Pharmacogenetically Driven Treatments for Alcoholism

Are We There Yet?

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Abstract

Pharmacogenetic analyses of treatments for alcohol dependence attempt to predict treatment response and side-effect risk for specific medications. We review the literature on pharmacogenetics relevant to alcohol dependence treatment, and describe state-of-the-art methods of pharmacogenetic research in this area. Two main pharmacogenetic study designs predominate: challenge studies and treatment-trial analyses. Medications studied include US FDA-approved naltrexone and acamprosate, both indicated for treating alcohol dependence, as well as several investigational (and off-label) treatments such as sertraline, olanzapine and ondansetron. The best-studied functional genetic variant relevant to alcoholism treatment is rs1799971, a single-nucleotide polymorphism in exon 1 of the *OPRM1* gene that encodes the μ -opioid receptor. Evidence from clinical trials suggests that the presence of the variant G allele of rs1799971 may predict better treatment response to opioid receptor antagonists such as naltrexone. Evidence from clinical trials also suggests that several medications interact pharmacogenetically with variation in genes that encode proteins involved in dopaminergic and serotonergic neurotransmission. Variation in the *DRD4* gene, which encodes the dopamine D₄ receptor, may predict better response to naltrexone and olanzapine. A polymorphism in the serotonin transporter gene *SLC6A4* promoter region appears related to differential treatment response to sertraline depending on the subject's age of onset of alcoholism. Genetic variation in *SLC6A4* may also be associated with better treatment response to ondansetron. Initial pharmacogenetic efforts in alcohol research have identified functional variants with potential clinical utility, but more research is needed to further elucidate the mechanism of these pharmacogenetic interactions and their moderators in order to translate them into clinical practice.

1. Background

Pharmacogenetics is the study of genetic variation and how it produces individual differences in medication response, particularly pharmacokinetic and pharmacodynamic differences.^[1,2] Although

the theoretical scope of pharmacogenetics includes all interactions between genetic variants and drugs regardless of their therapeutic value, there has been increased interest recently in identifying interactions that can be incorporated practically into individualized, targeted, better tolerated and

more effective pharmacological treatments. The explosion of research in medical genetics has spawned attempts across all fields of medicine to examine and scientifically explain the frequently observed differences in treatment effects and adverse reactions by attributing them to genetic variation. While these attempts have garnered attention and generated excitement, critics have also pointed out many limitations to the approach, and some have questioned pharmacogenetics' promise.^[3] In contrast, demonstrated applications in areas as diverse as warfarin treatment and asthma therapy have highlighted the potential for pharmacogenetics to change medical practice through the development and application of genetically informed, personalized treatments that optimize outcomes and limit side effects.^[4] In this paper we review the literature on pharmacogenetics relevant to the treatment of alcohol dependence, and describe state-of-the-art methods of pharmacogenetic research in this area.

There are currently three FDA-approved medications for the treatment of alcohol dependence: naltrexone (both oral and depot), acamprostate and disulfiram. A fourth drug, topiramate, has shown compelling evidence of efficacy in two randomized controlled trials and frequently is used 'off-label' for alcohol dependence.^[5,6] However, many patients do not respond to these medications, and side effects often limit usefulness.^[7,8] Efforts to try to predict treatment response and side-effect risk of specific medications for alcohol dependence treatment with pharmacogenetic analyses have begun. The opioid receptor antagonist naltrexone is the medication most studied in pharmacogenetic analyses of alcohol treatment trial data. Nalmefene, another opioid receptor antagonist that is similar to naltrexone but longer acting, is being developed for the treatment of alcohol dependence in Europe. Some medications, sertraline, ondansetron and olanzapine, have not demonstrated robust efficacy, but appear to have pharmacogenetic interactions that may explain variations in treatment response.^[7,9-11]

Two main pharmacogenetic study designs predominate in alcoholism research: analyses of medication treatment trials in outpatients, and clinical laboratory drug and alcohol administra-

tion studies (referred to as 'challenge' studies). The latter administer alcohol with or without medication to subjects with or without an alcohol use disorder (abuse or dependence) in controlled laboratory settings while measuring subjective, cognitive, behavioural and electrophysiological effects. In pharmacogenetic analyses of clinical treatment trials, published analyses run the gamut from *post hoc* pooled samples to *a priori* planned prospective analyses. We will examine both study designs, starting with challenge studies, and review major findings by medication and by the neurotransmitter systems upon which they act.

2. Pharmacogenetic Human Clinical Laboratory Studies

Pharmacogenetic challenge studies usually administer oral or intravenous (IV) alcohol to non-treatment seekers (typically either healthy social drinkers or heavy drinkers), often co-administering a pharmaceutical of interest. These studies seek to identify important differences in the subjective and physiological response to alcohol and to medications that comprise *endophenotypes* (also known as 'translational' or intermediate phenotypes), which are defined more narrowly than clinical behavioural phenotypes (such as 'alcohol dependence').^[12,13] Heritable biological endophenotypes may be more closely linked to underlying genetic variation than full clinical phenotypes. Challenge studies can help identify functional genetic variants with potential utility in treatment. Such studies also provide information critical to understanding the mechanism of action of pharmacogenetic interactions. A complete review of all the laboratory studies investigating the pharmacogenetics of alcohol response (i.e. to ethanol alone, or specific medications alone) is beyond the scope of this article, so we will focus instead on pharmacogenetic studies of the effects of medication co-administered with alcohol.

