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Review

Review

Variation in the Serotonin Transporter Gene and Alcoholism: Risk and Response to Pharmacotherapy

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Abstract

SLC6A4, the gene encoding the serotonin transporter protein (5-HTT), has been extensively examined as a risk factor for alcohol dependence (AD). More recently, variability in the transporter gene was identified to be a potential moderator of treatment response to serotonergic medications such as ondansetron and sertraline. There is an insertion-deletion polymorphism in the promoter region (5-HTTLPR) of the *SLC6A4*, with the most common alleles being a 14-repeat short (S) allele and a 16-repeat long (L) allele. The S allele has often been associated with AD. By contrast, the L allele has been associated with pharmacological responsiveness in some individuals with AD. Differences in clinical phenotype may determine the utility of the 5-HTTLPR polymorphism as a moderator of pharmacological interventions for AD. We review the AD typology and disease onset in the context of pharmacogenetic and genomic studies that examine the utility of 5-HTTLPR in improving treatment outcomes.

INTRODUCTION

Alcohol use disorders (AUD) are complex disorders that may be influenced by a wide variety of personal characteristics and psychiatric features. Twin studies have shown alcohol dependence (AD) to have a heritability of ~50–60% (Prescott and Kendler, 1999; Enoch and Goldman, 2001; Walters, 2012; Ducci and Goldman, 2012). Among the genetic components, many of the genes that may contribute to the risk of alcohol phenotypes encode components of the dopamine (DA), serotonin (5-HT), glutamate (GLU) and acetylcholine (ACh) neurotransmitter systems (Morozova *et al.*, 2014).

In this review we will focus on the reliability of AD typology with respect to disease onset and phenotypic heterogeneity before examining the pharmacogenomic evidence implicating sequence variability in the *SLC6A4* gene with the phenotype. The *SLC6A4* gene, encoding the serotonin transporter (5-HTT), has been associated with AD risk and differential treatment response.

ALCOHOL DEPENDENCE AND ALCOHOL USE DISORDER: THE DIAGNOSTIC CRITERIA FOR ASSESSING THE SEROTONIN TRANSPORTER AS A TARGET FOR INTERVENTION INTO ALCOHOLISM

Recently, the DSM-IV AD diagnostic criteria was replaced by the DSM-5 diagnostic criteria, alcohol use disorder (AUD). It would be inaccurate, however, to assume diagnostic portability across these criteria. Since there are little data available to determine whether the diagnosis of AD is applicable to the wider spectrum of AUD, for consistency, we retain the use of AD as a diagnostic descriptor. In addition, we use the term 'alcoholism', however imperfect it may be, in order to broadly refer to both DSM-IV and DSM-5 diagnoses.

EVALUATION OF THE CONTRIBUTION OF *SLC6A4* GENE VARIABILITY TO ALCOHOLISM

Interpretation of the available data regarding the contribution of *SLC6A4* variants to the etiology of AD remains a source of debate (Villalba *et al.*, 2015). As a result, we will examine the utility of making use of variability in the gene in the development of personalized treatments for AD with drugs such as serotonergic medications (Kenna *et al.*, 2012).

The *SLC6A4* gene was one of the first candidate genes to be examined in relation to the risk for AD. The serotonin transporter (5-HTT), which is encoded by the gene, is a 12 transmembrane spanning protein that helps to regulate mood by its re-uptake of serotonin from the synaptic cleft (Lesch *et al.*, 1996; Bevilacqua and Goldman, 2011). As such, the 5-HTT is a major target in the pharmacologic treatment of depression and anxiety by the serotonin reuptake inhibitor (SSRI)

and serotonin-norepinephrine reuptake inhibitor (SNRI) drugs (Lesch *et al.*, 1996; Bevilacqua and Goldman, 2011). Variation in *SLC6A4* is associated with traits such as anxiety and depression (Lesch *et al.*, 1996), which are also associated with AD (Feinn *et al.*, 2005; McHugh *et al.*, 2010).

