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SEROTONIN TRANSPORTER GENOTYPE BY ENVIRONMENT: STUDIES ON ALCOHOL USE AND MISUSE IN NON-HUMAN AND HUMAN PRIMATES

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Abstract

Much evidence indicates that gene-by-environment interactions (GxE) play a role in alcohol misuse. It has been proposed that interactions between serotonin and stress confer vulnerability for alcohol misuse. The present review examined studies of the interaction between the serotonin transporter linked polymorphic region (5-HTTLPR) genotype and stressful life events and alcohol-related phenotypes, in rhesus monkeys and humans. Ten studies were found that had investigated the interaction of 5-HTTLPR and various measures of stress and alcohol use or misuse, two studies of rhesus monkeys, and eight of humans. The results are contradictory. Important differences were reported in study samples, experimental designs, measures used to assess environmental variables, definitions and measurements of alcohol-related phenotypes, and in the statistical analyses. These differences may explain the contradictory results. Guidelines for future studies are suggested. Results are discussed in light of findings from molecular, non-human animal, and clinical studies. The review highlights the need for future studies examining associations of interactions between the serotonin transporter gene and environmental factors and alcohol misuse, especially in samples followed over time.

Keywords

• Alcohol • Alcohol use disorder • Association • Environment • Gene • Genotype • Interaction • Primates • Serotonin transporter • 5-HTTLPR

Introduction

Serotonin transporter linked polymorphic region

Serotonin is a neurotransmitter with a developmental role in the brain, from neurogenesis to differentiation of neurons in early life, and in the maintenance and plasticity of the brain in adulthood [1-3]. Termination of the serotonin signal at the synaptic level is regulated by the serotonin transporter (named 5-HTT or SERT or SLC6A4) through a reuptake mechanism [4]. In the mid-1990s, a polymorphism in the *5-HTT* gene promoter region [5], named serotonin transporter linked polymorphic region (5-HTTLPR), consisting of two common alleles made of an insertion/deletion at a 16 repeat motif with each repeat 22-23 base pair long, was shown to differentially regulate *5-HTT* transcription. The short (S) allele is associated with less (three fold) basal promoter activity compared to the long (L) allele, which leads to lower expression of *5-HTT* in individuals carrying the S allele [5]. Furthermore, 10 novel, but rarer, alleles have been discovered, along with an adenine/guanine (A/G) single nucleotide polymorphism (SNP) within the L allele, identifying two sub-genotypes, La and Lg [6]. This SNP, rs25531, was found to modify the transcriptional activity of *5-HTT,* with the L allele containing a G nucleotide substitution having equivalent activity to the S allele [7], and heterozygotes, LaLg, SLa and SLg, having intermediate activity compared to SS and LaLa [7]. Similarly, in Rhesus Macaque (*Macaca mulatta*) a polymorphism orthologous for the 5-HTTLPR, rh5-httlpr, with a similar functionality, has been identified [8,62]. Other laboratory animals such as mice lack the *5-httlpr* polymorphism [9], however partial *5-htt* knockout mice represent a rodent model for the 5-HTTLPR in humans. The links between this polymorphism and various mental disorders have been the subject of many investigations.

Serotonin transporter linked polymorphic region, gene-by-environment interactions, and alcohol misuse

To date several studies have investigated the association between 5-HTTLPR and alcohol use disorders and alcohol misuse, and results have not been consistent [10-17] While some studies failed to find any association [16,17], among the studies that show an association there is disagreement as to which allele (i.e. L or S) confers risk [10-15]. Finally a recent meta-analysis reported a significant, but modest, association between the S allele and alcohol dependence [18]. Alcohol use, misuse, abuse, and dependence index complex phenotypes that are likely to result from interactions between several genetic and environmental factors at specific times during the course of development. Further, these associations may be specific to patterns of alcohol use (use, misuse, abuse, dependence, binge drinking), and/or be dependent on age at first exposure to alcohol, and differ among males and females [11]. The differential susceptibility hypothesis proposes that specific genes act as plasticity factors so that individuals vary in their susceptibility to environmental influences [20,21]. Thus, some individuals have a higher risk of functioning poorly when challenged by stressful conditions, but they are also more likely to benefit from supportive environments [20-22].