2.1 Opioidergic Agents

The endogenous opioid system, especially the μ -opioid receptor, plays a critical role in alcohol dependence pathophysiology, and is thought to

alter mesolimbic dopaminergic neurotransmission and ultimately dopamine release in the nucleus accumbens.^[14,15] The most carefully examined opioidergic gene-related polymorphism is the A118G (a.k.a. Asn⁴⁰Asp, or rs1799971¹), a single-nucleotide polymorphism (SNP) in exon 1 of the *OPRM1* gene that encodes the μ -opioid receptor and leads to a nonsynonymous substitution of aspartate for asparagine in the amino terminus of the protein. Functional² consequences of the G allele of rs1799971 (hereafter referred to as the G allele) include altered response to alcohol and ligands, changes in baseline receptor levels and altered induction of downstream second-messenger molecules. Much evidence supports the hypothesis that the G allele is a functional polymorphism with complex physiological effects in humans, despite the fact that it may not contribute to the risk for developing substance dependence, at least for opioid, alcohol or cocaine dependence.^[16-18] A more recent meta-analysis examining association studies of rs1799971 with alcohol dependence analyzed Asian and Caucasian samples independently and found the G allele to be significantly associated with alcohol dependence in Asians but not Caucasians.^[19] A series of elegant *in vitro* studies has demonstrated that the rs1799971 G allele has functional consequences that affect the expression, as well as the binding and potency of the altered receptor (see Krosiak et al.^[18] for a review of molecular findings and Zhang et al.^[20] for a review of post-mortem findings). Positron emission tomography (PET) of acute alcohol administration effects has shown increased dopamine release in the ventral

striatum of men with the G allele, but not in those homozygous for the A allele, and similar results are seen in a 'humanized' mouse model with a cloned *OPRM1* gene substituted for the mouse μ -opioid-receptor gene.^[21]

In alcohol challenge studies, heavy drinkers with the G allele experienced a more intense 'high' from IV alcohol as well as greater subjective intoxication, stimulation, sedation and happiness.^[22] They also were more likely to report a family history of alcoholism and higher levels of craving when exposed to an alcohol stimulus than were A-allele homozygotes. However, cue-induced craving was paradoxically increased in G-allele carriers who were pretreated with naltrexone, but did not change craving in A-allele homozygotes.^[23] In Caucasians (European Americans) with the G allele, naltrexone produces a significantly greater blunting of subjective 'high' (stimulation, positive mood, craving, enjoyment) from alcohol co-administration than it does in those with the A allele.^[24] The G allele also alters neuroendocrine response to naloxone and alcohol co-administration,^[25,26] but may have functional effects that are specific to individuals of European ancestry. In a human challenge study examining cortisol response after an IV naloxone challenge (without alcohol co-administration), only European-ancestry subjects with the G allele showed significantly elevated cortisol levels.^[27] In another naloxone challenge, a sample of mostly Caucasian subjects with the G allele had a greater cortisol response to the naloxone challenge, but a blunted cortisol response to stress, compared with those homozygous for the A allele.^[28]

1 Historical names (A118G, Asn⁴⁰Asp) have been used commonly in the published literature. The term 'A118G' refers to the position of the variant nucleic acid in the exon, and 'Asn⁴⁰Asp' refers to the position of the nonsynonymous change substitution in the resultant peptide chain. We have chosen to identify this SNP by the reference sequence number (*rs*), corresponding with the system used by the National Center for Biotechnology Information (NCBI) in their database of single-nucleotide polymorphisms, dbSNP.

2 A functional polymorphism is one that alters gene function at any level. Some polymorphisms may have more profound physiological effects than others. Identification and verification of functional polymorphisms is the first step towards identifying genetic variants that are pharmacogenetically active. Sometimes, however, polymorphisms chosen based on an association with a related phenotype such as disease status and risk or via a haplotype-based analysis are not actually functional, but simply in linkage disequilibrium with the actual functional polymorphism responsible for the pharmacogenetic interaction (i.e. closely associated with the real functional polymorphism without actually being a functional polymorphism). Consequently, it is necessary to verify suspected functionality through molecular and challenge studies.

A more recent study examined the pharmacogenetics of naltrexone-modulated subjective response to IV alcohol administration, as well as cortisol and adrenocorticotrophic hormone (ACTH) response.^[29] Non-treatment-seeking, heavy-drinking East Asian Americans (of Korean, Chinese and Japanese ancestry) were given either naltrexone (25 mg for 2 days, then 50 mg for 2 days) or matching placebo then administered IV alcohol in a double-blind crossover study. Cortisol and ACTH levels were measured at three different levels of breath alcohol. Significant genotype-by-medication interactions between naltrexone and the rs1799971 G allele existed for measures of subjective intoxication and craving. Subjects with the G allele experienced more intoxication, greater sedation and greater reduction in craving with naltrexone and alcohol. Consistent with Hernandez-Avila et al.,^[27] they did not find a pharmacogenetic effect on cortisol or ACTH levels in East Asian Americans. The results suggest that the pharmacogenetic interaction of the G allele and naltrexone in Asian populations is somewhat different than in Caucasians; rather than causing a greater attenuation of the pleasurable or euphoric effects of alcohol,^[24] the allele increases the less desirable feelings and the less desirable sedation, and also does not activate the neuroendocrine stress response as has been observed in previous studies of European Americans. A previous study noted an increase in cue-elicited craving in a sample of mostly Caucasians with the G allele with naltrexone administration, which contrasts with the findings of Ray et al.^[24] in East Asian Americans.