In particular, an insertion-deletion polymorphism in the 5-HTT gene-linked promoter region (5-HTTLPR) has been associated with emotional traits and psychiatric disorders that commonly are seen in individuals with AD (Bevilacqua and Goldman, 2011). This polymorphism consists of a 20-23 base pair (bp) repeat of variable sequence (Nakamura *et al.*, 2000). Two common variants have been identified: the 14-repeat short (S) allele and the 16-repeat long (L) allele (Lesch *et al.*, 1996). At the behavioral level, the S allele has been linked to greater emotional arousal (Bevilacqua and Goldman, 2011). Evidence suggests that the L-allele results in higher serotonin transporter mRNA transcription: putatively due to the long single nucleotide polymorphism (SNP)—A-allele of rs25531. Subjects with the rs25531(A) allelic bi-alleles (i.e. L_AL_A) have the highest levels of transcription. In contrast, the long rs25531(G) (an A→G substitution) yields a protein with properties similar to those of the S allele carriers that have levels of lower transcriptional efficiency (Praschak-Rieder *et al.*, 2007).

The role of the 5-HTTLPR polymorphism in regulating serotonin transporter expression, post-synaptic serotonin reuptake, and neuronal activity (Ho *et al.*, 2012) in relation to mood disorders, susceptibility to addiction (Bevilacqua and Goldman, 2011) and treatment response (Johnson *et al.*, 2011) has been widely studied. The S allele has been associated with AD in many but not all study populations (Feinn *et al.*, 2005; McHugh *et al.*, 2010; Cao *et al.*, 2013; Villalba *et al.*, 2015).

Despite inconsistent findings in the literature regarding the association of the 5-HTTLPR with AD, there may be a trend in the data that suggests that the S allele may be more often associated with AD (Feinn *et al.*, 2005; McHugh *et al.*, 2010). The populations and methodologies described vary widely: making it difficult to directly compare many studies. A recent meta-analysis limited to matched case-control studies found no association of 5-HTTLPR alleles with AD (Villalba *et al.*, 2015). Furthermore, another meta-analysis reported an association of the S allele with AD in studies conducted with European, Asian and Mexican populations while the L allele was associated with AD in studies conducted with African populations (Cao *et al.*, 2013): possibly explaining why these population effects may vary with study design and inclusion criteria. These observations are intriguing in the context of pharmacogenetic studies showing that AD individuals homozygous for the L allele show a greater treatment response to some serotonergic medications (Johnson *et al.*, 2011).

As a result, we will focus on *SLC6A4* variability in the context of its potential for personalizing treatments for AD with drugs such as ondansetron (Kenna *et al.*, 2012). In order to do so, we will first review the reliability of alcohol dependence typology with respect to disease onset and prognosis. It is important to note that inclusion criteria based on the polythetic criteria defined by DSM-5 instead of the monothetic criteria for DSM-IV could have significant implications for genetic studies (Table 1).

DISEASE TYPOLOGY AND ONSET

There are many ways to categorize AD patients by typology (Leggio *et al.*, 2009). Categorizing AD by considering multivariate approaches into late (Type I) and early onset (Type II) disorders (e.g. Cloninger,

Table 1. Selected studies of alcohol dependence pharmacogenetics: association of serotonin transporter (SLC6A4) gene variant (5HTTLPR) with patient response to treatment with ondansetron

Ondansetron clinical trial design	Population	Efficacy	Ancestry	Number of patients	Gene marker	Outcome measure	Reference
0.5 mg Randomized; washout; crossover to 200 mg/day Sertraline; 3 weeks each	Non-treatment Seeking AD	Ondansetron decreased DDD; sertraline increased DDD	Unspecified	15	LL	DDD alcohol self-administration experiment (ASAE)	Kenna *et al.* (2009)
Double-blind placebo controlled 4 μg/kg twice daily 11 weeks	AD AD	2.63% decrease in DDD 16.99% abstinence	AD 84% EA 15.2% H	283	LL rs1042173	DDD; % days abstinent	Johnson *et al.* (2011)
4 μg/kg twice daily 11 week placebo controlled	AUD; No Axis I	DDD decreased	95% C; 5% H	19 treatment 22 placebo	LL mRNA	DDD	Seneviratne and Johnson (2012)
4 μg/kg twice daily 11 week placebo controlled	AD No Axis I	DDD decrease is predicted by L allele of SLC6A4 and markers in 5HT 3A; 5HT 3B	AD 84% EA 15.2% H	283	LL plus 4 other SNPs	DDD	Johnson *et al.* (2013)
0.5 mg Randomized; washout; crossover to 200 mg/day Sertraline; 3 weeks each	Non-treatment Seeking AD	Women with LL had decreased DDD in on ondansetron; Women with SS or SL had decreased DDD on Sertraline; Exacerbated when drug was reversed	5.2% H 1.3% F 20.8 AA 69% C 7.8 M	55 enrolled; 49 complete data	LL SLC6A4 DRD4 alleles	DDD alcohol self-administration experiment (ASAE)	Kenna *et al.* (2014a)

