

The Role of the Asn40Asp Polymorphism of the Mu Opioid Receptor Gene (*OPRM1*) on Alcoholism Etiology and Treatment: A Critical Review

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The endogenous opioid system has been implicated in the pathophysiology of alcoholism as it modulates the neurobehavioral effects of alcohol. A variant in the mu opioid receptor gene (*OPRM1*), the Asn40Asp polymorphism, has received attention as a functional variant that may influence a host of behavioral phenotypes for alcoholism as well as clinical response to opioid antagonists. This paper will review converging lines of evidence on the effect of the Asn40Asp SNP on alcoholism phenotypes, including: (i) genetic association studies; (ii) behavioral studies of alcoholism; (iii) neuroimaging studies; (iv) pharmacogenetic studies and clinical trials; and (v) pre-clinical animal studies. Together, these lines of research seek to elucidate the effects of this functional polymorphism on alcoholism etiology and treatment response.

Key Words: *OPRM1*, Pharmacogenetics, Genetics, Alcoholism, Naltrexone, Asn40Asp.

TWIN AND ADOPTION studies have shown the heritability of alcoholism to be as high as 50 to 60% (Kendler et al., 1997; Prescott and Kendler, 1999). The neuropathophysiology of alcoholism and its underlying genetic architecture are complex and remain largely elusive. In recent years, progress in “risk-gene” identification for alcohol use disorders (AUD) has occurred through the use of intermediate phenotypes (Ducci and Goldman, 2008; Hines et al., 2005), including the subjective effects of alcohol and/or alcohol cues on the neural pathways of reward. In that regard, the endogenous opioid system has been implicated in the pathophysiology of some aspects of alcoholism as it modulates the reinforcing effects of alcohol via activation of opioid receptors in the ventral tegmental area and nucleus accumbens, which enhances extracellular concentrations of dopamine (DA) in the mesolimbic pathway (Gianoulakis, 2009; Koob and Kreek, 2007; Kreek, 1996; Ramchandani et al., 2011). In light

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of the implication of endogenous opioids in alcohol-induced reward, several genetic association studies have focused on allelic variation in *OPRM1* as a plausible candidate locus for several alcoholism-related phenotypes.

In particular, a single nucleotide polymorphism (SNP) of *OPRM1*, the Asn40Asp SNP (rs17799971),¹ has received significant attention given molecular evidence that this locus codes for a protein site of glycosylation and, as such, can have functional significance. Specifically, this variant results in an amino acid change from asparagine to aspartic acid, which in turn is thought to increase receptor binding affinity for β -endorphin (Bond et al., 1998), although these effects may be cell line dependent. And, while this polymorphism appears to be functional, there is contradictory evidence regarding whether the minor allele (Asp40) is associated with a gain or loss of receptor function (Zhang et al., 2005).

Additional polymorphisms of *OPRM1* have also been examined. A study by Hoehe and colleagues (2000) identified a total of 43 biallelic variants of the gene, 40 of which were substitution SNPs and 3 insertion/deletion variants (Hoehe et al., 2000). Of those 43 variants, 24 were located in the 5'-regulatory region and only 6 in the coding region (including the Asn40Asp SNP). It was hypothesized that variants in noncoding regions could affect transcription regulatory motifs. As such, multiple *OPRM1* polymorphisms located in both coding and noncoding regions have been

¹Note that although this SNP is referred to in the literature, as well as this paper, as the Asn40Asp (or the A118G SNP), this designation has been recently updated in the public bioinformatics databases (ABI, NCBI, HapMap) as it has been determined that the mu-opioid receptor may contain an additional 62 amino acids. The new designation of this SNP on the NCBI Human Genome Assembly 36 is Asn102Asp (or A355G) (<http://www.ncbi.nlm.nih.gov/snp>).

studied to date. For example, the +17C/T SNP located in position +17C/T in exon 1 leading to the Ala6Val in the N-terminus has been associated with opioid and cocaine dependence in African Americans (Hoehe et al., 2000). However, this finding stands in contrast to previous reports (Bergen et al., 1997; Gelernter et al., 1999). Likewise, most of the case and control genetic association studies failed to identify additional *OPRM1* variants consistently associated with alcohol and drug dependence phenotypes.

More recent molecular studies have implicated 2 CpG sites in the promoter region of *OPRM1*, which demonstrated hypermethylation in opioid-dependent patients relative to controls (Nielsen et al., 2009). Hypermethylation reduces the expression of *OPRM1*, indicating a potential cause, or consequence, of opioid addiction (Nielsen et al., 2009). A molecular study examining the haplotype structure of *OPRM1* has found that the Asn40Asp SNP is part of a haplogroup that includes several variants in the distal 5' region, which may have regulatory potential (Levrán et al., 2011). Yet, another study seeking to characterize the functional significance of human variants of *OPRM1* revealed several polymorphisms involved in altered trafficking and signaling properties, which could impact a host of clinical phenotypes (Ravindranathan et al., 2009). These molecular findings have not been yet extended to alcoholism and while these developments offer opportunities for novel lines of inquiry, the vast majority of the research on *OPRM1* to date has focused on the Asn40Asp SNP, which is the primary focus of this review.

