.

Chen et al. (2004) Functional analysis of genetic variation in catechol-O-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. Am J Hum Genet 75(5):807-821.

Ciliax et al. (1999) Immunocytochemical localization of the dopamine transporter in human brain. The Journal of Comparative Neurology 409(1):38-56.

Cottrell et al. (2004) A real-time PCR assay for DNA-methylation using methylation-specific blockers. Nucleic Acids Research 32(1):e10.

Cross et al. (1994) Purification of CpG islands using a methylated DNA binding column. Nature Genetics 6:236-244.

Doucette-Stamm et al. (1995) Population genetic study of the human dopamine transporter gene (DAT1). Genet Epidemiol 12(3):303-308.

Eads et al. (2000) MethyLight: a high-throughput assay to measure DNA methylation. Nucleic Acids Research 28 (8):e32.

First et al. (2002) Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Research Version, Non-patient Edition. Biometrics Research, New York State Psychiatric Institute: New York.

Fuke et al. (2001) The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. Pharmacogenom J 1(2):152-156.

Furuichi et al. (1970) Chemical modification of tRNA-Tyr-yeast with bisulfite. A new method to modify isopentenyladenosine residue. Biochemical and Biophysical Research Communications 41(5):1185-1191.

Gait (ed.) (1984) Oligonucleotide Synthesis, A Practical Approach, IRL Press, Oxford, England, United Kingdom.

Gonzales & Weiss (1998) Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. J Neurosci 18(24):10663-10671.

Hames & Higgins (eds.) (1985) Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, DC, United States of America.

Hartwell et al. (2020) Systematic review and meta-analysis of the moderating effect of rs1799971 in OPRM1, the mu-opioid receptor gene, on response to naltrexone treatment of alcohol use disorder. Addiction 115(8):1426-1437.

Heinz et al. (2000) Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology 22(2):133-139.

Herman et al. (1996) Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. Proc Natl Acad Sci U S A 93:9821-9826.

Hillemacher et al. (2009) Promoter specific methylation of the dopamine transporter gene is altered in alcohol dependence and associated with craving. J Psychiatr Res 43(4):388-392.

Horvath (2013) DNA methylation age of human tissues and cell types. Genome Biol 14(10):R115.

Horvath et al. (2012) Aging effects on DNA methylation modules in human brain and blood tissue. Genome Biol 13(10):R97.

Illingworth et al. (2008) A novel CpG island set identifies tissue-specific methylation at developmental gene loci. PLoS Biol 6(1):e22.

Jasiewicz et al. (2015) DAT1 methylation changes in alcohol-dependent individuals vs. controls. J Psychiatr Res 64:130-133.

Joehanes et al. (2016) Epigenetic Signatures of Cigarette Smoking. Circ Cardiovasc Genet 9(5):436-447.

Jonas et al. (2014a) Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis. JAMA 311(18):1889-1900.

Jonas et al. (2014b) Genetic polymorphisms and response to medications for alcohol use disorders: a systematic review and meta-analysis. Pharmacogenomics 15(13):1687-1700.

Jones (2012) Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet 13(7):484-492.

Jorgensen et al. (2006) Engineering a high-affinity methyl-CpG-binding protein. Nucleic Acids Research 34:e96.

Lachman et al. (1996) Human catechol-O-methyltransferase pharmacogenetics: description of a functional polymorphism and its potential application to neuropsychiatric disorders. Pharmacogenetics 6(3):243-250.

Laird (2003) The power and the promise of DNA methylation markers. Nature Reviews 3:253-266.

Lin et al. (2020) An analysis of the effect of mu-opioid receptor gene (OPRM1) promoter region DNA methylation on the response of naltrexone treatment of alcohol dependence. Pharmacogenomics J.

Maisel et al. (2013) Meta-analysis of naltrexone and acamprosate for treating alcohol use disorders: when are these medications most helpful? Addiction 108(2):275-293.

Matsumoto et al. (2003) Catechol O-methyltransferase mRNA expression in human and rat brain: evidence for a role in cortical neuronal function. Neuroscience 116(1):127-137.

Maunakea et al. (2010) Conserved role of intragenic DNA methylation in regulating alternative promoters. Nature 466(7303):253-257.

Melnikov et al. (2005) MSRE-PCR for analysis of gene-specific DNA methylation. Nucleic Acids Research 33(10):e93.

Methods in Enzymology, Academic Press, Inc., Cambridge, Massachusetts, United States of America.

Murphy et al. (2005) Site-specific cytosine methylation in S-COMT promoter in 31 brain regions with implications for studies involving schizophrenia. Am J Med Genet B Neuropsychiatr Genet 133B(1):37-42.

Nieratschker et al. (2014) Epigenetic alteration of the dopamine transporter gene in alcohol-dependent patients is associated with age. Addict Biol 19(2):305-311.

PCT International Patent Application Publication No. WO 97/46705.

Pidsley et al. (2013) A data-driven approach to preprocessing Illumina 450K methylation array data. BMC Genomics 14:293.