AD, alcohol dependent; AUD, alcohol use disorder; DDD, drinking severity expressed as drinks per drinking day; ASAE, alcohol self-administration experiment, 5HTTLPR, serotonin transporter (SLC6A4) gene promotor polymorphism; LL, homozygous for long 5HTTLPR allele; SL, heterozygous for 5HTTLPR allele; SS, homozygous for short 5HTTLPR allele; *5HT1A*, serotonin receptor type 3A gene; *5HT3B*, serotonin receptor type 3A gene; *DRD4*, dopamine D4 receptor gene; AA, African American; F, First Nations American Indian; H, Hispanic; EA, European American; C, Caucasian; W, white; M, mixed ancestry.

1987; Babor *et al.*, 1992; Lesch *et al.*, 1996; Bevilacqua and Goldman, 2011) is a pragmatic and validated approach. Type I patients develop AD later in life and are more likely to suffer from comorbid DSM Axis I disorders such as major depression and anxiety (Devor and Cloninger, 1989; American Psychiatric Association, 2000). Others have categorized age of onset of alcoholism dichotomously as early (≤25 years old) vs late onset (Johnson, 2000).

By contrast, Type II AD patients are defined as those with earlier onset (often before age 20 years), which may reflect the presence of an Axis II DSM personality disorder—most notably, antisocial personality disorder (Schuckit, 1973; Hesselbrock *et al.*, 1985; Devor and Cloninger, 1989; Bevilacqua and Goldman, 2011; Chen *et al.*, 2011). Early-onset AD, however, is not consistently found in families known to include persons with Axis II disorders (Hill, 1992; Chen *et al.*, 2011). As a result of the heterogeneity present in AD patients, many studies describe AD patients with respect to DSM comorbidity (Chen *et al.*, 2011).

ALCOHOL DEPENDENCE TYPOLOGY AND 5-HTT GENE-ENVIRONMENT INTERACTIONS

Given the interplay of the 5-HT and DA systems and its role in novelty seeking and reward, 5-HT gene regulatory regions have been suggested as candidates for vulnerability for AD (Kweon *et al.* 2005; Johnson *et al.*, 2008; McHugh *et al.*, 2010). Alcohol use, misuse, abuse, dependence and binge drinking involve complex phenotypes that are likely to result from gene by environment (G × E) interactions at specific times during temporal development (Kenna *et al.*, 2012). These associations may be related to patterns of drinking, and may be dependent on age at first alcohol exposure, changes in hormones across the lifespan, and differ between gender and ethnicity (Buchmann *et al.*, 2010). Further, specific genes act as plasticity factors that contribute to inter-individual variability in susceptibility to environmental influences (Caspi *et al.*, 2003; Boyce and Ellis, 2005). There is evidence that the L and S alleles of the 5-HTTLPR gene are such plasticity alleles associated with increased sensitivity to environmental factors (Beaver and Belsky, 2012). While most studies demonstrate an association between 5-HTTLPR and AD, the association with specific alleles may vary with AD subtype, type of drinking behavior, co-morbid diagnoses, age of onset and ethnicity.

For example, in contrast with the results of some meta-analyses (Cao *et al.*, 2013), a study of AD inpatients in Korea reported that L-carriers had a significantly increased risk of AD (Kweon *et al.*, 2005) when the association between the 5-HTTLPR and AD was assessed. The researchers also noted a gene-dose effect. The gene-dose effect suggested that patients with L/L alleles had an even greater risk of AD compared to those with heterozygous variants. In addition, it was reported that patients with a family history of AD had a significantly higher frequency of the L-allele and L-carrying patients with AD had a significantly younger age of onset (Kweon *et al.*, 2005). Others have also demonstrated that reduced functional 5-HTT uptake in L-carriers, not S/S carriers, is due to alcohol's effect on 5-HTT gene expression and that 5-HTT expression varies with current and lifetime alcohol consumption in people with the L genotype alone, resulting in more severe and chronic drinking (Johnson *et al.*, 2008).