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The S allele of the serotonin transporter gene has been suggested as a typical plasticity allele associated with increased sensitivity to environmental factors [22]. Notably, the S allele of 5-HTTLPR in interaction with negative life events was found to be related to higher levels of self-reported depression symptoms [23], a finding which has been recently confirmed in a meta-analysis [24]. Several environmental stressors, including childhood maltreatment (physical, sexual, and emotional abuse, neglect), exposure to domestic violence, parents' divorce, and poverty, have been proposed to be risk factors for alcohol use disorders [25,26]. There are several results indicating an association between 5-HTTLPR and the stress response system in humans and monkeys [27]. The first evidence that an interaction of 5-HTTLPR and stress was associated with alcohol use and misuse was shown in monkeys by Barr *et al*. in 2003 [28], and in a sample of adolescents by Nilsson and colleagues in 2005 [29]. Subsequently, a number of studies have attempted to replicate these results.

Aim

The present review examines the literature on the associations of interactions between 5-HTTLPR genotype and environmental factors and alcohol use and misuse in both non-human and human primates. The studies reviewed have adopted different methodologies and the results are contradictory, thereby limiting clear conclusions. Thus, this comprehensive review provides a systematic summary of findings in order to facilitate further investigations of gene by environment (GxE) interactions, and most specifically of the role of 5-HTTLPR and stress in conferring vulnerability for alcohol misuse.

Method

Selection of studies

A systematic PubMed search was performed with combinations of the following words: "5-httlpr / serotonin transporter / slc6a4", "non-human primates / rhesus macaques", "alcohol / ethanol", "alcohol use / misuse", "gene", and "environment". Original peer-reviewed articles, in English, published by December 2012, were selected. Subsequently, the articles were screened by hand. Studies that estimated the association of an interaction between 5-HTTPLR and an environmental factor with alcohol use were selected. Studies assessing only the association of 5-HTTPLR with alcohol use were excluded. Studies were grouped into those that examined non-human primates and those that examined humans.

Statistical analyses

No statistics were computed due to the heterogeneity of the measures used to assess environmental factors and alcohol use.

Results

Ten studies estimating the associations of an interaction between the 5-HTTLPR genotype and an environmental factor with alcohol use and misuse were found, two that examined rhesus monkeys [28,30] and eight that examined humans [29,31-37]. Descriptions of the studies are presented in Tables 1 and 2.

Gene-by-environment interactions in non-human primates

The studies of non-human primates are described in Table 1. The two studies were performed in the same laboratory. One study included 57% females [28], and the other study investigated only females [30]. The weighted mean age was 3.47 years, which may be considered as young adolescence. The weighted frequencies for the genotypes were 71% LL and 29% LS. The SS genotype was rare