In summary, Caucasians with the G allele of rs1799971 experience greater intoxication and pleasure from alcohol, increased activation and dopaminergic neurotransmission in the ventral striatum, increased cue-elicited craving and an altered neuroendocrine response. In this group, naltrexone likely produces greater attenuation of the endophenotype-related increases in pleasurable experiences and may help normalize neuroendocrine response to alcohol (see Ray^[30] and Ray et al.,^[31,32] for a more detailed review). In Asians with the G allele of rs1799971, the pharmacogenetic response to naltrexone may be dif-

ferent, characterized by greater intoxication, greater sedation and greater reduction in craving from alcohol, which in theory could possibly increase satisfaction with low doses of alcohol and/or aversively deter heavier drinking.^[29]

2.2 Serotonergic Agents

Kenna and colleagues^[33] studied the serotonin 5-HT₃ receptor antagonist ondansetron and the selective serotonin reuptake inhibitor (SSRI) sertraline in a small (n=21), within-subjects, laboratory alcohol self-administration study in non-treatment-seeking, alcohol-dependent subjects. Subjects completed three double-blind, self-administration laboratory sessions; each after receiving 3 weeks of one of the medications (ondansetron 0.25 mg twice daily, sertraline titrated to 200 mg daily or placebo). When subjects were genotyped for the bi-allelic form of the 5HTTLPR *SLC6A4* polymorphism (see section 3.2), a significant genotype-by-medication group effect was found. At the first laboratory session, subjects homozygous for the long (L) allele treated with ondansetron self-administered less alcohol in the laboratory and also drank less during the week before the laboratory session, compared with subjects homozygous for the L allele taking sertraline. Subjects homozygous for the L allele taking sertraline drank more during the week before the first laboratory session compared with baseline and other timepoints. Eighty-six percent of subjects homozygous for the L allele had early-onset alcoholism. A significant order effect for medications may have obscured other findings, and the small sample size is a limitation.

2.3 Dopaminergic Agents

The dopamine D₄ receptor gene has a variable number of tandem repeats (VNTR) polymorphism in exon 3, with the most common variants being two, four and seven repeats,^[34] the last of which has functional consequences.^[35-37] Olanzapine, a D₂/D₄ receptor blocker, has been tested in a laboratory challenge paradigm by Hutchison and colleagues^[38] to see whether it reduces craving for alcohol and whether the *DRD4* VNTR polymorphism moderates this effect. Olanzapine

reduced cue-elicited craving for alcohol, and craving after consumption of alcohol. Subjects with the seven-repeat *DRD4* variant showed significantly higher craving for alcohol after consuming alcohol than they did after consuming a non-alcoholic beverage; those with the shorter repeats did not.^[39] In a separate study examining for an olanzapine-*DRD4* VNTR interaction, olanzapine reduced craving at baseline regardless of genotype, but reduced craving after cue exposure and alcohol consumption only for carriers of the long variant (i.e. seven repeats or more) of *DRD4*.^[40]

2.4 GABAergic/Glutamatergic Agents

GABA_A receptors mediate several behavioural effects of alcohol.^[41-43] Variation in the gene encoding the GABA receptor α -2 subunit (*GABRA2*), which mediates the anxiolytic effects of benzodiazepines,^[44] may be associated with alcohol dependence.^[45] *GABRA2* SNPs have also been associated with variation in subjective alcohol response.^[46,47] One oral alcohol administration laboratory study examined whether *GABRA2* alleles and finasteride administration affected subjective response to alcohol on the ascending and descending limb of the alcohol intoxication curve, as measured with the Biphasic Alcohol Effects Scale (BAES) and other alcohol subjective response scales.^[48,49] Finasteride is a 5- α steroid reductase inhibitor that limits conversion of progesterone to the neuroactive steroids allopregnanollone and tetrahydrodeoxycorticosterone, which act as positive allosteric modulators of the GABA_A receptor. Subjects homozygous for the A allele of *GABRA2* reported greater stimulant and gastrointestinal subjective effects of alcohol than those with the G allele, which is over-represented in the alcohol-dependent^[50] and finasteride-attenuated stimulant and anaesthetic effects of alcohol in the A homozygotes alone.

Glutamate antagonists such as topiramate have received empirical support for the treatment of alcohol dependence.^[51] Topiramate has a number of effects, including blockage of voltage-gated sodium channels, inhibition of carbonic anhydrase, potentiation of neurotransmission at

a non-benzodiazepine site on GABA_A receptors and antagonism of AMPA and kainate glutamate receptors.^[7] The combination of GABA facilitation (which inhibits mesocortical dopamine release) and glutamate antagonism is thought to explain the effects of topiramate of decreasing craving,^[5] ameliorating withdrawal symptoms^[52] and blunting alcohol response.^[53] Unfortunately, side effects of topiramate frequently contribute to high rates of discontinuation in clinical trials and limit its usefulness in clinical practice.^[6]

One attempt to explain these side effects of topiramate through pharmacogenetic analysis in a challenge study of topiramate examined three SNPs of the glutamate receptor subtype 5 (*GluR5*) gene (*GRIK1*) for their relationship to side effects and alcohol response,^[54] chosen based on evidence of their association with alcohol dependence.^[55] Topiramate did not reduce drinking in subjects with any of the three SNPs during the 5-week medication titration, but rs2832497, a SNP located in exon 9 of *GRIK1*, was associated with more severe topiramate-related side effects even when controlling for serum levels, suggesting a possible pharmacodynamic-pharmacogenetic interaction. However, the results (reported as $p < 0.05$) were not corrected for multiple comparisons.