This paper will review converging lines of evidence on the effect of the Asn40Asp SNP on alcoholism phenotypes, including the following: (i) genetic association studies; (ii) behavioral studies of alcoholism; (iii) neuroimaging studies; (iv) pharmacogenetic studies and clinical trials; and (v) pre-clinical animal studies. Together, these lines of research seek to elucidate the effects of this polymorphism on alcoholism etiology and treatment response.

GENETIC ASSOCIATION STUDIES

Several genetic association studies have tested the relationship between the Asn40Asp SNP and substance use disorders, particularly alcoholism and opioid dependence. The results, however, are inconsistent, and while some investigations have found support for the association between this SNP, as well as other intronic *OPRM1* SNPs (Kranzler et al., 1998), and alcohol or opioid dependence (Bart et al., 2005; Schinka et al., 2002; Tan et al., 2003; Town et al., 1999), other studies have failed to report an association (Bergen et al., 1997; Crowley et al., 2003; Franke et al., 2001; Gelernter et al., 1999; Shi et al., 2002), including haplotype-based analyses (Luo et al., 2003). A family-based association study by Xuei and colleagues (2007) examined multiple SNPs of the mu (*OPRM1*) and delta opioid receptor (*OPRD1*) genes, as well as genetic polymorphisms in the genes encoding for their endogenous ligands (*POMC* and *PENK*). Results did not support associations between these genes and alcohol depen-

dence (Xuei et al., 2007). However, other family-based association analyses showed an effect of genes encoding the kappa-opioid receptor (*OPRK1*) and its ligand (*PDYN*) with regard to alcohol dependence liability (Xuei et al., 2006).

While the vast majority of the studies have focused on adult samples, a recent study examining AUD in a sample of adolescents reported a significantly higher frequency of the Asp40 allele among youth with AUD (51.9%), as compared to non-AUD controls (16.3%) (Miranda et al., 2010). Asp40 carriers in this sample were more likely to endorse drinking motives related to the enhancement of positive affect, which in turn mediated the association between *OPRM1* genotype and AUD status (Miranda et al., 2010).

To integrate the case and control genetic studies, a meta-analysis was completed including 22 articles and over 8,000 individuals across multiple drugs of abuse (primarily alcohol and opioid dependence) (Arias et al., 2006). This meta-analysis failed to support an association between the Asn40Asp SNP and substance dependence. These results were not moderated by substance of abuse (i.e., alcohol vs. opioid) or study population (Arias et al., 2006). This meta-analysis, as well as previous population studies (Gelernter et al., 1999), also highlighted the allele frequency imbalance for this polymorphism across population groups. The estimated minor allele (Asp40) frequency is 3 to 16% in Caucasians, 0 to 5% in African Americans, and 25 to 47% in Asian populations. Ethnicity effects were also found in a very recent study of DNA methylation in the *OPRM1* promoter region in lymphocytes of heroin-dependent individuals, such that African Americans displayed a lower degree of methylation between cases and controls (Nielsen et al., 2010). Taken together, at this time, the results of association studies do not support the Asn40Asp SNP as a risk factor for alcoholism and other substance use disorders. Nevertheless, it remains puzzling that some studies found support for an association while others did not. Further elucidation of additional moderating variables, such as sex, environmental differences, ethnicity, and epistatic effects, may allow us to reconcile these disparate findings.

Although the etiological implication of this polymorphism is unclear on the basis of association studies, a series of preclinical, experimental, and pharmacogenetic studies have provided varying levels of empirical support for the effect of this polymorphism in a host of alcohol-related phenotypes, including treatment outcome with opioid antagonists. These experimental and pharmacogenetic studies are reviewed next.

BEHAVIORAL STUDIES OF ALCOHOLISM

A number of human laboratory studies employing behavioral pharmacology and experimental designs have examined the effect of the Asn40Asp SNP in a host of alcohol-related phenotypes, including alcohol craving, subjective intoxication, stress-induced drinking, and appetitive action-tendencies. These studies offer an advantage to case-control genetic

studies in that experimental phenotypes are putatively closer to the neurobiology of alcoholism (Ray et al., 2010b,c) and laboratory designs can more easily control extraneous variables. Specifically, experimental approaches allow for manipulation and assessment of critical components of alcoholism that are more proximal to the underlying neurobiology of the disorder, such as mechanisms of alcohol-induced reward (“liking”) and craving (“wanting”) (Ducci and Goldman, 2008). These traits may in fact represent endophenotypes or intermediate phenotypes for alcoholism (Hines et al., 2005). Moreover, given that alcohol dependence is highly heterogeneous in terms of both clinical phenomenology and causal pathways, more focused studies may be more effective at parsing discrete translational phenotypes and their underlying genetic bases. Ultimately, however, the utility of such studies hinges upon their ability to effectively inform clinical practice, such as disorder etiology and treatment outcome.