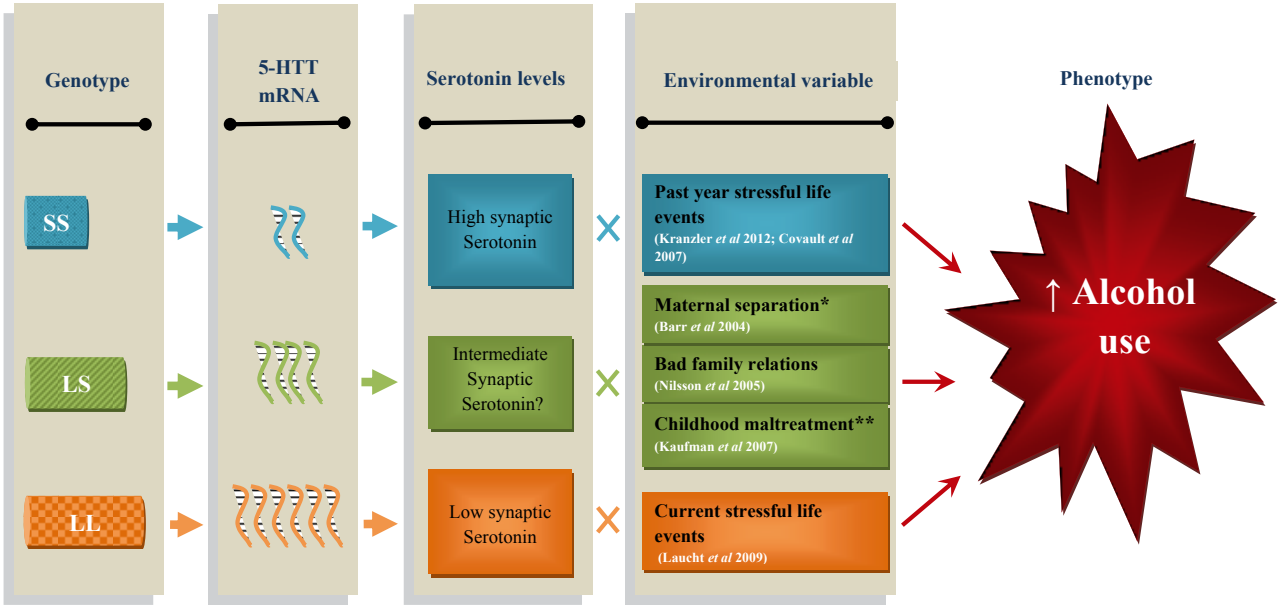

Figure 1. Do GxE studies contribute to understanding the role of serotonin in alcohol misuse?
* no individuals with the SS genotype were included in the analyses
** no individuals with the SS genotype reported a history of early experimentation with alcohol

Table 1. Studies of the association of alcohol misuse with an interaction between rh-5-HTTLPR genotypes and psychosocial factors

Author, year	Sample N (% females)	Age years (± SD)	Genotypes	Environmental factor	GxE effect	Statistical method	Comments
Barr C. *et al.* 2004 [30]	32 (100%)	3.4 (± 1)	LL, LS	Mother or peer reared	LS* peer rearing → higher preference for alcohol in females.	ANOVA	Sex effect; SS genotype not included
Barr C. *et al.* 2003 [28]	123 (57%)	3.5 (±0.2)	LL, LS	Mother or peer reared	LS* peer rearing → higher sensitivity to ethanol	Mann-Whitney U test and ANOVA	SS genotype not included

and hence it was excluded from the analyses. All animals were in cages during the experiments. Both studies compared maternal rearing to peer rearing with no maternal care for the first six months of life monkeys [28,30]. Sensitivity to alcohol intoxication [28] and preference for alcohol consumption [30] were measured. Analysis of variance was used to estimate group differences [28,30]. In one study, peer reared monkeys carrying the LS genotype showed increased sensitivity to alcohol intoxication compared to LL carriers [28]. No sex effect was detected. In the subsequent study, higher alcohol preference was observed among peer reared females carrying the LS genotype compared to those carrying the LL genotype [30].

Gene-by-environment interaction in human primates

Recruitment and designs

Studies recruited participants through flyers, advertisements, emails [34,36], questionnaires on youth psychosocial health [29,31,33,35], and from treatment programs [32,37]. Four of the eight studies of humans were longitudinal [31-33,35], and four cross-sectional [29,34,36,37]. One study was a family based study design [32].