Rand et al. (2002) Conversion-specific detection of DNA methylation using real-time polymerase chain reaction (ConLight-MSP) to avoid false positives. Methods 27:114-120.

Rein et al. (1998) Identifying 5-methylcytosine and related modifications in DNA genomes. Nucleic Acids Research 26(10):2255-2264.

Sambrook et al. (2001) Molecular Cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, United States of America.

Sasaki et al. (2003) Bisulfite conversion-specific and methylation-specific PCR: a sensitive technique for accurate evaluation of CpG methylation. Biochemical and Biophysical Research Communications 309:305-309.

Schacht et al. (2013) Interacting effects of naltrexone and OPRM1 and DAT1 variation on the neural response to alcohol cues. Neuropsychopharmacology 38(3):414-422.

Schacht et al. (2017) Predictors of Naltrexone Response in a Randomized Trial: Reward-Related Brain Activation, OPRM1 Genotype, and Smoking Status. Neuropsychopharmacology 42(13):2640-2653.

Shiraishi et al. (1999) Isolation of DNA fragments associated with methylated CpG islands in human adenocarcinomas of the lung using a methylated DNA binding column and denaturing gradient gel electrophoresis. Proceedings of National Academy of Sciences, USA 96:2913-2918.

Sobell & Sobell (1992) Timeline follow-back: A technique for assessing self-reported alcohol consumption. In: Allen JP, Litten RZ (eds) Measuring alcohol consumption: Psychosocial and biochemical methods. Humana Press: Totowa, NJ, pp 41-72.

Stout et al. (1994) Ensuring balanced distribution of prognostic factors in treatment outcome research. J Stud Alcohol Suppl 12:70-75.

Swift-Scanlan et al. (2014) Comprehensive interrogation of CpG island methylation in the gene encoding COMT, a key estrogen and catecholamine regulator. BMC Med Genomics 7:5.

Tenhunen et al. (1994) Genomic organization of the human catechol O-methyltransferase gene and its expression from two distinct promoters. Eur J Biochem 223(3):1049-1059.

U.S. Patent Application Publication Nos. 2018/0369238, 2018/0371542.

U.S. Patent No. 7,425,415.

Ursini et al. (2011) Stress-related methylation of the catechol-O-methyltransferase Val 158 allele predicts human prefrontal cognition and activity. J Neurosci 31(18):6692-6698.

Weerts et al. (2013) Influence of OPRM1 Asn40Asp variant (A118G) on [11C]carfentanil binding potential: preliminary findings in human subjects. Int J Neuropsychopharmacol 16(1):47-53.

Weerts et al. (2017) Independent and Interactive Effects of OPRM1 and DAT1 Polymorphisms on Alcohol Consumption and Subjective Responses in Social Drinkers. Alcohol Clin Exp Res 41(6):1093-1104.

Wiers et al. (2018) Methylation of the dopamine transporter gene in blood is associated with striatal dopamine transporter availability in ADHD: A preliminary study. Eur J Neurosci 48(3):1884-1895.

Wiers et al. (2015) Effects of depressive symptoms and peripheral DAT methylation on neural reactivity to alcohol cues in alcoholism. Translational psychiatry 5:e648.

Zeschnigk et al. (2004) A novel real-time PCR assay for quantitative analysis of methylated alleles (QAMA): analysis of the retinoblastoma locus. Nucleic Acids Research 32(16):e125.

Zhang et al. (2012) Hypermethylation of OPRM1 promoter region in European Americans with alcohol dependence. J Hum Genet 57(10):670-675.

It will be understood that various details of the presently disclosed subject matter can be changed without departing from the scope of the presently disclosed subject matter. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of limitation.

CLAIMS

What is claimed is:

1. A method for predicting naltrexone response in a subject with an alcohol use disorder (AUD), the method comprising, consisting essentially of, or consisting of performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject.

2. The method of claim 1, wherein the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the SLC6A3 gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene.

3. The method of claim 2, wherein the one or more regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of SEQ ID NOs: 2-10.

4. The method of claim 2, wherein the one or more regions of the SLC6A3 gene comprise one or more of SEQ ID NOs: 12-20.

5. The method of claim 2, wherein the one or more regions of the COMT gene comprise one or more of SEQ ID NOs: 28-38.

6. The method of claim 2, wherein the SLC6A3 VNTR comprises one or more of SEQ ID NO: 22-25.

7. The method of any one of the preceding claims, wherein the methylation status of at least two and optionally all three of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined.

8. The method of any one of the preceding claims, wherein the methylation statuses of at least one region of an OPRM1 gene and at least one region of an SLC6A3 gene and/or a COMT gene are determined.

9. The method of claim 8, wherein the at least one region of the OPRM1 gene is selected from the group consisting of nucleotide positions 274, 277, 357, and 419 of SEQ ID NO: 1, and further wherein:

(i) the at least one region of the SLC6A3 is selected from the group consisting of nucleotide position 576 and 1102 of SEQ ID NO: 11, nucleotide position 1102 of SEQ ID NO: 11, and nucleotide position 46 of SEQ ID NO: 21; and/or

(ii) the at least one region of the COMT gene is selected from the group consisting of nucleotides 46 and 107 of SEQ ID NO: 27.