3. Clinical Pharmacogenetic Treatment Trials

3.1 Opioid Antagonists

One of the first pharmacogenetic analyses of alcohol treatment trials was a retrospective pooled analysis of available samples from three studies examining the interaction of the rs1799971 G allele with medication.^[56] One study compared naltrexone 50 mg daily with placebo, while the other two trials compared naltrexone 100 mg daily with placebo; administered for varying periods, between 11 weeks and 9 months. All three studies measured outcomes of relapse to heavy drinking, a survival analysis of time to relapse to heavy drinking and the interaction of the rs1799971 genotype by medication group. Naltrexone improved treatment outcomes compared with placebo in the pooled sample. While the presence or

absence of the variant allele did not moderate the effect of naltrexone on relapse to heavy drinking (when tested as an interaction between genotype and medication group for all subjects) or abstinence (no alcohol use at all), the survival analysis found a significantly longer time to relapse in naltrexone-treated subjects carrying the minor³ G allele (Wald=4.22, 1 degree of freedom, odds ratio [OR]=2.79 [95% CI 1.05, 7.41], $p=0.040$), and a greater proportion of naltrexone-treated subjects carrying the minor G allele who did not relapse to heavy drinking (when tested only within the naltrexone-treated subjects; Wald=4.05, 1 degree of freedom, OR=3.52 [95% CI 1.03, 11.96], $p=0.044$).

Gelernter et al.^[57] looked for pharmacogenetic effects of several opioid-receptor gene polymorphisms (including rs1799971, see table I for all SNPs examined) in an analysis of a large subgroup of subjects from the VA Cooperative Study of naltrexone (vs placebo) for the treatment of alcohol dependence in treatment-seeking subjects.^[66] In this subset, naltrexone was significantly better than placebo for preventing relapse to any heavy drinking (the dichotomous main treatment outcome), but no opioid-receptor polymorphism moderated the effect of naltrexone on relapse rates. That a secondary survival analysis and a general linear model of other continuous drinking outcome variables were also negative suggests validity of these negative results.

The COMBINE (Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence) study was a large, randomized, placebo-controlled, multi-site clinical trial that examined the use of naltrexone, acamprosate or both versus placebo, combined with either medical management or a behavioural intervention based on adherence to treatment goals and medications, with or without a specialized behavioural intervention (combined behavioural intervention [CBI], which is based on motivational enhance-

ment and cognitive-behavioural techniques).^[67] In a prospective planned analysis of a subset of participants in the COMBINE study who were treated with naltrexone in conjunction with medical management (MM) with or without CBI, no significant genotypic moderation of the effect of naltrexone was observed in the aggregate group (naltrexone vs placebo). However, when the group of subjects receiving the concomitant CBI was removed, the presence of the G allele decreased heavy drinking days ($p<0.04$) and non-significantly increased abstinent days ($p=0.07$, although pairwise comparison between groups was significant; $p<0.01$ to 0.03). A higher percentage of subjects with abstinence or a controlled, non-risky drinking pattern, and low ratings of alcohol-related consequences also had the G allele ($p=0.005$, OR=5.75, 95% CI 1.88, 17.54).^[58,59]

A pharmacogenetic analysis of a large clinical trial of nalmefene (only used by the subjects on days when drinking seemed imminent rather than daily) for treating alcohol dependence examined a subset of subjects from the original study population who were willing to provide a DNA sample for interactions with opioid-receptor gene variants (see table I for specific SNPs^[60,68]). In this randomized, double-blind, placebo-controlled trial, subjects received nalmefene and brief therapy (BRENDA⁴) for 7 months, with the primary outcome measure being heavy drinking days per week analyzed with a longitudinal hierarchical multiple-regression model. Despite a significant effect of nalmefene on drinking outcomes in the pharmacogenetic subset, no SNP had any significant interaction with medication. In this study, subjects were instructed to use nalmefene in a 'targeted' fashion, meaning that they should take it a couple of hours before times when they were at high risk for drinking, instead of taking it every day at the same time. Subjects in the nalmefene group took nalmefene on average 35% of study days, which is reasonable for targeted treatment.

³ The variant 'minor' G allele of rs1799971 is less frequent than the A allele. However, its frequency varies by population, and approaches 50% in some Asian populations.^[16]

⁴ Biopsychosocial evaluation, Report to the patient on assessment, Empathic understanding of the patient's situation, Needs collaboratively identified by the patient and treatment provider, Direct advice to the patient on how to meet those needs, Assess reaction of the patient to advice and adjust as necessary for best care.