Samples

The eight studies of humans are described in Table 2. Sample sizes ranged from 51 to 2043 individuals. Six studies included samples recruited in the community [29,31,33-36]; one study examined a clinical sample of families of probands recruited in a multi-site alcohol treatment program [32], and one assessed a sample of children removed from their parents because of maltreatment [37]. Females constituted, on average, 54.5% of the samples in seven studies. One study did not mention the proportion of females in the sample [32]. The weighted mean age of the samples was 18.4 years for 3 studies [34,36,37]. The study by Nilsson *et al.* included two different age groups, 16 and 19 years [29]. Three studies were longitudinal: one followed a sample from 15 to 22 years [31]; another followed a sample from 15.5 to 24 years [35]; and the final study followed participants from age 15 to 19 [33]. One study did not report the age of participants [32]. In six of the eight human studies, participants were Caucasian [29,33-35,38], two studies included individuals of African descent, Hispanic heritage, and Caucasians [31,37]. One study investigated a sample with participants only of African heritage (African, Afro-Carribean, and African-American) [36].

Dependent and independent variables

The genotyping techniques used were polymerase chain reaction (PCR) followed by sequence detection in four studies [29,34,36], and PCR followed by gel electrophoresis in three studies [33,35,37]. Two studies did not report the method used [31,32]. The weighted mean frequencies of the genotypes were 18.3% SS, 45.9% LS, 35.8% LL, and were consistent with the Hardy Weinberg equilibrium, except in the female sample of Vaske *et al.* [31]. Two studies also assessed the SNP rs25531 within the 5-HTTLPR [33,36], with the following weighted mean genotype frequencies 24.8% L'L' (L_AL_A), 52% L'S' (L_AS, L_AL_G), 23.2% S'S' (L_GS, L_GL_G, SS).

Environmental factors were assessed by interviews [29,31,32], questionnaires [33-36] or both [37]. Several environmental factors were investigated: past year negative life events [34,36], current life stress [33], childhood neglect [31], childhood maltreatment [37], adult family attachment [35], and overall family conditions [29]. Dick *et al.* assessed relationship stressors, being divorced, separated or widowed, and health stressors, perceived poor or good health in past one year [32]. Thus, two studies assessed stress in early life [31,37], while the others measured stress later in life and closer to the assessment of alcohol use.

The definitions and measures of the dependent variables varied greatly across studies. Four studies used questionnaires to assess alcohol use and misuse [34-37] while others used interviews [29,31-33]. Studies assessed alcohol use, binge drinking, intention to drink later the same day, frequency of alcohol drinking, and alcohol related problems. In one study, the dependent variable was categorical [31], in two other studies it was dichotomous [35,37], whereas in most other studies alcohol use was indexed by a continuous variable [33, 34,36].

Statistical analyses

The most common statistical method used to assess GxE was a regression model, specifically linear regression [33], least square regression [34], logistic regression [35], and negative binomial regression [31]. One study used both a general linear model and binary logistic model [29], one used generalized estimating equation model [37], while another one used generalized linear model with binomial probability distribution and logit link function [36]. Dick *et al.* used a pedigree disequilibrium test within each stress-group [32].

Table 2. Studies of the association of alcohol misuse and an interaction between 5-HTTLPR and SNP rs25531 genotypes with psychosocial factors
AAQ: Adult Attachment Questionnaire, ASPD: Antisocial personality disorder, AUDIT: Alcohol use disorders identification test; BD II: Beck depression inventory revised; CAGE: Cutting down, Annoyance by criticism, Guilty feeling and Eye-openers, CIS-R: Clinical Interview Schedule, Revised, CPI-So: Socialization Index of the California Psychological Inventory; DDQ: Daily Drinking Questionnaire, ICD-10: International Statistical Classification of Diseases and Related Health Problems; LESS: Life Events Scale for Students; MEL: Munich Events List (stressful life events); PEI: Personal Experience Inventory, POMS: revised version of the Profile of Mood States; TLFB: timeline follow-back (for heavy drinking) RAPI: Rutgers alcohol problem index.