10. The method of any one of claims 8 and 9, wherein a positive response to naltrexone is predicted if the subject has a combination of methylation values that are:

(a) lower than 0.147 with respect to nucleotide position 27 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.651 with respect to nucleotide position 576 of SEQ ID NO: 11 and/or lower than 0.648 with respect to nucleotide position 1102 of SEQ ID NO: 11 and/or lower than 0.089 with respect to nucleotide position 46 of SEQ ID NO: 21; and/or

(b) lower than 0.147 with respect to nucleotide position 27 of SEQ ID NO: 1 and/or lower than 0.157 with respect to nucleotide position 277 of SEQ ID NO: 1 and/or lower than 0.126 with respect to nucleotide position 357 of SEQ ID NO: 1 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.587 with respect to nucleotide position 46 of SEQ ID NO: 27 and/or lower than 0.546 with respect to position 107 of SEQ ID NO: 27.

11. The method of any one of the preceding claims, wherein the genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

12. The method of any one of the preceding claims, further comprising converting the isolated genomic DNA with bisulfite prior to performing or having performed the one or more methylation assays.

13. A method for treating a subject with an alcohol use disorder (AUD), the method comprising, consisting essentially of, or consisting of:

(a) performing or having performed one or more methylation assays on a genomic DNA sample isolated from the subject to determine the methylation status of one or more regions of the isolated genomic DNA, wherein the one or more regions of the isolated genomic DNA are subsequences of a gene selected from the group consisting of a mu opioid receptor (OPRM1) gene, a catechol-O-methyltransferase (COMT) gene, and a dopamine transporter (SLC6A3) gene, and further wherein the methylation status of the one or more regions of the isolated genomic DNA determined is predictive of naltrexone response in the subject; and either

(b1) treating the subject with an effective amount of naltrexone if the methylation status of one or more regions of the isolated genomic DNA is predictive of the subject advantageously responding to the naltrexone; or

(b2) treating the subject with an effective amount of a non-naltrexone agent, optionally wherein the non-naltrexone agent is selected from the group consisting of acamprosate, topiramate, fluoxetine, ondansetron, or any combination thereof.

14. The method of claim 13, wherein the one or more regions of the isolated genomic DNA assayed comprise, consist essentially of, or consist of the promoter of the OPRM1 gene, the promoter of the COMT gene, the promoter of the CSLC6A3 gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene.

15. The method of claim 14, wherein the one or more regions of the OPRM1 gene include 130 nucleotides upstream and 600 nucleotides downstream of the OPRM1 transcription start site (TSS); optionally comprising one or more of SEQ ID NOs: 2-10.

16. The method of claim 14, wherein the one or more regions of the SLC6A3 gene comprise one or more of SEQ ID NOs: 12-20.

17. The method of claim 14, wherein the one or more regions of the COMT gene comprise one or more of SEQ ID NOs: 28-38.

18. The method of claim 14, wherein the SLC6A3 VNTR comprises one or more of SEQ ID NOs: 22-25.

19. The method of any one of claims 13-18, wherein the methylation status of at least two and optionally all three of the promoter of the OPRM1 gene, the promoter of the COMT gene, and the 40-base-pair variable number tandem repeat (VNTR) polymorphism in the 3' untranslated region of SLC6A3 gene are determined.

20. The method of any one of claims 13-19, wherein the methylation statuses of at least one region of an OPRM1 gene and at least one region of an SLC6A3 gene and/or a COMT gene are determined.

21. The method of claim 20, wherein the at least one region of the OPRM1 gene is selected from the group consisting of nucleotide positions 274, 277, 357, and 419 of SEQ ID NO: 1, and further wherein:

(i) the at least one region of the SLC6A3 is selected from the group consisting of nucleotide position 576 and 1102 of SEQ ID NO: 11, nucleotide position 1102 of SEQ ID NO: 11, and nucleotide position 46 of SEQ ID NO: 21; and/or

(ii) the at least one region of the COMT gene is selected from the group consisting of nucleotides 46 and 107 of SEQ ID NO: 27.

22. The method of any one of claims 20 and 21, wherein a positive response to naltrexone is predicted if the subject has a combination of methylation values that are:

(a) lower than 0.147 with respect to nucleotide position 27 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.651 with respect to nucleotide position 576 of SEQ ID NO: 11 and/or lower than 0.648 with respect to nucleotide position 1102 of SEQ ID NO: 11 and/or lower than 0.089 with respect to nucleotide position 46 of SEQ ID NO: 21; and/or

(b) lower than 0.147 with respect to nucleotide position 27 of SEQ ID NO: 1 and/or lower than 0.157 with respect to nucleotide position 277 of SEQ ID NO: 1 and/or lower than 0.126 with respect to nucleotide position 357 of SEQ ID NO: 1 and/or lower than 0.488 with respect to nucleotide position 419 of SEQ ID NO: 1 in combination with lower than 0.587 with respect to nucleotide position 46 of SEQ ID NO: 27 and/or lower than 0.546 with respect to position 107 of SEQ ID NO: 27.