Similarly at odds with some meta-analyses, when a G × E interaction hypothesis was tested in Hispanic AD individuals, subjects with the L/L or L/S genotype would have higher craving for alcohol due to lower 5-HT neurotransmission than subjects with the S/S genotype (Ait-Daoud *et al.*, 2009). In addition, craving in L-carriers also increased with an earlier age of onset of problem drinking, an effect opposite of that reported by individuals with the S/S genotype (Ait-Daoud *et al.*, 2009). While a significant main effect was reported for genotype and alcohol cue, as well as an interaction among genotype, age of onset of problem drinking, and tryptophan depletion, L-carriers reported higher craving for alcohol. This effect decreased under tryptophan depletion: suggesting that decreased auto-inhibition of 5-HT neuronal firing leads to lowered alcohol craving for individuals with the L-genotype.

Given the notion that the association between AD and 5-HTTLPR seems significant, it must also be kept in mind that 5-HTTLPR frequencies are also known to vary dramatically with ethnicity (e.g. Kweon *et al.*, 2005) and a meta-analysis with a broader ethnic sample yielded conflicting results (McHugh *et al.*, 2010). A meta-analysis of 22 studies ($n = 8{,}050$) investigated the association between 5-HTTLPR and a clinical diagnosis of AD. A significant association between AD and the S-allele was reported, with an even greater effect seen in those with the S/S genotype. It was also reported that the study size influenced the reported effect size, with larger studies finding relatively smaller effects (McHugh *et al.*, 2010).

CO-OCCURRING DIAGNOSES, AGE OF ONSET AND DRINKING SUBTYPE

The relationship between AD and 5-HTTLPR has been analyzed with respect to co-occurring diagnoses and age of onset or drinking subtype. These findings are summarized in Table 2. For example, a study of heavy drinking young adults by Tartter and Ray (2011) demonstrated that heavier alcohol use was associated with depressive symptoms in L/L homozygotes but not among S-carriers. These results also indicated a main effect of 5-HTTLPR genotype such that L allele homozygotes had significantly more alcohol problems and more drinks consumed over a 30-day period than S allele carriers. This study offers support for the L allele as a risk for alcohol use problems when depressive symptoms are present. These results, however, contrast with those reported by Feinn *et al.* (2005) from a meta-analysis of data from 17 studies consisting of 3489 AD individuals and 2325 controls. In this study the frequency of the S-allele was significantly associated with AD. Additionally, the association was even greater among AD individuals with either a co-morbid psychiatric condition or an early-onset or more severe Type II alcoholic subtype (see Feinn *et al.*, 2005).

The genetic factors influencing age of onset of drinking and subsequent progression to AD are complex (Pagan *et al.*, 2006; Kendler *et al.*, 2008; van der Zwaluw *et al.*, 2012). Many studies have found that the *5-HTTLPR* S allele is a risk factor for insidious onset of AD (Greenberg *et al.*, 2000; Lesch, 2005; Olsson *et al.*, 2005). In particular, the low-activity *5-HTTLPR* S allele was associated with the initiation of alcohol use in young patients (Pagan *et al.*, 2006; Bevilacqua and Goldman, 2011) with affective and anxiety disorders which are associated with the putative Type I AD classification (Lesch, 2005; Olsson *et al.*, 2005).

Although Type I AD may itself be heterogeneous, there is evidence from neuroimaging studies that the S allele affects several brain structures that are engaged in anxiety and mood disorders. The S allele is associated with reduced gray matter volume in limbic brain areas, in the prefrontal cortex and in structures connecting the amygdala to prefrontal areas that may predispose to AD (Lesch, 2005; Hariri and Holmes, 2006; Frodl *et al.*, 2008). This may result in changes in neural connectivity between the prefrontal cortex and the amygdala during emotional processing (Pezawas *et al.*, 2008) that may reflect some aspects of the physiological basis of Type I AD.