First author, year	N (% Females)	Ethnical background	Age (years ± SD)	Genotypes	Environmental factors	Phenotype	GxE effect	Statistical method	Comments
Kranzler H. *et al.*, 2012 [36]	393 (58%)	African	20.1 (± 1.6)	Biallelic LL (57%), LS (36%), SS (7%) Triallelic L'L' (24%), L'S' (49%), S'S' (27%)	LESS	Risk of any drinking, heavy drinking	S' (S and Lg) * past year stressfull life events → higher alcohol consumption in females	General linear model with Binomial probability distributions and 'Logit link' function	
Vaske J. *et al.*, 2012 [31]	2,403 twins and their siblings (52%)	Caucasian Black and Hispanic	Wave I 15(±4) Wave II 17(±4) Wave III 22 (± 2.9)	LL (34%), LS (46%), SS (20%)	Childhood neglect, criminal behaviour	Alcohol and substance use (negative consequence questionnaire)	none	Negative binomial regression	failure of Hardy Weinberg's equilibrium in females
Laucht M. *et al* 2009 [33]	309 (54%)	European (Caucasian)	longitudinal 19 (n.i.)	L'L' (L_AL_A) (26%) L'S' (L_AS, L_AL_G) (56%), S'S' (L_GS, L_GL_G, SS) (19%)	MEL	TLFB	L'L' * current stressful life events → Higher frequency of binge drinking in men	Linear regression model	
Covault J. *et al.* 2007 [34]	295 (54%)	Caucasian	18.7 (± 0.8)	LL (31%), LS (49%), SS (20%)	LESS	Web based questionnaire on drinking	SS * past year negative life experiences → higher intension to drink and frequency of alcohol consumption	Least squares regression analysis	significant especially in women
Dick *et al.* 2007 [32]	882 (n.i.)	Caucasian	n.i.	n.i.	SSAGA	DSM III-R for alcohol dependence	none	Pedigree disequilibrium test	
Kaufman *et al.* 2006 [37]	127 (58%)	27% European American, 28% African American, 17% Hispanic and 28% biracial	12,5 (± 2.3)	LL (44%), LS (45%), SS (11%)	Childhood maltreatment	PEI	LS * childhood maltreatment → higher vulnerability to early alcohol use	Generalized estimated equation model	SS genotype present in 11% of sample which did not reported any history of alcohol use
Olsson C. *et al.* 2005 [35]	752 (59%)	Caucasian	Wave 0 to V 15.5 (n.i.) Wave VI 20.5(n.i.) Wave VII 24 (n.i.)	LL (32%), LS (48%), SS (20%)	CIS-R worry and anxiety subscale, AAQ	Binge drinking	S * secure attachment → less binge drinking	logistic regression	rather an association by subgroups, than a GxE effect
Nilsson K. *et al.* 2005 [29]	200 (59%)	Caucasian	16 or 19 (n.i.)	LL (31%), LS (46%), SS (21%)	Mental and psychosocial health screening questionair (SALVe)	Alcohol consumption questions from ESPAD	LS * unfavourable family conditions → higher alcohol consumption	General linear model and non parametric test	

Gene-by-environment interactions

Results of human studies were contradictory as to which genotype interacts with what environmental factor to confer vulnerability for differing measures of alcohol misuse (Table 2). Six of eight studies identified a GxE association (75%) [29,33-37]. Nilsson *et al.* found that carriers of the SL genotype who reported bad family relations also reported 12 to 14 fold higher alcohol consumption [29]. Kaufman *et al.* found that the S allele in interaction with childhood maltreatment was associated with early alcohol use. However, in this study, carriers of the SL genotype showed the greatest vulnerability to early alcohol use, and no one with the SS genotype reported a history of early experimentation with alcohol [37]. Olsson *et al.* found that young adults reporting secure attachment to their families when growing up who carried the S allele were protected against binge drinking [35]. One study of students by Kranzler *et al.* [36] reported that S' (S or Lg) in interaction with stressful life events was associated with a propensity to drink only in women, while another study of students by Convault *et al.* [34] reported that an interaction between the S allele and past year negative life events was associated with elevated levels of drinking especially among females. The sixth study, Laucht *et al.* [33], showed that an interaction between the L'L' genotype and current life stress was associated with binge drinking among males but not females [33]. Two studies detected no GxE associated with alcohol use [31,38].