23. The method of any one of claims 13-22, wherein the genomic DNA is isolated from a cell selected from the group consisting of blood cells, optionally peripheral blood mononuclear cells, and buccal cells, and/or from a biological sample containing cells, optionally, blood, saliva, cerebrospinal fluid, and/or any fraction or component thereof.

24. The method of any one of claims 13-23, further comprising converting the isolated genomic DNA with bisulfite prior to performing or having performed the one or more methylation assays.

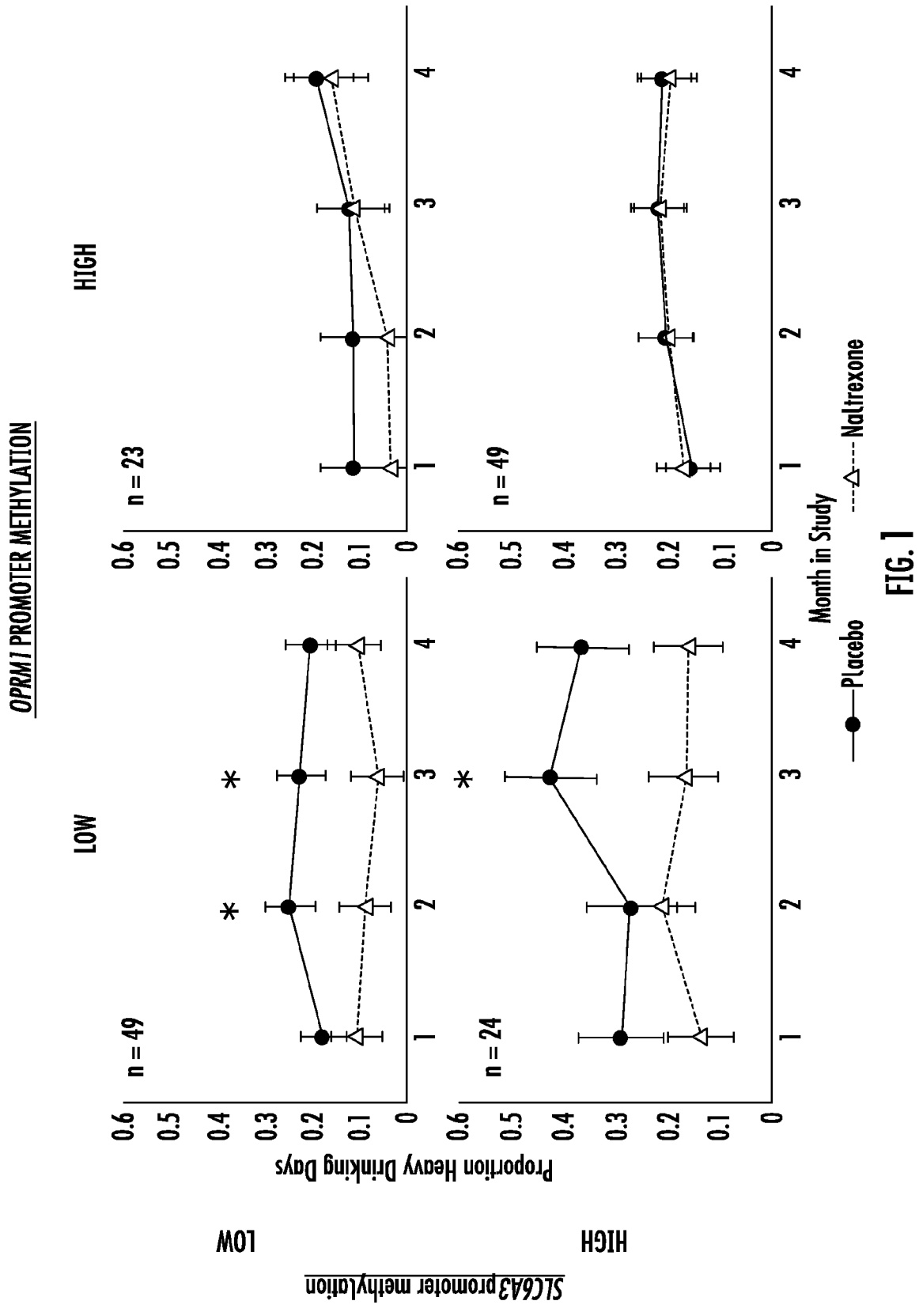
OPRM1 PROMOTER METHYLATION
LOW
HIGH
n = 49
n = 23
n = 24
n = 49
Proportion Heavy Drinking Days
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
*
Month in Study
Placebo
Naltrexone
SLC6A3 promoter methylation
LOW
HIGH