Table 2. Selected studies examining co-occurring personality endophenotypes of alcoholics with respect to the serotonin transporter (SLC6A4) gene variant (5HTTLPR)

Phenotype	Population	Assessment	Number of patients	Gene marker	Association	Reference
Anxiety: high risk attachment settings	Cohort of young Australians followed from 14 to 24 years of age	Adolescent anxiety and alcohol use outcomes	2032	S allele	Inversely associated with binge drinking in those securely attached	Olsson *et al.* (2005)
Childhood neglect	Add Health study of twins, nontwin sibs of twins, full sibs, half sibs, and nonrelated sibs	Eight questions: negative consequences from alcohol past year	2403	SS	Gene-environment correlation females	Vaske *et al.* (2012)
Lower socialization scores	Alcohol dependence	California Psychological Inventory	862	LL	Associated with poor socialization in males	Herman *et al.* (2011)
Axis II Personality disorders	Type II, Early onset; International Classification	Self-rating depression scale (SDS); Self-rating anxiety scale (SAS)	70	L allele 10 repeat SNP (STin2)	Higher extraversion and lower harm avoidance scores in carriers of the S-10 haplotype	Reese *et al.* (2010)
Depressive symptoms	Heavy drinking young adults	Beck depression inventory	72	LL homozygotes	Drinks/day; drinks/drinking day (DDD) over a 30 day period	Tartter and Ray (2011)

Although Axis I DSM disorders (i.e. major mental disorders, autism, learning disorders and substance abuse disorders) are commonly identified in individuals suffering from Axis II DSM (i.e. personality and intellectual disorders), the early onset of addiction may be more strongly associated with the development of Axis II DSM personality disorders (Reese *et al.*, 2010). Furthermore, the 5-HTTLPR polymorphism has been associated with personality disorders unrelated to AD (Blom *et al.*, 2011). Among early-onset patients, those carrying both the L allele and the 10 repeat allele of the intron 2 variable number of tandem repeats polymorphism (STin2) may have a higher odds ratio (OR = 2.52) of developing AD. Homozygosity of the L allele has also been associated with the high alcohol tolerance (Reese *et al.*, 2010).

5-HTTLPR GENE VARIANT AND SSRI EFFICACY

AUDs frequently are accompanied by both Axis I and Axis II disorders; however, there is substantial heterogeneity both within and between studies with respect to inclusion criteria (Feinn *et al.*, 2005; Cao *et al.*, 2013). For example, in the study by Thompson *et al.*, 45% of patients genotyped were diagnosed with at least one co-occurring axis Axis I disorder (Thompson *et al.*, 2000). Before discussing the genetic moderation of pharmaceutical interventions into AD, therefore, it is worth briefly reviewing the research into the pharmacogenetics of disorders such as major depression, and Axis II disorders, such as antisocial personality disorder.

Studies of the moderating effects of 5-HTTLPR on the efficacy of AD treatments have yielded contradictory results. Depressed patients with or without a concurrent diagnosis of late-onset AD were more likely to respond to SSRIs if they carried the L allele (Serretti *et al.*, 2007; Muhonen *et al.*, 2011). Conversely, patients with early-onset AD who were homozygous for the L allele and had relatively high levels of anxiety, increased their drinking when treated with sertraline, while L homozygotes receiving placebo significantly reduced their drinking intensity (Kranzler *et al.*, 2011). These results support earlier work showing that, whereas early-onset AD individuals drink more when treated with sertraline, late-onset individuals appear to decrease their drinking (Pettinati *et al.*, 2004). Phenotypic differences may explain these divergent treatment outcomes. As a result, additional pharmacogenetic studies that carefully phenotype participants are needed to guide clinical care.

Because many studies have found the S allele to be associated with AD concurrent with an Axis I disorder (Thompson *et al.*, 2000; Feinn *et al.*, 2005; McHugh *et al.*, 2010; Herman and Balogh, 2012), it seems paradoxical that treatment response to SSRIs may be greater in patients with one or two L alleles. This phenomenon may result from the increased expression of 5-HTT associated with the L allele and present opportunities for the development of SSRI drugs with improved transporter binding. Early-onset patients, for example, were initially found to respond better to the $5HT_3$ antagonist ondansetron. The presumed explanation for that effect was that treatment with the $5HT_3$ antagonist overcomes the tendency for the 5-HTT expressed by the L allele to be refractory to alcohol's effects on serotonin (Johnson *et al.*, 2011; Kuehn, 2011; Ait-Daoud, 2012).