Table 1. Major findings from pharmacogenetic analyses of treatment trials for alcohol dependence

Author, year	N ^a	Population	Medications (daily dose)	Length of exposure	Genetic variants examined	Population ancestry ^b	Psychosocial treatments	Outcome measures	Pharmacogenetic findings
Anton et al., ^[58] 2008	604 (307) ^c	AD	Naltrexone 100 mg	16 weeks	rs1799971 (haplotype analysis by Oroszi et al., ^[59] 2009)	EA	CBI: up to 20 sessions, MM: 9 sessions	% heavy-drinking days, abstinent days, 'good clinical outcome'	Positive pharmacogenetic effect on % heavy-drinking days and abstinent days, good clinical outcome, but only when subgroup of 297 subjects receiving CBI therapy was removed
Arias et al., ^[60] 2008	272	AD	Nalmefene 20 mg	7 months	(OPRM1) rs1799971, rs648893 (OPRD1) rs1042114, rs2234918, rs678849 (OPRK1) rs963549	Finnish Caucasian	BRENDA: 9 sessions	Heavy-drinking days/week, abstinent days/week	Negative
Coller et al., ^[61] 2011	100	AD	Naltrexone 50 mg	12 weeks	(OPRM1) rs1799971	Australian Caucasian	CBT: 6 sessions	Drinks/week (grams of ethyl alcohol)	Negative, but no placebo control
Geleinter et al., ^[57] 2007	213	AD	Naltrexone 50 mg	13 weeks	(OPRM1) rs1799971, rs17180961 rs648893 (OPRD1) rs1042114, rs2234918, rs678849 (OPRK1) rs963549	EA and AA	Varied; individual counselling and 12-step facilitation	Rate of relapse to heavy drinking	Negative. Subjects also received 12-step and other psychosocial interventions
Hutchison et al., ^[11] 2006	64	AD	Olanzapine 5 mg	12 weeks	DRD4 VNTR	Mostly Caucasian	2 brief therapy sessions	Drinks total, drinks/drinking day	Positive; long variant carriers drank fewer drinks per drinking day and fewer drinks total when taking olanzapine
Johnson et al., ^[9] 2011	283	AD	Ondansetron 8 µg/kg	11 weeks	LL/LS/SS (5-HTTLPR) rs1042173 (5-HTTLPR) SLC6A4	84.8% W, 15.2% H	CBT: 11 sessions (weekly)	Drinks/drinking day, % days abstinent	Positive for subjects homozygous for the long (L) allele of rs1042173. Those with a concomitant TT genotype had increased treatment response

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Table 1. Contd

Author, year	N ^a	Population	Medications (daily dose)	Length of exposure	Genetic variants examined	Population ancestry ^b	Psychosocial treatments	Outcome measures	Pharmacogenetic findings
Kim et al., [62] 2009	32	AD	Naltrexone 50mg	12 weeks	(OPRM1) rs1799971	Korean Asian	CBT: 4 sessions	Relapse to heavy drinking, survival analysis	Positive for genotype; however, no placebo control and only 32 subjects included due to lack of adherence
Kranzler et al., [10] 2011	134	AD	Sertraline 200 mg	12 week	5-HTTLPR tri-allelic SLC6A4	EA	CBT: 9 sessions	Heavy-drinking days/week, drinking days/week	Positive for genotype by medication by age of onset of alcoholism
Mitchell et al., [63] 2007	25	HD, NTS	Naltrexone 50mg	1 week	(OPRM1) rs1799971	EA	None	Drinks/week	Negative
Ooteman et al., [64] 2009	52: naltrexone; 56: acamprosate	AD	Naltrexone 50mg Acamprosate 1.3 g/2.0 g	21 days	A+118G (OPRM1) D2+1403D1 (DRD1) Taql A1/A2 (DRD2) C2664T (GRIN2B) T+1519C (GABRA6) C+1412T (GABRA2) G+3145A (GABRA2) (GABRAB2)	Dutch (not given)	None	VAS, craving, relapse	Negative, but no placebo control
Oslin et al., [56] 2003	130	AD	Naltrexone 50mg, 100 mg	Varied, 11 weeks up to 9 months	(OPRM1) rs1799971	EA	MM, BRENDA, CBT	Relapse to heavy drinking, survival analysis for heavy drinking	Pooled analysis of three treatment trials. Positive for G allele with longer heavy drinking
Tidey et al., [65] 2008	115-DRD4, 107-OPRM1	HD, NTS (63% AD)	Naltrexone 50mg	3 weeks	(OPRM1) rs1799971 DRD4 VNTR	Mostly EA	None	% heavy-drinking days, cue-elicited craving, subjective measures	No effect for rs1799971 but the L allele of the DRD4 VNTR reduced heavy drinking days in the naltrexone group

a Number of genotyped subjects actually included in the analysis.

b Ancestry is listed here as it was reported in the corresponding study.

c After subjects who had received specialized behavioural therapy were removed, this remaining subset was used in the pharmacogenetic analysis.

AA = African American; AD = alcohol dependent; CBI = combined behavioural intervention; CBT = cognitive behavioural therapy; EA = European American; H = Hispanic; HD = heavy drinkers; MM = medical management; NTS = nontreatment seeking; VAS = Visual Analog Scale; VNTR = variable number of tandem repeats; W = White.

However, this strategy may have limited observable pharmacogenetic effects. Subjects were not required to commit to any specific drinking goal (e.g. abstinence, cutting down or controlled drinking). While the amount of therapy provided was not extensive and is not as specific as cognitive behavioural therapy (CBT), it is possible that the nine sessions of BRENDA therapy could have interfered with finding a significant pharmacogenetic effect via a 'ceiling effect'. A similar explanation was proposed by Anton et al.,^[58] who found no effect when subjects who received the CBI were included in the analysis.