FIG. 1

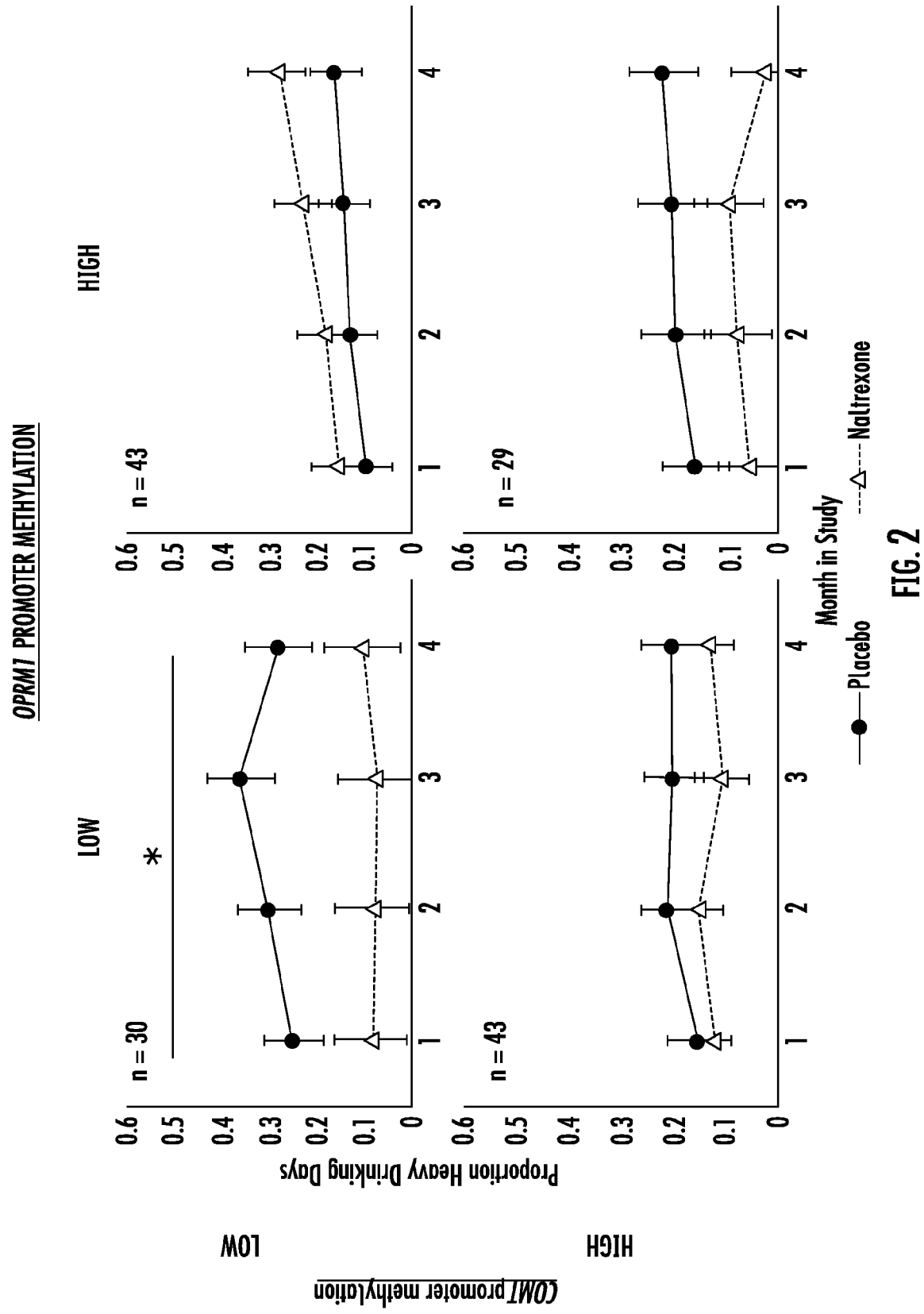
OPRM1 PROMOTER METHYLATION
LOW
HIGH
n = 30
n = 43
n = 43
n = 29
*
Proportion Heavy Drinking Days
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Month in Study
Placebo
Naltrexone
COMT promoter methylation
LOW
HIGH