Discussion

The present review examined studies of interactions between 5-HTTLPR and various measures of stress and their association with alcohol use and misuse.

Summary of gene-by-environment findings in non-human studies

Non-human primates such as rhesus macaques are the ideal choice for GxE studies because: (1) they live in complex social structures as do humans; (2) their rearing environments can be experimentally manipulated; and (3) they are genetically very close to humans, with 93% similarity, and carry the 5-httlpr polymorphism as humans and other simian primates [8,39,40]. Other laboratory animals such as mice lack the 5-httlpr polymorphism [9], and although partial *5-htt* knock-out (+/-) mice would be a good translational model, we found no published GxE study on alcohol. To date, there are two studies with monkeys of rh5-httlpr GxE in relation to alcohol use, both were conducted by the same research group and both obtained similar results. The findings indicated that the interaction between the S, less active, allele and peer rearing was associated with high alcohol preference [30], and with an increased sensitivity (higher degree of intoxication) to alcohol [28]. However, the SS genotype is rare in monkeys and consequently it was not included in the animal studies. Thus, it is presently unknown whether the presence of an additional S allele would increase the association of the interaction with stress that confers vulnerability for increased drinking and sensitivity to alcohol [28,30].

Summary of gene-by-environment findings in human studies

Studies of humans have produced conflicting results [29,33-37], or failed to find any association [31,32]. Among the studies that showed an association of alcohol use with an interaction of 5-HTLLPR and a measure of environmental stress, results are inconsistent as to the allele or genotype that was involved. In fact, three GxE studies found the low activity variants (S and Lg) to be associated with alcohol use [34-36], while two studies found the LS genotype to be associated with alcohol use, but neither SS nor LL [29,37]. However it is possible that the lack of association with the SS genotype in these two latter studies [29,37] was simply due to limited statistical power resulting from the small number of individuals carrying the SS genotype. Another study found an association with the L allele, the high activity variant [33]. Thus it remains to be determined whether it is the SS genotype, or the LS genotype, or a specific dosage of the S allele that confers vulnerability when carriers are exposed to stress and whether the GxE interactions differ by sex.

Methodological differences across studies

As highlighted by the present review, findings of an association between alcohol use and misuse in humans and an interaction of 5-HTTLPR and stress are contradictory. Moreover, the interpretation and generalizability of the results is limited by differences across studies in: (1) sample characteristics; (2) measures of alcohol use and misuse; (3) definitions and measures of stress; (4) procedures for genotyping; and (5) statistical analyses.

1. In the studies reviewed, sample characteristics differed markedly and there was no evidence that samples were representative of the populations from which they were recruited. For example, O'Malley *et al.* noted that college students differ from peers who do not attend college in alcohol use. Similarly, this study observed that alcohol use differed by sex, ethnicity, region of residence, and family conditions [41].

Sex is an important factor both from a social and a biological point of view. Olsson *et al.* showed that females reported more insecure attachment and related ruminative and somatic anxiety [35], and Vaske *et al.* showed that males were more often neglected in childhood than females [31]. Further, DNA methylation at selected cytosine-phosphate-guanine sites (CpG) of the 5-HTTLPR was reported to be higher among females than males [42]. Only three studies analyzed GxE interactions separately among males and females and two found a sex difference [33,34,36], as has been shown for other genetic markers [43]. Ethnicity is another important factor as differences have been reported in the frequencies of the 5-HTTLPR genotype and in alcohol use. One study found that alcohol misuse was more common among Caucasians than among African Americans and Hispanics [31]. These findings are in agreement with another study of college students that found a trend suggesting that Caucasians drink heavily, Blacks drink little, and Hispanics show intermediate levels of drinking [41].