An open-label study examined the rs179971 G-allele effects in Korean Asian subjects who took naltrexone 50 mg daily concurrent with CBT.^[62] Although the rates of relapse to heavy drinking between groups was non-significant, a trend showed subjects with the A allele were about 10.6 times more likely to relapse to heavy drinking ($p=0.072$). Survival analysis showed that subjects with at least one G allele took significantly longer to relapse to heavy drinking than those without; a harm reduction of 13.6 ($p=0.014$). The trial was limited by lack of a placebo group.

A brief double-blind, placebo-controlled, counterbalanced, crossover trial of naltrexone versus placebo in non-treatment-seeking heavy social drinkers gave 1 week of naltrexone 50 mg daily or placebo to each subject to assess the effect of the G allele on drinks per week.^[63] Naltrexone significantly reduced drinking, but there was no difference between subjects carrying the G or A alleles.

Another study examined the pharmacogenetics of naltrexone versus placebo in a 3-week, double-blind, randomized trial of non-treatment-seeking heavy drinkers.^[65] The G allele did not alter the effects of naltrexone on the percentage of heavy drinking days, but the L allele of the *DRD4* VNTR was associated with reduced heavy drinking days in the naltrexone group [$F(1, 94)=8.08$, $p<0.01$, for the interaction between naltrexone and genotype, and $F(1, 34)=10.20$, $p<0.01$, when examined within L allele carriers]. These results suggest that 'downstream' dopaminergic effects from genetic variants take precedence over opioid receptor gene variation in

naltrexone treatment. A positive family history of alcoholism did not moderate heavy drinking days, although in combination with naltrexone it did moderate the time to second drink. Prior studies have shown family history to be one of the best predictors of naltrexone response, so ultimately, for pharmacogenetics to be useful clinically in predicting response to medication, it must be a better predictor of alcoholism than family history.^[69,70] The study also included ecological momentary assessments (data on alcohol response in the natural environment, similar to a challenge study but not in the controlled laboratory setting), which showed no difference between genotypes on the effects of naltrexone on urge to drink or subjective intoxication. However, subjects with the G allele showed increased vigor and positive mood with drinking and drank more.^[71]

A recent Australian study, Collier et al.,^[61] examined the effect of rs179971 on treatment outcomes in a prospective uncontrolled trial of naltrexone plus CBT in Caucasian alcohol-dependent subjects, and found no difference between G-allele carriers and A-allele homozygotes. The lack of a placebo control makes it difficult to rule out the possibility of a false-negative study, as a specific medication effect was not isolated.

One relapse-prevention study tested the hypothesis that naltrexone response would be associated with allele variations in genes coding for dopaminergic and opioidergic proteins, and that acamprosate response would be associated with glutamatergic and GABAergic genes.^[64] 126 treatment-seeking and non-treatment-seeking Dutch alcoholics were randomly assigned to either naltrexone 50 mg daily or acamprosate 1.3–2.0 g daily and exposed to alcohol craving cues both before and after 21 days of medication, with alcohol craving measured by the Visual Analog Scale (VAS). The efficacy of acamprosate on craving reduction was enhanced depending on the C-allele frequency of the *GABRA6* gene, whereas the efficacy of naltrexone was enhanced depending on the frequency of the T allele. A1-allele homozygotes of the TaqIA *DRD2* polymorphism did better with acamprosate; those with the A2 allele did better with naltrexone. Heterozygotes with both alleles fared equally well

with both medications. G-allele carriers of the *OPRM1* polymorphism also did better with naltrexone, but this finding fell short of statistical significance. Different polymorphisms appeared to have an additive effect on craving. However, the authors did not correct for multiple comparisons, and used a significance cut-off of $p < 0.01$, therefore these results should be interpreted with caution. Treatment seekers were also not separated from non-treatment seekers.

In summary, only one large prospective, placebo-controlled, clinical-trial-based pharmacogenetic analysis supports a clinically significant interaction between a genetic variant (i.e. rs1799971, the G allele) and an opioid antagonist medication.^[58] These findings were significant only after a large number of genotyped subjects that also received a specialized behavioural intervention were removed, suggesting that this form of concomitant treatment can obscure pharmacogenetic effects in trials. In the face of the other negative studies examining rs1799971 and opioid antagonists, it seems premature to declare this variant a predictor of clinical response. The significant interaction between the L allele of the *DRD4* VNTR and naltrexone in the absence of a significant finding for rs1799971 is intriguing, and suggests that possibly, genetic variants at loci other than *OPRM1* might influence response to naltrexone, and should be investigated further as well as controlled for in opioid receptor antagonist trials.