FIG. 2

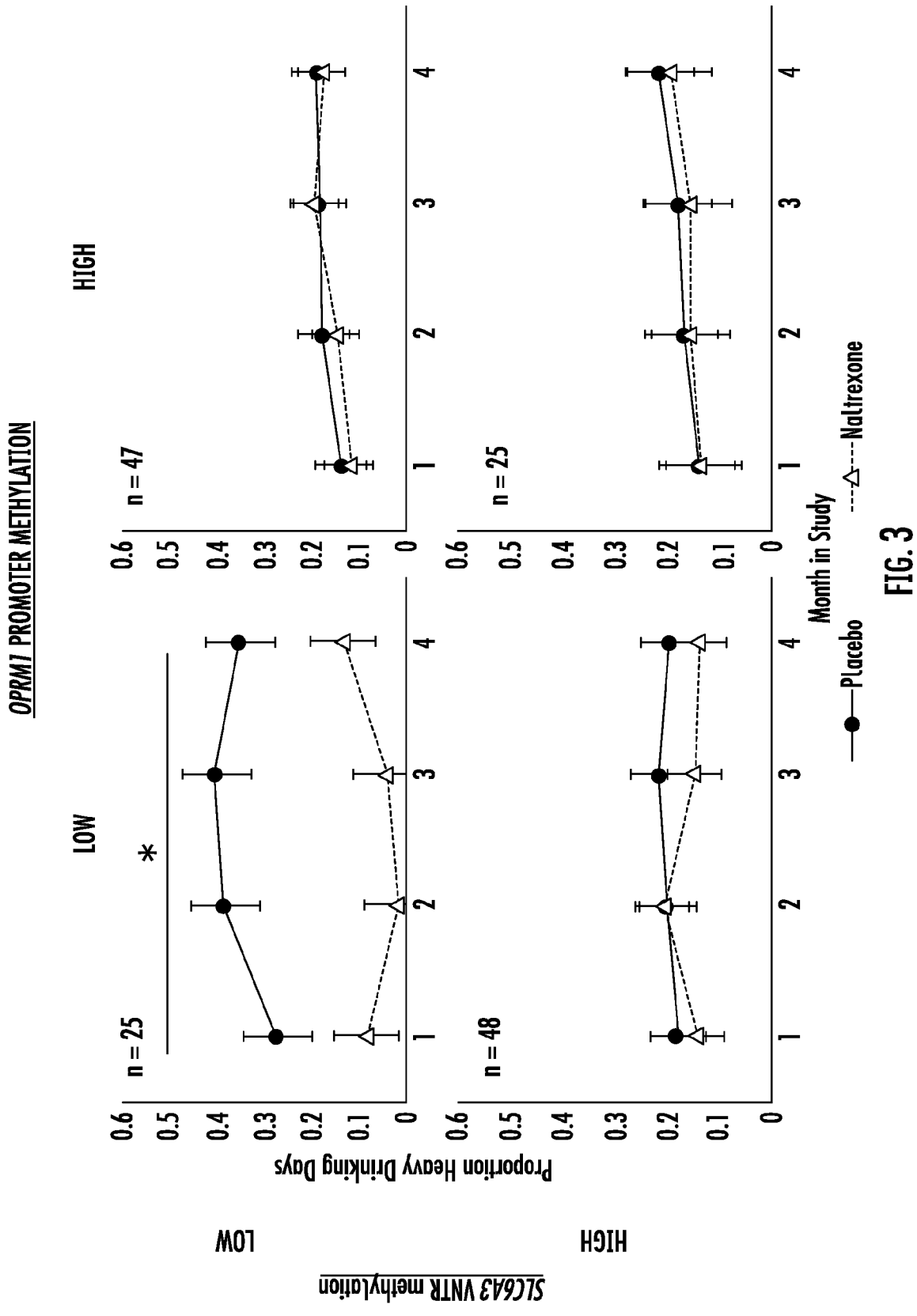
OPRM1 PROMOTER METHYLATION
LOW
HIGH
n = 25
n = 47
n = 48
n = 25
*
Proportion Heavy Drinking Days
0.6
0.5
0.4
0.3
0.2
0.1
0
1
2
3
4
Month in Study
Placebo
Naltrexone
SLC6A3 VNTR methylation
LOW
HIGH

FIG. 3

OPRM1 Promoter

5′ –
ttgcccagtgaagagacctactccttggatcg[1]ctttgcg[2]caaaatccacccctttttccct
cctccctcccttccagcctccgaatcccgcatggcccacgctcccctcctgcagcggtgcgg
ggcaggtGATGAGCCTCTGTGAACTACTAAGGTGGGAGGGGGCTATACG[3]CAGAGGAGAATGT
CAGATGCTCAGCTCGGTCCCCTCCG[4]CCTGACG[5]CTCCTCTCTGTCTCAGCCAGGACTGGTTT
CTGTAAGAAACAGCAGGAGCTGTGGCAGCG[6]GCG[7]AAAGGAAGCGGCTGAGGCGCTTGGAACC
CGAAAAGTCTCGGTGCTCCTGGCTACCTCGCACAGCGGTGCCCGCCCGGCCG[8]TCAGTACCAT
GGACAGCAGCGCTGCCCCCACGAACGCCAGCAATTGCACTGATGCCTTGGCG[9]TACTCAAGTT
GCTCCCCAGCACCCAGCCCCGGTTCCTGGGTCAACTTGTCCCACTTAGATGGCAACCTGTCC
GACCCATGCGGTCCGAACCGCACCGACCTGGGCGGGAGAGACAGCCTGTGCCCTCCGACCGG
CAGTCCCTCCATGATCACGGCCATCACG[10]ATCATGGCCCTCTACTCCATCGTGTGCGTGGTG
GGGCTCTTCGGAAACTTCCTGGTCATGTATGTGATTGTCAG-3′