A series of studies focusing on behavioral mechanisms of alcohol reward have shown that compared to Asn40 homozygotes, Asp40 carriers report greater subjective reinforcement from alcohol consumption in the laboratory (Ray and Hutchison, 2004) and in the natural environment, measured using ecological momentary assessment (EMA) methods (Ray et al., 2010d). EMA data revealed that Asp40 carriers consumed more alcohol per drinking episode and that although craving was positively associated with alcohol use, in general, among Asp40 carriers, craving was less strongly related to alcohol use as compared to Asn40 homozygotes (Ray et al., 2010d). These studies are consistent with the hypothesized role of endogenous opioids as mediators of the reinforcing effects of alcohol and suggest that favorable phenotypes to probe for the effect of the Asn40Asp SNP may involve assays of the rewarding effects of alcohol.

Additional experimental studies have found that male carriers of the Asp40 allele report higher levels of alcohol craving following alcohol cue exposure, as compared to homozygotes for the Asn40 allele (van den Wildenberg et al., 2007). Likewise, alcohol-dependent Asp40 carriers reported higher alcohol intake and greater craving in response to stress, as compared to Asn40 homozygotes (Pratt and Davidson, 2009). Similar genotype effects on stress-reactivity were not reported in a study of heavy drinkers; however, higher cue-induced alcohol craving was observed among Asp40 carriers (Ray, 2011). More recently, a laboratory study of metyrapone, a stressor which blocks glucocorticoid production in the adrenal cortex, revealed that Asp40 carriers displayed a blunted adrenocorticotrophic hormone (ACTH) response to this medication suggesting a greater tonic inhibition of the hypothalamic–pituitary–adrenal (HPA) sites and less cyclical glucocorticoid inhibition among Asp40 carriers (Ducat et al., in press). A laboratory study showed that Asp40 carriers displayed stronger automatic approach tendencies for alcohol and suggested that such approach bias may not be alcohol-specific, as it was observed for other appetitive stimuli (Wiers et al., 2009). A self-report study in a sample of American Indians showed that Asp40 carriers reported expecting a more

intense subjective response to alcohol (e.g., feeling buzzed, dizzy, drunk) as compared to Asn40 homozygotes, which in turn was correlated with lower alcohol use (Ehlers et al., 2008).

In summary, a number of experimental paradigms have afforded a more comprehensive test of the effects of this polymorphism on a host of alcohol-related phenotypes. Results suggest that this variant may be associated with greater alcohol-induced reward, which is in turn consistent with the role of the opioidergic system in the hedonic properties, or liking, of alcohol as well as natural rewards (Robinson and Berridge, 1993). Inconsistencies in the literature may be associated with factors such as sample characteristics (e.g., heavy drinkers vs. alcohol-dependent samples), which in turn may serve as a proxy for stages of alcohol-related problems. Specifically, to the extent that this polymorphism is related to the reinforcing effects of alcohol, current neurobiological theories of addiction suggest that positive reinforcement is most salient early in the transition from heavy drinking to dependence, whereas late stage alcoholism is characterized primarily by negative reinforcement processes (Koob and Kreek, 2007). Nevertheless, such models of addiction have not been sufficiently translated to human samples and the transition from positive to negative reinforcement remains poorly understood in clinical populations.

NEUROIMAGING STUDIES

A few studies have examined the Asn40Asp SNP using neuroimaging methods. A study by Filbey and colleagues (2008a) employed a novel functional magnetic resonance imaging (fMRI)-based alcohol taste-cue paradigm to elicit alcohol craving and to activate the mesocorticolimbic circuitry underlying the phenotypic expression of craving using a sample of heavy drinkers. This study compared Asp40 carriers to Asn40 homozygotes on hemodynamic response to the alcohol taste-cue task and found that Asp40 carriers had greater fMRI BOLD response in the mesocorticolimbic areas (i.e., ventral striatum, ventromedial prefrontal cortex, and orbitofrontal cortex) before and after a priming dose of alcohol, relative to control cues, and as compared to Asn40 homozygotes (Filbey et al., 2008b). This study supported the association between the Asn40Asp SNP and the neural basis of alcohol-induced reward.