3.2 Serotonergic Agents

The serotonin transporter gene (*SLC6A4*) has two abundant functional alleles within its promoter region (5-HTTLPR), designated L or short (S) on the basis of the number of copies of an imperfect repeat sequence contained within, also referred to as the 'bi-allelic' form of the polymorphism.^[72] This 44-base-pair repeat insertion polymorphism has been associated with a wide range of psychiatric disorders such as anxiety,^[73,74] major depression,^[75] obsessive-compulsive disorder,^[76] improved antidepressant response^[77] and alcohol dependence.^[78,79] Serotonin transporter availability does not differ between alco-

hol misusers and healthy normal subjects who carry the S allele; however, raphe serotonin transporters are significantly reduced in alcoholics homozygous for the L genotype.^[80] However, this may be a result of rather than a cause of chronic alcohol intake, as other studies have found no association between serotonin transporter genotype and alcohol dependence.^[81,82]

Another SNP in the 3'-untranslated region of *SLC6A4*, rs1042173 (T/G), is also associated with the severity of alcohol consumption,^[83] and the TT genotype may work synergistically with the LL genotype in the promoter region to increase its effects. Another SNP, rs25531 (an A → G nucleotide change), located specifically in the L repeat region in the 5-HTTLPR region of the *SLC6A4* gene, is thought also to be functional, with a 'tri-allelic form' of the minor G-allele variant (L_G) causing the L allele to function similar to the lower-activity S allele. Blockade of postsynaptic 5-HT₃ receptors with the serotonin-receptor antagonist ondansetron reduced drinks per drinking day and increased percentage of days abstinent more than placebo in alcohol-dependent subjects homozygous for the L allele, and those with a concomitant TT genotype had increased treatment response.^[9] Those without the LL or LL/TT genotype showed no benefit from ondansetron. This study is notable for its use of stratification by genotype at the 5-HTTLPR region as part of the urn randomization procedure, as well as using ancestry-informative markers to control for ancestry in the statistical analysis.

Kranzler et al.^[10] prospectively studied the moderation of sertraline treatment for alcohol dependence by genotype (the tri-allelic form of the 5-HTTLPR polymorphism) and age of onset of alcoholism (early or late) in a randomized placebo-controlled trial. Among the L' homozygotes (L' meaning the L_A , or higher expression allele), those with early-onset alcoholism had more drinking and heavy drinking days per week with sertraline compared with placebo, and those with late-onset alcoholism had significantly fewer drinking and heavy drinking days per week with sertraline compared with placebo. In subjects with at least one S allele or L_G allele, both early- and late-onset alcoholics changed little over time

with regard to drinking behaviour, with the late-onset group drinking slightly more on sertraline versus the placebo group at the end of treatment. These results suggest that the drinking of many subjects does not improve or even worsens when they take sertraline, but a subset of subjects (L' homozygotes with late-onset alcoholism) may show substantial improvement. For L' homozygotes, the beneficial effect of sertraline on drinking days persisted 3 months after treatment for those with late-onset alcoholism.^[84] However, the increased drinking observed during treatment for L' homozygotes with early-onset alcoholism was no longer significant at this timepoint.^[84]

3.3 Dopaminergic Agents

In a 12-week clinical trial of olanzapine 5 mg daily, Hutchison and colleagues^[11] found craving was attenuated specifically in subjects with the seven-repeat (L) variant of *DRD4*, who also drank fewer drinks per drinking day and fewer drinks in total when taking olanzapine. Drinking remained unchanged in those with the shorter alleles.

4. Future Directions

4.1 Advances in Translational Genomics

Pharmacogenetic studies and candidate gene choice may be better informed in the future by genome-wide pharmacogenomic methods such as *genome-wide expression*, which measures the amount of expressed RNA in samples with high-throughput assay technology and has been used to predict antidepressant response.^[85-87] The effects of pharmaceuticals on gene expression can also be studied with gene expression techniques, although the choice of tissue sample affects results, and it is unlikely that human brain tissue samples will be obtained in any pharmacogenomic experiments. Post-mortem tissue bank samples are limited, and are confounded by factors related to phenotypic characterization and unknown exposures to various medications and drugs. The validity of using proxy tissue samples (e.g. blood cells) or cell lines to study and predict response to pharmaceuticals in alcoholism research has yet to be determined. Genome-wide

association methods (i.e. the use of high-throughput assays to study many genetic polymorphisms at loci across the genome) have been applied in smoking-cessation pharmacogenetics, but because thousands or even hundreds of thousands of SNPs are tested, they require a very large sample size (preferably >1000) to compensate for statistically required corrections for multiple comparisons, a size rarely seen in multisite alcohol-dependence treatment trials.^[88]

Pharmacoeugenomics is the study of epigenetic modifications that affect response to drug therapy. Epigenetic modifications can silence genes, reducing or eliminating the pharmacogenetic effects of functional variants and posing a threat to the validity of pharmacogenetic studies by obscuring functionality.^[89] Epigenetic therapies that affect DNA methylation, histone acetylation or both may be able to optimize pharmacogenetic interactions of medications such as naltrexone. Translational research in pharmacoeugenomics may improve our understanding of pharmacogenetic trial data, particularly for the *OPRM1* gene, which may be subject to addiction- and opioid-related pharmacoeugenetic interactions.^[90] As with expression studies, limitations of tissue specificity and uncertain correlations between observations in peripheral tissue and actual changes in the brain may apply to pharmacoeugenomics as well.