FIG. 4A

CpG site #	Illumina ID	Chromosomal position	Gene feature	Mean methylation (SD)
1	cg22370006	154360344	TSS200	0.142 (0.049)
2	cg14262937	154360351	TSS200	0.156 (0.049)
3	cg06649410	154360483	5′ UTR	0.222 (0.036)
4	cg23143142	154360521	5′ UTR	0.083 (0.015)
5	cg23706388	154360528	5′ UTR	0.067 (0.014)
6	cg05215925	154360587	5′ UTR	0.147 (0.021)
7	cg14348757	154360590	5′ UTR	0.157 (0.035)
8	cg12838303	154360670	5′ UTR	0.126 (0.027)
9	cg22719623	154360732	1st exon	0.488 (0.027)
10	cg15085086	154360894	1st exon	0.128 (0.069)

FIG. 4B

SLC6A3 Promoter

5′ –
tgcctccccagtagctgggactacaggtgtgggccaccatgcctggctaatttttttttcaaagtcagggttttgccatgtggcccaggctggtctcaaactcctggcc
ct
caagagatcctccttcctcggcctcccagagtgctgggattacaagcgtgagccctcactcc
tggcctgtgtatttttaatatacctgaacatccattctctctgtgtgttttatttaacagcc
tcccttagtcacctgcaaagtcttttccttgggagactgtttcctcaaccctgctgctctgg
ggccaagccctggctcactccttttttattgaaacctgtgccatggagataatagggggtagag
agatcccttctgtggcagccactgacacactacagcttcgaggtggcacatccccctctcct
gaagtcccctcacctccctggcgatgaagtcccacccctgatgggaggtggtgtcaggaggc
cttcaggtggtcaggccaggagggctccaccctgaggaatgggaccagtgccctcataaaac
agaccccggagagctctccccagcccctagcg[1]tggggagatacagggagagaactgtctgca
accccgaagcggccctcaccagacacagagtcggccaggccttggcctcgggacaccggaac
cgttagaactgaaggcttctgtgtgagcccccaggctgtggagtttttgtcatggcagccc
caggggtcactaggctcccacttgattccaactcagcgtgaagtcacagccctgagtgcct
tctgcctgggtgccagccccggagccggggagcggggggagcgggggcggggagggggagtgg
tggtgtgcggggagtgcggggcgggcgcaggggtggggcaccgcgctgcgggcgggtactg
cggagtcaggcaccaagggtccctgcctccctcactgctgagcgcgggctgcaggctggaat
ggctggagagccccagggctcgcctggacgcccagggcagggtgctcacgggagcatcgagg
gtacacgggggaggaacgccggggttcgggcgaccctaggggcgacgcacagagctgggcg[2]
gccactcacctcggtgccttctaaggacctggacatcctgggccttggcggcctgggggctc
cattcctccgcgcgctgaatggaagaaatcccgcccgggcatctcggaaggaaagcctcgga
gtccattcggccctggagccggataccaaccgccagggctttccaggcccgtcccgggaaat
ggttttcttaggcgagtgcgaggcgggcccctcggttccgatgcaggcg[3]cactagatgccgg
caaggcggggactaggcctaggggacctcggtcgcctcgaggtcgcggagaccccaaggcca
cggaaggacccgcgtctccgcagcccgcacgccgggaagcgtgcagagtcctcggcggggtc
ccgagcccgctggtcagagcgtggagcggcggggtgggagggacgtggtccccagagcgcgg
ggccaccgtaggggcccctgatggggagggagggaagggtcggcccgacggggtcccagcag
ttccccgcgcgcagccgctcggctccctccccgtccagctgggagccgccagccctgggcgt
ccgaagatagcgggtgcccggggcagcccccaggggtgcgggcgagggcgcagggcggccca
gacagttcccgcgtggaaggcgcccgtctagatccgcgacgtctcggaccccccaggccccg
caccccgtgtccgaggctccgggacgcgcaggacagtggagccgtggccgccgcttgctccc
agccatctgcgtccgggaggcgggggcgggggcgcggcccgggggaggtgaggaggaggagcc
aggacgcgagggcgaccccgtcggcgggagggcgggggcgggggcg[4]gaccctgtctactggata
agagcccg[5]aggccg[6]aggctGAGACCG[7]CCCAGCG[8]CTGCGGAGCGGGAGGGGAGGCTTCGCG
GAACGCTCTCGGCGCCAGGACTCGCGTGCAAAGCCCAGGCCCGGGCGGCCAGgtgaggccag
cgtcgctcgcggcatcggggcgccccgctccttccgcagaccccgaagtggggcgcaggggc
ggggggccggggaccgggcacagtctggggtccccgcg[9]tcccgcagaccgcgccgtctccaaa
gtcgccaacagtcgcgggtgccgagcgccccccgatagcgccacatgggaccctgaggccgt
ccgaggcgcgag-3′