A recent report combined a placebo-controlled intravenous alcohol administration with positron emission tomography methodology to examine the striatal DA response to alcohol in social-drinking male Asp40 carriers and Asn40 homozygotes (Ramchandani et al., 2011). Using 11C-raclopride, this study showed that Asp40 carriers displayed a more potent striatal DA response to alcohol as compared to individuals who were homozygous for the Asn40 allele. In conjunction, the 2 neuroimaging studies described above have supported the biological plausibility of this polymorphism as a determinant of alcohol-induced reward, both in terms of hemodynamic response (Filbey et al., 2008b) and DA release in the

striatum following alcohol exposure. A functional neuroimaging study of social pain has implicated the Asp40 allele with greater neural response to a social rejection task, as well as with greater dispositional levels of social rejection sensitivity (Way et al., 2009). Together, these studies have begun to elucidate the neural bases of differential response to alcohol conferred by this polymorphism and have suggested that these mechanisms may not be alcohol-specific and instead may apply to a host of psychological domains.

PHARMACOGENETIC STUDIES AND CLINICAL TRIALS

The first pharmacogenetic reports of the Asn40Asp SNP were from studies of naloxone, an opioid antagonist often used to provide a pharmacological challenge of the HPA axis. These initial studies have shown that the Asp40 allele was associated with greater cortisol response to opioid receptor blockade, via naloxone (Wand et al., 2002). The effects of this polymorphism on cortisol response to naloxone were independently replicated (Hernandez-Avila et al., 2003) and shortly after, the first report of naltrexone pharmacogenetics was published (Oslin et al., 2003). Oslin and colleagues (2003), in a combined retrospective analysis of 3 distinct clinical trials, showed that the Asn40Asp polymorphism was associated with clinical response to naltrexone among alcohol-dependent patients. Specifically, individuals with at least 1 copy of the Asp40 allele had lower relapse rates and longer time to return to heavy drinking when treated with naltrexone, as compared to Asn40 homozygotes (Oslin et al., 2003). As these findings emerged, influential reviews have highlighted the potential clinical utility of this polymorphism as a pharmacogenetic predictor of response to naltrexone, 1 of only 3 pharmacotherapies approved by the FDA for the treatment of alcoholism (Oroszi and Goldman, 2004; Oslin et al., 2006). Importantly, the Asn40Asp SNP was advanced as an a priori genetic moderator of clinical response to naltrexone in the Combining Medications and Behavioral Interventions for Alcoholism (COMBINE) Study, a large multisite trial allowing for a retrospective analysis of clinically meaningful pharmacogenetic effects (Goldman et al., 2005).

Results from the COMBINE Study showed that carriers of the Asp40 allele receiving naltrexone plus medication management (MM; a brief behavioral platform) reported a significantly greater decrease in heavy drinking days, compared to homozygotes for the Asn40 allele. In addition, while 87% of carriers of the Asp40 allele were classified as having a good clinical outcome to naltrexone plus MM, only 55% of individuals who were homozygous for the Asn40 allele were classified as good responders, suggesting a clinically meaningful predictive utility for this polymorphism (Anton et al., 2008). Importantly, the 2 genotype groups did not differ in their response to MM plus placebo indicating that the clinical effects were unique to naltrexone response. These findings were further supported by haplotype-based analyses of the COMBINE Study data set, which implicated the Asn40Asp

SNP as the single *OPRM1* locus predictive of naltrexone response regarding the good clinical outcome variable (Oroszi et al., 2009). While these results are encouraging, it should be noted that in both the Oslin and colleagues (2003) study and the COMBINE Study (Anton et al., 2008), patients were not randomized to treatment based on genotype. Instead, the analyses were conducted in a post hoc retrospective fashion.

Additional studies have examined the Asn40Asp SNP and naltrexone pharmacogenetics. A controlled laboratory study of naltrexone found that Asp40 carriers reported greater naltrexone-induced blunting of alcohol "high," as compared to placebo and to individuals who were homozygous for the Asn40 allele (Ray and Hutchison, 2007). This study suggested a biobehavioral mechanism by which naltrexone may be differentially effective among Asp40 carriers, such that these individuals may be more sensitive to the reinforcing effects of alcohol and in turn more responsive to the dampening of the alcohol "high" afforded by naltrexone. Secondary analyses from this laboratory study found that naltrexone selectively elevated GABAergic neurosteroid levels among Asp40 carriers, indicating a potential neurosteroid contribution to the differential effects of naltrexone among Asp40 carriers (Ray et al., 2010a). A recent placebo-controlled study has replicated the naltrexone-induced blunting of alcohol "euphoria" among Asp40 carriers after a priming dose of alcohol in a sample of social drinkers (Setiawan et al., 2011). These effects were only seen in women and were not extended to a progressive ratio paradigm, such that naltrexone did not decrease motivation to work for additional alcoholic beverages.