To what extent individual differences associated with treatment response reflect the moderating effects of the 5-HTTLPR polymorphism will determine its potential to guide treatment decisions. Ultimately, these findings will be interpreted in the context of genome-wide association studies (GWAS). Concurrent improvements in phenotyping could also permit greater accuracy both the definition of AD and its prognosis in co-occurring psychiatric disorders.

ENHANCING THE EFFICACY OF MEDICATIONS TO TREAT ALCOHOL DEPENDENCE

The moderating role that the 5-HTTLPR polymorphism can play in the clinical treatment of AD is exemplified by studies of ondansetron. An early study reported improvement in non-treatment seeking patients receiving ondansetron (Kenna *et al.*, 2009). More recently, the same group reported the result of a placebo-controlled mixed two-factor design in which AD persons with LL or SS/SL genotypes were treated with both ondansetron and sertraline. The

subjects receiving ondansetron were found to consume fewer drinks per drinking day (DDD) if they carried the LL genotype. No reduction was noted in patients with SS/SL alleles receiving sertraline in an alcohol self-administration experiment (ASAE) (Kenna *et al.*, 2014a).

In view of these results, it is possible that ondansetron may be effective in treating AD patients without Axis I disorders (Johnson *et al.*, 2011). Two polymorphisms in *SLC6A4*, the 5-HTTLPR and rs1042173 (A→T) in the 3′-untranslated region of the gene, were shown to moderate the response to ondansetron (Johnson *et al.*, 2011). Further study showed that the LL genotype and 5′-HTTLPR mRNA expression levels may be useful biomarkers for ondansetron response (Seneviratne and Johnson, 2012). Potential mechanisms of these effects may reflect the greater expression of the transporter associated with the L allele and its inverse relationship with serotonin reuptake and paroxetine binding (Kuehn, 2011). Recently, Johnson *et al.* (2013), in a secondary analysis of the previously described ondansetron trial (Johnson *et al.*, 2011), found that polymorphisms in the genes encoding the $5HT_3$ receptor, *HTR3A* (rs1150226 and rs1176713) and HTR3B (rs17614942), also moderated the response to ondansetron with respect to DDD, percentage of heavy drinking days, and days abstinent. Further, combining the relevant genotypes in HTR3A and HTR3B with those in *SLC6A4* increased the proportion of individuals of European descent who showed a robust response to ondansetron from 20 to 34% (Johnson *et al.*, 2013).

One interpretation of these findings is that ondansetron targets post-synaptic 5-HT receptors because receptor expression is constitutively higher in AD individuals with the LL genotype of the *5-HTT*. Blockade of over-expressed 5-HT receptors by ondansetron, therefore, may markedly reduce alcohol consumption (Johnson *et al.*, 2011). This may reflect the downstream effects of altered synaptic neurotransmitter concentrations and/or the capacity of alcohol to induce receptor sensitization in the serotonin system (Johnson *et al.*, 2011). Altered receptor expression or desensitization has been reported in a variety of systems in response to prolonged agonist exposure (Thompson *et al.*, 2006, 2008, 2014).

Johnson first showed that ondansetron may be effective in 35% of AD patients (Johnson *et al.*, 2011). Although patients in these studies were screened to exclude those with Axis I disorders other than nicotine and alcoholism (Johnson *et al.*, 2011; Ait-Daoud *et al.*, 2012), there was no such exclusion for Axis II disorders. We can only suggest that a proportion of these patients may have an underlying Axis II disorder: the implications of which remain unknown. An early definition of early-onset AD, however, implies that persons with Axis II disorders may react differently to alcohol's effects on serotonin or have serotonergic dysregulation associated with antisocial traits (Devor and Cloninger, 1989). In any event, the greater efficacy of certain drugs in persons carrying the L allele, with its relatively higher level of transporter expression, may reflect the role of genetics in transporter expression.

Variability in the results of association studies of the 5-HTTLPR polymorphism with AD and pharmacotherapy may reflect the genetic heterogeneity of the study samples. This may be the result of an epigenetic process involving the interaction of 5-HTTLPR alleles with patients' environment and genetic background (Zhang *et al.*, 2013). Pharmacogenetic studies that consider a variety of AD endophenotypes may better match AD typology with specific treatment (Kenna *et al.*, 2012). For example, alcoholism risk may be better described by five endophenotypes, including alcohol-metabolizing enzymes, the level of response to alcohol, neuronal or behavioral disinhibition, independent Axis I major psychiatric disorders and the opioid system (Schuckit, 2002). Moreover, various additional traits have been investigated in epidemiological research as potential endophenotypes for AD, including those related to endocrine measures, electrophysiology, personality, and drinking behavior (Hines *et al.*, 2005).