FIG. 5A

SLC6A3 3′ UTR

5′ –

AGGAGCGTGTCCTATCCCCGGACGCATGCAGGGCCCCCACAGGAGCG[10]TGTCCTATCCCCGG
ACGCATGCAGGGCCCCCACAGGAGCATGTCCTATCCCTGGACGCATGCAGGGCCCCCACAGG
AGCGTGTACGACCCCAGAACGCATGCAGGGCCCCCACAGGAGCG[11]TGTACTACCCCAGGACG
CATGCAGGGCCCCCACTGGAGCGTGTACTACCCCAGGACGCATGCAGGGCCCCCACAGGAGC
GTGTCCTATCCCCGGACCGGACGCATGCAGGGCCCCCACAGGAGCGTGTACTACCCCAGGAC
GCATGCAGGGCCCCCACAGGAGCGTGTACTACCCCAGGATGCATGCAGGGCCCCCACAGGAG
CGTGTACTACCCCAGGACGCATGCAGGGCCCCCAT-3′

FIG. 5B

CpG site #	Illumina ID	Chromosomal position	Gene feature	Mean methylation (SD)
Promoter				
1	cg16180821	1446969	TSS1500	0.651 (0.065)
2	cg13202751	1446443	TSS1500	0.242 (0.038)
3	cg14502484	1446208	TSS1500	0.086 (0.029)
4	cg05030481	1445593	TSS200	0.05 (0.012)
5	cg27037018	1445567	TSS200	0.03 (0.016)
6	cg04210284	1445561	TSS200	0.034 (0.005)
7	cg12882697	1445549	5′ UTR	0.089 (0.016)
8	cg04598517	1445542	5′ UTR	0.14 (0.026)
9	cg27348223	1445354	5′ UTR	0.036 (0.013)
3′ UTR				
10	cg15600751	1394054	3′ UTR	0.648 (0.039)
11	cg16392193	1393934	3′ UTR	0.591 (0.04)

FIG. 5C

COMT P2 PROMOTER

5′-
gacaaggcacccagccccagtttccccacctgggaaggggggctacttgtggctagaagcagcccg[1]gactcctgagcaagactagaccaagaggccg[2]gtatg
t
ggacacccccg[3]cgtgggcacccccacggggacaccctggccaccgccgcgcggacaccctcacgaggacaccccggccgccg[4]cggacacctaccgcgg
ggacg[5]ccccgaccccatcctacctgctgcgccccgcgccgcgccccgcaccccg[6]cccgccacggcctgcgtccg[7]ccaccggaagcgccctcctAATC
CCCGCAGCG[8]CCACCGCCATTGCCGCCATCGTCGTGGGGCTTCTGGGGCAGCTAGGGCTGCCCGCC
GCGCTGCCTGCGCCGGACCGGGGCGGGTCCAGTCCCGGGCGGGCCGTCGCGGGAGAG-3′

FIG. 6A

COMT P1 PROMOTER

5′-
aaggctggcatttctgaaccttgcccctctgcaaacacaaggggggcg[9]atggtggcactccaagcaaaggggcgtgtgggtgctgcaggaggaggcacagagca
c
tggcg[10]cccctcccctcccg[11]ccctgcagATGCCGGAGGCCCCGCCTCTGCTGTTGGCAGCTGTGTTGCTGGGCCT
GGTGCTGCTGGTGGTGCTGCTGCTGCTTCTGAGGCACTGGGGCTGGGGCCTGTGCCTTATCGGCTG
GAACG[12]AGTTCATCCTGCAGCCCATCCACAACCTGCTCATGGGTGACACCAAGGAGCAGCGCATC
CTGAACCACGTGCTGCAGCATGCGGAGCCCGGGAACGCACAGAGCGTGCTGGAGGCCATTGACAC
CTGAACCACGTGCTGCAGCATGCGGAGCCCGGGAACGCACAGAGCGTGCTGGAGGCCATTGACAC
CTACTGCGAGCAGAAGGAGTGGGCCATGAACGTGGGCGACAAGAAAG-3′

FIG. 6B

CpG site #	Illumina ID	Chromosomal position	Gene feature	Mean methylation (SD)
P2 promoter				
1	cg17810098	19929066	TSS200	0.035 (0.009)
2	cg23268677	19929087	TSS200	0.050 (0.013)
3	cg15834517	19929114	TSS200	0.055 (0.03)
4	cg24899205	19929184	TSS200	0.057 (0.013)
5	cg07019740	19929205	TSS200	0.034 (0.006)
6	cg11032634	19929254	TSS200	0.031 (0.026)
7	cg03205258	19929274	TSS200	0.028 (0.021)
8	cg12175949	19929286	1st Exon	0.061 (0.014)
P1 promoter				
9	cg06346307	19949965	5′ UTR	0.587 (0.045)
10	cg22546130	19950026	5′ UTR	0.546 (0.046)
11	cg23601416	19950040	5′ UTR	0.54 (0.042)
12	cg01335087	19950166	Body, 5′ UTR	0.862 (0.074)

FIG. 6C