Given the known minor allele frequency imbalance across ethnic groups, extension of these findings to diverse samples is warranted. To that end, a placebo-controlled study of naltrexone among Korean alcohol-dependent patients found that, among treatment adherent individuals, Asp40 carriers reported longer time to relapse, compared to Asn40 homozygotes (Kim et al., 2009). These results were not statistically significant in the intent-to-treat analysis and only reached statistical significance when medication noncompliant patients were excluded from the analysis. As with the preclinical literature discussed later, there is evidence of sex-specific effects of this polymorphism in a Korean sample, such that the Asp40 allele was overrepresented in women with alcohol dependence versus controls, but not in men (Kim, 2009). This is relevant as some clinical trials have reported sex-specific effects, with men showing a better clinical response to naltrexone (Garbutt et al., 2005; Kranzler et al., 2009), although these differences may be a function of reduced sample size and/or the endpoint drinking variable selection (Baros et al., 2008).

A laboratory-based study of naloxone comparing individuals of European ancestry to those of Asian ancestry found that the effects of the Asn40Asp polymorphism on naloxone-induced HPA-axis activation were restricted to individuals of European ancestry (Hernandez-Avila et al., 2007). A reanalysis of the COMBINE Study data set focusing exclusively on African American participants did not support the efficacy of naltrexone (Ray and Oslin, 2009). However, power to

detect this effect was low, increasing the risk of type II error. Moreover, the lack of naltrexone effect might be explained by the low Asp40 allele frequency among African Americans (approximately 7% in the COMBINE Study). Ancestry-specific effects remain a critical area of investigation for naltrexone pharmacogenetics as well as etiological studies. Population genetic issues are not restricted to the Asn40Asp SNP. For example, the Ala6Val polymorphism is more frequent among African Americans (12%) than in individuals of European ancestry (<1%) and Hispanics (3%) (Gelernter et al., 1999). Ethnic differences have been shown to affect haplotype construction for *OPRM1* leading to diverse haplotype structures found for a primarily Caucasian sample (Oroszi et al., 2009), as compared to a sample of American Indians (Ehlers et al., 2008). While it remains unclear how alcohol-dependent African Americans and those in other ethnic groups respond to naltrexone overall, population effect and allele frequency considerations might be of particular importance as the field of pharmacogenetics (and genomics) progresses. These issues may have important implications for health disparities in the era of personalized medicine (Tate and Goldstein, 2004).

As with the other lines of inquiry reviewed herein, pharmacogenetic studies of naltrexone and *OPRM1* are far from conclusive and nonreplications have been reported. Most notably, a large clinical trial did not find support for the moderating effect of Asn40Asp polymorphism on clinical response to naltrexone in a sample of male veterans who were actively involved with Alcoholics Anonymous and intensive psychosocial treatments (Gelernter et al., 2007). In the COMBINE Study, patients who received a more sophisticated psychosocial intervention showed no effect of naltrexone and no *OPRM1* pharmacogenetic interaction, suggesting that robust psychosocial interventions may obscure pure pharmacological, as well as pharmacogenetic, effects. Another possible explanation for the discrepancies in clinical trials may be the high likelihood of type II error, especially if naltrexone is not observed to have an overall effect. If one does not observe a pharmacogenetic interaction and there is no main effect of naltrexone, one cannot effectively discriminate between alternative interpretations: one being that there is no effect of *OPRM1* variation on naltrexone response and the second being that the study failed to identify a naltrexone effect because of extraneous factors (e.g., study population, powerful ancillary treatment, high dropout rate, etc.). Yet, another plausible explanation for the mixed findings is that the effect size of this pharmacogenetic interaction is small. There is increasing recognition in behavioral genetics that psychiatric disorders may be due to multiple genes of small effect size (Plomin et al., 2009). Applying a similar polygenic framework to pharmacogenetic studies would suggest a relatively small effect size for this particular pharmacogenetic finding, which in turn can account for the mixed findings. In fact, a recent review of genetics of alcoholism argued that there may be a modest pharmacogenetic effect of the Asp40 allele on response to

naltrexone and that large prospective studies are needed to more accurately estimate the effect size of this interaction (Gelernter and Kranzler, 2009).

Likewise, results of behavioral/laboratory studies of naltrexone pharmacogenetics have not been completely consistent. For instance, a placebo-controlled study of nontreatment seekers found that Asp40 carriers treated with naltrexone reported *greater* cue-induced craving for alcohol, than Asn40 homozygotes, and as compared to placebo (McGeary et al., 2006). Further analyses of the same sample failed to support a pharmacogenetic effect on measures of alcohol use and urge to drink in the natural environment, using EMA (Tidey et al., 2008). Similar null findings were reported for measures of cue-reactivity in a mixed sample comprised of both nontreatment-seeking and treatment-seeking alcohol-dependent individuals (Ooteman et al., 2009). A small neuroimaging study examined the pharmacogenetics of naltrexone on delay discounting ($n = 19$) and did not support the pharmacogenetic effect of the Asn40Asp SNP of *OPRM1* on impulsive decision-making as well as activation of its underlying neurocircuitry (Boettiger et al., 2009). Such null findings highlight the need to cautiously evaluate the empirical evidence before pharmacogenetic prescriptions can be made regarding naltrexone for alcoholism. As highlighted by Gelernter and colleagues (2007), attention to additional opioid genes, such as those encoding kappa and delta receptors, which are also targeted by naltrexone, yet to a lesser degree than mu receptors, represents an important avenue for future research.