THE ROLE OF EPIGENETIC PROCESSES IN SEROTONIN TRANSPORTER PHARMACOLOGY

The 5-HTTLPR polymorphism may contribute to a variety of AD phenotypes. For example, chromosome 17q11-q12, the region to which *SLC6A4* maps, has been found to harbor genes that may contribute to the risk of AD with co-occurring antisocial traits (Gizer *et al.*, 2012). Although the region also contains other candidate genes (Gizer *et al.*, 2012), the *SLC6A4* gene is maybe the best functional candidate. The evaluation of this locus should take into account the role of epigenetic processes. Along with micro-RNA mediated effects and histone acetylation, the DNA methylation process that is implicated in epigenetic modifications may change promoter activity and influence the expression of 5-HTTLPR alleles in the development of AD (Zhang *et al.*, 2013). A methylation pattern at many genes, including the *SLC6A4* loci, has been identified (Philibert *et al.*, 2008). DNA methylation, therefore, seems to be associated with AD in a population-specific way. AD predisposition could result from an intricate interaction of genetic variation as well as epigenetic modifications (Zhang *et al.*, 2013).

Low socialization scores found in psychopathic personalities have also been associated with the L allele irrespective of the presence of AD —especially in male subjects (Glenn, 2011). This relationship has been found to hold for male alcoholics in some (Herman *et al.*, 2011), but not all, cohorts (Vaske *et al.*, 2012). In this context, it may be relevant that psychopathic traits, including adult substance abuse, appear to be associated with the impact of adverse childhood experiences (Beach *et al.*, 2011; Glenn, 2011; Herman *et al.*, 2011; Vaske *et al*, 2012). Evidence that epigenetic processes, reflecting G X E interaction, underlie the phenotype may be found in the methylation of *SLC6A4* (Beach *et al.*, 2011). The identification of methylated loci, therefore, may be evidence of a susceptibility signature for AD (Zhang *et al.*, 2013) that influences the expression of 5-HTTLPR alleles.

GENDER DIFFERENCES AND ONDANSETRON RESPONSE

Gene–gene interactions are of growing importance in accounting for how the subjective response to alcohol endophenotype (Ray *et al.*, 2010) may differ between the sexes (Skowronek *et al.*, 2006). The continuum of subjective responses to alcohol's effects may result from its ability to stimulate or inhibit various neural pathways (Ray *et al.*, 2010). For example, there is a probable influence of dopamine (DA) and 5-HT polymorphisms on impulsivity in infants (Auerbach *et al.*, 2001) and temperament in adults (Varga *et al.*, 2012) that may be consistent with the risk for establishing a substance use disorder in adulthood (Skowronek *et al.*, 2006). The dopamine D4 (DRD4) 7-repeat allele has been associated with greater smoking and drinking involvement in boys. By contrast, in girls, an interaction was reported between the other alleles of the DRD4 and the LL allele of 5-HTTLPR (Skowronek *et al.*, 2006). Serotonin may have a regulatory role over DA neurons (Johnson, 2000), suggesting that serotonergic dysfunction alters DA function and DA-mediated behavior; although polymorphisms in other dopaminergic genes, such as the dopamine

transporter (DAT1) (Vandenbergh *et al.*, 2000), inevitably influence the outcome.

Dopamine and 5-HT are both linked to novelty and impulsivity that vary between Axis II traits and Axis I disorders (Holmboe *et al.*, 2011). In alcoholics, impulsivity, novelty seeking (Evren *et al.*, 2012) and negative affect (Gizer *et al.*, 2012) are linked to increased risk for craving and relapse to drinking. While beyond the scope of this review, the data suggest that personality traits associated with DRD4 and 5-HTTLPR polymorphisms could predispose some individuals to alcoholism and that these individuals may have a distinct reaction to AD pharmacotherapy.