2. Measures of alcohol use and misuse vary across studies, even though instruments such as the self-report Alcohol Use Disorder Identification Test (AUDIT) that have been

validated in many countries are available. Further, questionnaires and structured/semi-structured interviews may provide different results. For example, in one study adolescents reported higher alcohol consumption on interview than on questionnaires, but neither the interview nor the questionnaire estimates correlated with biological measures (phosphatidylethanol and fatty acid ethyl esters) of alcohol use [44]. Moreover, symptoms of depression should be considered as a confounding factor given that depression symptoms are often co-morbid with alcohol use disorders [45], and that the interaction of 5-HTTLPR and stress may increase risk of depression and consequently alcohol misuse [24]. Only one study has assessed whether current depression symptoms moderate the association of genotype and alcohol use and no effect was detected [34].

3. The definitions and measures of stress vary across studies, as do the time during development when the stress occurred, the severity and the duration. As shown by Covault *et al.* and Kranzler *et al.* [34,36], the use of similar measures of psychosocial stress and alcohol use lead to consistent results, despite differences in the ethnicity of the samples. Similarly, two studies [29,37] reported that measures of stress within the family, poor family relations or childhood maltreatment, in interaction with LS genotype were associated with increased alcohol consumption or a lower age at first use of alcohol. Measures such as the Adult Attachment Questionnaire (AAQ) [35] and self-reports of family relationships [29] yielded different results, as shown by the findings that high secure attachment [35] and bad family conditions were associated with high risk drinking [29].

4. Different genotyping methods were used in the studies reviewed, among which the most common was polymerase chain reaction (PCR) followed by gel electrophoresis. However, the target promoter region of the 5-HTT has a high GC content and is difficult to genotype. Indeed, the L allele is sometimes hard to detect with gel electrophoresis, and heterozygotes can be genotyped as SS [46]. Hence, to ensure validity, results need to be confirmed using another unrelated technique. This was not done in any of the studies that were reviewed. Furthermore, a more robust functional bases for the activity of 5-HTT has been indicated in the tri-allelic system of 5-HTTLPR and rs2553, but only two studies assessed the tri-allelic genotype of 5-httlpr and SNP rs25531 [33,36]. However, it is also important to note that the LgLg genotype is present among only ~0.9% of the population [46]. Results of a recent study show that the "Lg" allele has no effect on 5-HTT mRNA transcription [42], in contrast with previous results [7]. Thus, the role of this polymorphism requires further exploration.

5. Statistical methods to assess GxE effects have been debated. Commonly, main and interaction effects are estimated using general linear models (GLM), as in linear regression analyses. The GLM is ideal when the dependent variable presents a Gaussian distribution, either interval or ratio, and preferably when the predictor variables also show similar distributions. However, in most studies of alcohol use the dependent variable is on an ordinal scale that does not present a Gaussian distribution. Most commonly a log-transformation is used to correct the distribution of the dependent variable. Other studies use cut-off scores to create a dichotomous dependent variable and logistic regression models to estimate associations. One advantage of logistic regression models is that they are more assumption-free than linear models. However, the use of logistic regression models to test GxE interactions has recently been questioned. The main effects of the genetic and the environmental factors and of the interaction term may be described as exponential or multiplicative. Therefore some authors have stated that there is no interaction when additive effects on a relative risk, relative rate, or relative odds scale are detected [47]. GxE occur when the effect of exposure to an environmental factor on a dependent variable is conditional on a specific genotype [48]. Such interaction effects are larger than the sum of the effects of the genetic and environmental risk factors. The relative excess in risk due to interaction (RERI) test has been suggested as a useful estimate distinct from additive effects on a relative risk [47].

Neuropsychobiology of the serotonin transporter linked polymorphic region and alcohol use

The exact mechanism by which GxE interactions are associated with alcohol use remains elusive. Findings from murine *5-htt* knock-out, clinical, and molecular studies may contribute to furthering understanding of the role of serotonin in alcohol use and help unravel discrepancies in the results of studies of GxE.