In summary, the empirical data provide some evidence that the Asn40Asp SNP is associated with a differential subjective response to alcohol and as a predictor of clinical response to naltrexone. These findings have been met with considerable enthusiasm as well as a healthy level of skepticism. As stated by O'Brien (2008), if supported, "the results of such pharmacogenetic studies could change clinical practice so that the selection of medication could be based on genotype rather than guesswork" (p. 133). Personalized medicine is the ultimate goal of pharmacogenetic studies, and the field of alcoholism has received attention for its strong efforts toward personalized care (Kranzler and Edenberg, 2010; Kuehn, 2009). It appears that considerable work remains to be performed before the promise of targeted therapies may be realized for naltrexone. Nevertheless, the biological and clinical plausibility of this line of research is rather compelling in the case of naltrexone and the Asn40Asp SNP of *OPRM1*. A summary of the human studies of *OPRM1* and alcoholism phenotypes is presented in Table 1.

PRECLINICAL STUDIES

Given the inconsistencies in the human literature described previously, as well as inconsistent results regarding the functional properties of the Asn40Asp variant in heterologous expression systems, a number of preclinical cellular and animal models have been developed to gain insight into the

Table 1. Summary of Human Behavioral Experiments and Clinical Trials of the Asn40Asp and Alcoholism Phenotypes

First author	<i>n</i>	Study sample	Approach	Key findings
Wand (2002)	39	Healthy men	Naloxone administration, pharmacogenetic	Asp40 carriers showed greater cortisol response to naloxone
Hernandez-Avila (2003)	30	Healthy men and women	Naloxone administration, pharmacogenetic; PC	Asp40 carriers showed greater peak cortisol response to naloxone; no differences in ACTH
Oslin (2003)	130	Alcohol-dependent, European descent	Naltrexone treatment, clinical, pharmacogenetic; PC	Asp40 carriers had lower relapse rates and longer time to return to heavy drinking when treated with naltrexone
Ray (2004)	38	Heavy drinkers	Alcohol administration, human laboratory	Asp40 carriers reported greater subjective intoxication upon alcohol administration
McGeary (2006)	93	Heavy drinkers, most alcohol dependent	Naltrexone and cue-reactivity, pharmacogenetic; PC	Asp40 carriers treated with naltrexone reported <i>greater</i> cue-induced alcohol craving
Ray (2007)	40	Heavy drinkers	Naltrexone and alcohol challenge, pharmacogenetic; PC	Asp40 carriers reported greater naltrexone-induced blunting of alcohol "high"
Gelernter (2007)	215	Alcohol-dependent, male Veterans	Naltrexone treatment, clinical, pharmacogenetic; PC	There was no significant pharmacogenetic effect of the Asn40Asp SNP on response to naltrexone
Hernandez-Avila (2007)	29	Individuals of Asian and European ancestry	Naloxone administration, pharmacogenetic; PC	Asp40 carriers reported greater cortisol response to naloxone; only in individuals of European ancestry
van den Wildenberg (2007)	108	Male heavy drinkers	Cue-exposure, human laboratory	Asp40 carriers reported greater cue-induced craving for alcohol
Anton (2008)	604	Alcohol-dependent, European descent	Naltrexone treatment, clinical, pharmacogenetic; PC	Asp40 carriers showed an increase in days abstinent, a decrease in heavy drinking days, and were more likely to be classified as having a good clinical outcome when treated with naltrexone
Tidey (2008)	107	Heavy drinkers, most alcohol dependent; nontreatment seekers	Naltrexone and ecological momentary assessment (EMA), pharmacogenetic; PC	OPRM1 genotype did not moderate the effects of naltrexone on drinking out comes, urge to drink, or subjective intoxication (EMA)
Ehlers (2008)	251	American Indians; on average, heavy drinkers	Expected subjective intoxication (self-report)	Asp40 carriers reported expecting a more intense subjective intoxication after consuming 2 to 3 drinks
Filbey (2008a,b)	73	Heavy drinkers	Alcohol taste cue, fMRI study	Asp40 carriers showed greater activation of the mesocorticolimbic areas upon alcohol cue exposure
Kim et al. (2009)	32	Alcohol-dependent, Koreans	Naltrexone treatment, clinical, pharmacogenetic; PC	Asp40 carriers, who were medication compliant, had longer time to relapse and lower relapse rates
Pratt (2009)	74	Alcohol dependent	Stress induction, human laboratory	Asp40 carriers reported higher alcohol intake and greater subjective craving in response to stress
Wiers (2009)	108	Male heavy drinkers	Approach-avoidance task, human laboratory	Asp40 carriers showed greater automatic approach tendencies for alcohol and other appetitive stimuli
Ooteman (2009)	63	Alcohol dependent	Naltrexone and cue-reactivity, pharmacogenetic	No significant pharmacogenetic effect
Boettiger (2009)	19	Alcohol-dependent patients and controls	Naltrexone and delay discounting, fMRI study; PC	No significant pharmacogenetic effect on risky decision making and associated neural activation
Ray (2010d)	112	Heavy drinkers, (61% alcohol dependent)	EMA study	Asp40 carriers reported higher vigor and lower negative mood following alcohol use in the natural environment; consumed more alcohol per episode
Ray (2011)	64	Heavy drinkers	Stress induction and cue-exposure, human laboratory	Asp40 carriers reported greater cue-induced alcohol craving; no differences in stress reactivity
Setiawan (2011)	40	Social drinkers	Alcohol priming, progressive ratio paradigm; PC	Naltrexone reduced "euphoria" after priming dose among women and Asp40 carriers
Ramchandani (2011)	38	Male social drinkers	Alcohol administration, positron emission tomography study	Asp40 carriers showed a more potent striatal dopamine response to alcohol administration
Ducat (in press)	48	Healthy volunteers	Metyrapone administration; pharmacogenetic	Asp40 carriers had a more modest rise and significantly lower ACTH levels