4.2 Potential Major Confounders in Pharmacogenetic Treatment Trials for Alcoholism

Some of the mixed results of initial pharmacogenetic treatment trials for alcoholism (particularly for rs1799971) may be due to variation in research methodologies. Five major problems potentially confound pharmacogenetic research efforts:

1. *Exposure of both medication and placebo groups to effective therapies such as CBT in clinical trials risks introducing a 'ceiling effect'.* This includes the CBI treatment used for some subjects in Anton et al.,^[67] the varied interventions in Krystal et al.^[66] and possibly the BRENDA therapy used by Karhuvaara et al.^[68] The medical

management therapy used in the COMBINE trial may be the minimal, and thus best, therapeutic interaction to use with subjects in pharmacogenetic studies in order to prevent this. Brief behavioural compliance enhancement therapy (BBCET) is similar to medical management and may be a reasonable alternative.^[6]

2. *Failure to control for genetic background and racial ancestry, or population substructure or both.* This will be discussed further below.

3. *Failure to analyze gene-gene interactions and epistatic influences,* which can silence genes and reduce observed pharmacogenetic effects.

4. *Lack of a placebo control,* which prevents isolation of a specific medication effect, makes it difficult to discern a pharmacogenetic effect. A negative main effect of the medication may obscure a positive pharmacogenetic finding.

5. *Failure to stratify across medication groups based on genotype or to enrich the sample for minor alleles or variants in order to optimize study power.* Power can be limited by the low naturally occurring frequency of variant alleles, but can be increased by oversampling for the allele of interest, thus reducing type-II error.

Other methodological questions include the choice of the best outcome measures, and whether subjective measures are sensitive enough to detect pharmacogenetic effects.^[57] Advances in alcohol consumption measurements such as 24-hour non-invasive monitoring and more sensitive quantitative biochemical markers could be helpful. A subject's stated treatment goal (e.g. abstinence vs cutting down) may also affect findings. Age of onset of alcoholism (early or late) interacts with sertraline response for alcohol dependence, and so should be investigated as a moderator in future trials. Two positive studies for naltrexone pharmacogenetic association with rs1799971 used higher daily doses of naltrexone than other negative studies (100 mg vs 50 mg), thus medication dose and inadequate exposure may have contributed to mixed findings seen in naltrexone pharmacogenetic trials. Only one published alcoholism treatment trial optimized power (reducing the possibility of type-II error) by utilizing stratification or enrichment (oversampling) based on genotype, although another stratified

trial is underway (Oslin,^[91] from www.clinicaltrials.gov [NCT00831272]).^[9] Large samples are required for pharmacogenetic studies, especially when examining gene-gene interactions.

Although several of the initial negative pharmacogenetic treatment trial studies reported above had large sample sizes for clinical trials, they may have been underpowered for finding a pharmacogenetic effect owing to the large number of uninformative subjects who did not have a variant allele.^[57] The effect of epigenetic status (i.e. impact of DNA methylation and histone acetylation on gene expression) on pharmacogenetic interactions relevant to alcoholism treatment as well as pharmacoeepigenetic effects of mediations to treat alcohol dependence remains unstudied. Several types of behavioural therapies were used across studies, including minimal medical management, BRENDA^[92] and CBT.

Given that pharmacogenetic interactions associate genotype with a complex phenotype (medication response), population stratification within the sample could confound results.^[93,94] Alcohol pharmacogenetic studies have typically used self-reported racial classifications, and more scientifically rigorous evaluation of subjects' ancestry is usually lacking. For example, it may be a reasonable assumption that the Finnish Caucasian sample from Arias et al.^[60] is an ancestrally homogeneous sample, but since recruitment was carried out across multiple sites in Finland, failure to measure ancestry scientifically makes the degree of ancestral homogeneity speculative. Samples recruited in the US using self-reported ancestry and racial identification are even more suspect, especially studies combining subjects under the classifications of 'European American' and 'East Asian American'. Ancestry informative markers (AIMs), sets of SNPs that help to determine quantitatively the degree to which a subject's genome is represented by different ancestral populations, are a possible partial solution to this problem. AIMs can measure and control for stratification, and can be applied statistically as a covariate in outcome analysis.^[95,96] Failure to rigorously assess ancestry may confound results.

5. Conclusions

Initial pharmacogenetic efforts in alcohol research have identified functional variants associated with drinking-related translational phenotypes and possibly clinical pharmacotherapeutic response, which ultimately could lead to pharmacogenetically driven treatment optimization. However, given the paucity of studies and lack of consistent findings, *we are not there yet* in terms of translating experimental pharmacogenetic personalized therapies for alcoholism to clinical practice. One explanation for mixed findings, particularly for rs1799971, is that really there is no clinically significant pharmacogenetic treatment effect even though there are functional effects. Alternatively, we may not have enough knowledge about mechanisms of action and other moderating factors to unlock the full potential of pharmacogenetic interactions. The most promising findings point to interactions of a genetic variant in *OPRM1* (specifically rs1799971) with naltrexone, the *5HTTLPR* tri-allelic polymorphism with sertraline and the *DRD4 VNTR* with olanzapine and naltrexone. More challenge studies to elucidate the mechanism of action of these pharmacogenetic interactions in alcoholism research are warranted, as well as more clinical treatment trials to replicate or refute initial positive findings. Genome-wide expression studies and pharmacoeigenomic research may help to inform the next generation of pharmacogenetic studies.

The next generation of pharmacogenetic treatment trials should improve upon methodology used in the first generation. Pharmacogenetic studies should use placebo controls, and measure ancestry with AIMs. Power to detect effects should be maximized by oversampling and stratification based on relevant genotype. In order to develop further pharmacogenetic treatment for alcoholism, a substantial amount of translational research remains to be done.

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