An investigation of the interaction between gender, the 5-HTTLPR and DRD4 alleles, and serotonergic pharmacotherapy evaluated the efficacy of both ondansetron and sertraline in non-treatment seeking alcoholics (Kenna *et al.*, 2014b). This study, based on results reported by Skowronek *et al.* (2006) with adolescents, confirmed an interaction between the 5-HTTLPR the DRD4 and serotonergic medications that was gender specific. While in male subjects, there were no differences in drinking, women with the L/L 5-HTTLPR and DRD4 ≥ 7-repeat allele receiving ondansetron and women with the S/S, S/L 5-HTTLPR but the DRD4 < 7-repeat allele receiving sertraline, had a significant reduction in both naturalistic and bar-lab drinking.

One explanation for these gender differences may result from neuroendocrine-dependent changes in gene expression (Munafò *et al.*, 2005). There are strong and consistent differences in sex and stress hormones that effect drinking (Mendelson and Mello, 1988). Consistent with these observations are the gender-specific responses to treatment such as those reported for sertraline in AD women (Pettinati *et al.*, 2004) that are particularly evident in those with specific polymorphisms (Roache, 2012).

CONCLUSIONS

There is growing evidence of the importance of genetic and epigenetic factors in the development and maintenance of AD. Epigenetic mechanisms lead to functionally related modifications of the genome as they induce alterations in gene expression which can influence the phenotypic outcome. These alterations can have, for example, particular age or gender-specific effects on the subjective effects to alcohol.

Although the 5-HTTLPR S allele has been more commonly associated with AD than the L allele, there is considerable genetic heterogeneity associated with the AD phenotype. The S allele association may be attributable to the development of alcohol use throughout adolescence and young adulthood (Pagan *et al.*, 2006; Bevilacqua and Goldman, 2011) in patients with comorbid Axis I disorders (Type I AD) consisting of affective and anxiety disorders (Olsson *et al.*, 2005). By contrast, patients with early onset, Type II AD, often comorbid with Axis II personality disorders, more often carry the L allele (Blom *et al.*, 2011). There is evidence that those homozygous for the L allele may have higher alcohol tolerance (Reese *et al.*, 2010).

In this context, treatment efficacy examined with respect to 5-HTT genotype seems contradictory. Depressed patients with and without concurrent diagnosis of late-onset AD may be more likely to respond to SSRIs if they carry the L allele (Serretti *et al.*, 2007; Muhonen *et al.*, 2011). Conversely, early-onset patients who were homozygous for the L allele and had relatively high levels of anxiety, were reported to drink more when receiving treatment with sertraline. By contrast, patients who were homozygous for the L allele were found to reduce their drinking significantly when they received placebo (Kranzler *et al.*, 2011).

With respect to ondansetron response, the LL genotype may be a useful biomarker for drug response (Seneviratne and Johnson, 2012). The serotonin transporter alleles may be associated with cue-induced alcohol craving (Ait-Daoud *et al.*, 2012) and genotyping may be an effective way to enhance treatment in AD patients who lack Axis I disorders (Johnson *et al.*, 2011). However, since many studies fail to consider Axis II disorders, this may be the subject of future studies of the putative serotonin dis-regulation present in some forms of AD.

The variable efficacy of treatments for AD that target the 5-HTT, therefore, may reflect: (a) the heterogeneity in genetic backgrounds of the AD population, (b) an epigenetic process involving the interaction of the 5HTT alleles with a person's environment and genetic background (Zhang *et al.*, 2013), and (c) other loci that influence the etiology of AD. Interventions for AD may more appropriately match AD typology with specific treatment if these effects are considered. Attempts to optimize pharmaceutical interventions will benefit from a consideration of the multicultural variability of 5-HTTLPR polymorphism with AD (Cao *et al.*, 2013). The availability of SNPs as proxy markers for the 5-HTTLPR polymorphism (Vinkhuyzen *et al.*, 2011) will also be useful in replicating 5-HTT data in a larger genome-wide investigation. The goal of this research is to optimize drug therapy by identifying genetic factors that predict who is more likely to respond or not to respond to certain pharmacotherapies: thereby matching patients to medications on the basis of genetic factors. Future studies using the revised diagnostic criteria for AUD are also likely to improve our understanding of the relationships between genes and treatments.

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CONFLICT OF INTEREST STATEMENT

None declared.

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