PC is used to denote placebo-controlled studies of naltrexone.

ACTH, adrenocorticotrophic hormone; fMRI, functional magnetic resonance imaging.

effect of the Asn40Asp variant in humans using controlled experimental systems. A nonhuman primate ortholog of the Asn40Asp SNP has been identified and 2 mouse models have been generated to address the effects of this polymorphism on phenotypes used to model alcoholism.

Mouse Models

Initial studies demonstrated that the Asp40 variant increases the binding affinity of β endorphin to 3-fold higher than that of the Asn40 human mu opioid receptor (hMOR) in AV-12 cells and results in higher potency for the activation of G protein-coupled potassium channels (Bond et al., 1998). Other studies reported no differences in agonist binding, functional coupling, or desensitization (Befort et al., 2001; Beyer et al., 2004). Using an allelic expression assay, Zhang and colleagues (2005) found a 1.5-fold reduction in allele-specific mRNA expression and a 10-fold reduction in protein levels with the Asp40. More recent data supported this claim, showing lower surface receptor expression, decreased forskolin-induced cyclic AMP activation, and lower agonist-induced MOR activation with the Asp40 allele (Kroslak et al., 2007). Discrepancies in the in vitro findings establish the rationale for generating a mouse model to examine the pharmacological, molecular, and behavioral significance of this polymorphism. A knock-in mouse model harboring the equivalent point mutation in the mouse, the A112G, which alters the same amino acid coding from an asparagine to aspartic acid at position 38 (Asn38Asp; N38D), was recently developed (Mague et al., 2009). Studies of this novel mouse model suggested that mice carrying this SNP (A112G) demonstrated phenotypic similarities to humans carrying the Asp40 allele, such as reduced *OPRM1* mRNA expression, lower *OPRM1* receptor protein levels, and reduced morphine-induced analgesia (Mague et al., 2009). Importantly, this study highlighted sex-dependent effects and suggested that the functional consequences of this SNP cannot be evaluated as a simple loss or gain of function and instead may be circuitry-dependent. A second approach to model this SNP generated 2 mouse lines that expressed humanized receptors with either the Asn or Asp variant (Ramchandani et al., 2011). In this study, mice with the Asp variant demonstrated a 4-fold greater peak striatal DA response to alcohol administration compared to mice with the Asn variant. Together, these preclinical models have allowed for the replication of important phenotypes across species and these translational efforts provide a promising avenue for future studies, including animal models of alcohol intake, alcohol reward, and naltrexone's effects on alcohol intake.

Nonhuman Primate Models

Rhesus macaques (*Maccaca mulatta*) offer a unique opportunity for translational research given the presence of a non-synonymous SNP (*OPRM1* C77G) resulting in an amino acid change (Arg26Pro), which in turn was found to confer a

3.5 increase in binding affinity for β -endorphin (Miller et al., 2004). This polymorphism is functionally similar to the Asn40Asp SNP in humans. Studies have shown that male macaques carrying the *OPRM1* 77G allele displayed increased alcohol-induced stimulation as well as higher alcohol preference and consumption, compared to *OPRM1* 77C homozygotes (Barr et al., 2007). The sex-specificity of the effect is contrary to the mouse models, where female carriers of the G112 allele displayed an altered behavioral response to acute morphine withdrawal (Mague et al., 2009). It is hypothesized that hormonal modulation may have an effect on this phenotype and could be experimentally manipulated in preclinical models to further investigate a sex by genotype interaction of this SNP.

Further studies of this polymorphism in rhesus macaques showed that carriers of the *OPRM1* 77G allele exhibited higher levels of attachment behavior in infancy, marked by persistent distress vocalizations in response to maternal separation and increased preference for maternal contact upon reunion (Barr et al., 2008). More recently, a study of naltrexone effects on alcohol preference suppression in rhesus macaques suggested that following naltrexone administration, carriers of the 77G allele significantly decreased their alcohol preference, whereas 77C homozygotes were unaffected by naltrexone treatment with regard to alcohol preference (Barr et al., 2010). This finding, which suggests that this polymorphism drives reward-dependent drinking behavior, was recently replicated in an independent sample of animals trained to self-administer alcohol and subsequently treated with naltrexone. Results revealed that animals with the G77G genotype showed greater naltrexone-induced reductions in alcohol self-administration as compared to those with C/C or C/G genotypes (Vallender et al., 2010). In short, studies of rhesus macaques have been consistent with the human findings, particularly those from the experimental studies. As with the rodent models, the nonhuman primate studies offer a unique opportunity for translational research on the molecular, pharmacological, and behavioral significance of this polymorphism in humans.

CONCLUSIONS AND FUTURE DIRECTIONS

A critical point to be highlighted in this review is how fast the empirical data are accumulating on the effect of the Asn40Asp variation on alcoholism-related phenotypes, including responses to opioid antagonists. This growing body of research underscores the enthusiasm in the field for finding specific genes and endophenotypes that might better predict the risk for developing alcohol dependence and better identify treatment responders. Despite this enthusiasm, it must be recognized that many clinical and laboratory studies are small in size and that some have not randomized individuals to conditions (such as naltrexone or placebo medication) based on a priori ascertainment of *OPRM1* genotypes. As clearly noted in Table 1, samples at different stages of alcohol use and misuse have been tested across studies

(e.g., social drinkers, at risk drinkers, nontreatment-seeking alcoholics, etc.). Differences in the gender and ethnic composition of the sample pose yet another obstacle to replication, as the genetic effects may be small and moderated by gender and/or ethnicity. And, while animal studies have been creative, technically demanding, and highly promising, some of the preclinical models lend support to the human condition only by correlation and circumstantial evidence (e.g., by showing differential DA elevation in the ventral striatum based on *OPRM1* genotype).

There has not yet been a clinical trial in which moderate to severe alcohol-dependent patients are prospectively randomized to naltrexone or placebo based on *OPRM1* genotype. Such a study represents a critical step toward the FDA adoption of genetic tests and ultimately of a pharmacogenetic indication for naltrexone (Lesko and Zineh, 2010). Currently, there are a few genomic biomarkers used in the drug labels of psychiatric medications, most referring to the cytochrome P450 2D6 (*CYP2D6*) gene, which predicts drug metabolism and availability. As noted by Lesko and Woodcock (2004), most of the pharmacogenetics research to date has focused on drug safety, rather than efficacy, and the integration of genetic tests into routine clinical practice remains a major challenge, even for well-established biomarkers. While much research remains to be performed to determine whether a pharmacogenetic indication is warranted, it is useful to consider the overall status of pharmacogenetics research, including federal-regulatory issues.

In conclusion, several steps have yet to be undertaken to advance this line of research. Specifically, agreement on the definition of patient populations is greatly needed along with replication and consistency in the clinical laboratory and clinical treatment arenas. Controlled prospective clinical pharmacogenetic trials need to be conducted using such definitions. Rodent and nonhuman primate models that have the greatest face validity for alcohol dependence should also be used in pharmacogenetic exploration and translation. The application of recent molecular findings regarding DNA methylation and additional functional *OPRM1* variants offers a novel avenue for elucidating the neuropsychiatric implication of genetic variation in this gene. Finally, understanding how opioid genes interact with other relevant genes (epistasis), and gene products, such as DA receptors and transporters, for example, is likely to be a fruitful area of exploration. The effect of population heterogeneity, regarding sex and ethnicity, might also be crucial in understanding the pharmacogenetic effects of *OPRM1* and other genes. In conclusion, while much progress has been made to elucidate the molecular, behavioral, and clinical effects of the Asn40Asp SNP on alcoholism etiology and treatment, much work remains to be performed before these findings may be translated into clinical practice. Limitations notwithstanding continued pursuit of this line of inquiry holds great promise to optimizing clinical care for patients suffering from alcoholism, as well as to further understand pathways of risk and recovery.

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