Serotonin plays a pivotal role in brain development (2), and postnatally *5-HTT* knockout mice, especially females [49], show decreased cell apoptosis [50], and mice deficient in monoamine oxidase A gene (Maoa) display elevated levels of brain serotonin, and defective barrel formation in somatosensory cortex [1]. Moreover, heterozygous knock-out mice, *5-HTT* (+/-), who received good maternal care showed increased levels of serotonin [51], while maternal separation was associated with lower expression of *5-HTT* and serotonin receptors in male rats [52]. Knocking out the *5-HTT* gene in rats and mice results in decreased alcohol intake [53], and increased sensitivity to the sedative effects of alcohol [54]. In order to better understand 5-HTTLPR-by-stress effects on alcohol use, studies examining ethanol intake and binge-like drinking of partial *5-htt* (+/-) knockout mice exposed to environmental stressors, such as maternal separation, may be potentially useful.

Clinical studies indicate that within the monoaminergic systems, the serotonergic system is the most affected by alcohol use [55], and pharmacological challenges show dampened serotonergic neurotransmission following excessive alcohol use [56]. Single-photon emission computed tomography of alcohol dependent patients has shown reduced levels of 5-HTT in brain [57], consistent with studies of rats who prefer alcohol [58]. However, a recent study showed increased mRNA expression in lymphoblasts of patients with a history of alcohol dependence [43]. More recently, reduced serotoninergic function, assessed as prolactin response to citalopram induced inhibition of serotonin reuptake, was associated with experiencing maltreatment in childhood, especially among alcohol

dependent patients [59]. Thus, paradoxically, S allele has been associated *in vitro* with higher serotonin levels, while alcohol use disorders have been associated with lower serotonin levels in the brain or peripherally [3]. However, no association between 5-HTTLPR and concentration of the serotonin metabolite 5-HIAA in cerebrospinal fluid (CSF) was found in healthy adults [60]. Interestingly, a non-significant trend was identified among individuals under treatment for alcohol misuse disorders such that S carriers displayed lower 5-HIAA in CSF than LL carriers [61], and a study of primates showed lower 5-HIAA in CSF of LS compared to LL animals but only among those who had been peer-reared [62].

Studies of the simultaneous association between the 5-HTTLPR polymorphism and level of the respective protein, and variability in the number of neurons which express the transporter, are lacking [3], thereby limiting conclusions regarding the functional aspects of how this polymorphism regulates serotonin levels and the consequences for alcohol use. So far, only one study using single photon emission computed tomography brain imaging reported a non-significant trend for lower 5-HTT availability in LS compared to LL healthy adults [63]. However, as observed in the case of *MAOA gene*, MAOA expression levels assessed in transfected cell line and autopsy samples do not correlate with each other or with *MAOA*-uVNTR genotype [64]. Similarly, another study with autopsy samples found no association between *MAOA*-uVNTR and gene expression or enzyme activity [65]. It is tempting to speculate that the S allele, that renders decreased availability of 5-HTT transcript, may show neurobiological and behavioural effects similar to those observed among *5-HTT* knockout mice. Knocking out *Maoa* in early development has a strong impact on serotonin levels, which may normalize with age [66], and a similar effect that dissipates with age may also occur with 5-HTT. Additionally, the time during development when GxE interactions are measured, and/or occur, may influence the results. Moreover, the difference in neuronal cell populations in males and females that have been observed in *5-HTT* knockout mice [49], may explain sex differences reported in studies of the association between alcohol use and interactions of the 5-HTTLPR with stress.

To further understanding of the psychological and neurobiological underpinnings of alcohol misuse, it is thus necessary to integrate molecular studies into GxE studies, as elegantly discussed by Szyf when writing about how environments "talk" to genes [67]. As seen in animal studies, environmental factors like maternal care affect the methylation of DNA and thereby regulate gene expression. Importantly, this mechanism can be reversed [68]. Alcohol exposure causes changes in epigenetic make-up, as shown, for example, by rats injected with ethanol who displayed increased histone acetylation [69]. Recently, human studies have investigated epigenetic modifications of specific DNA regions associated with alcohol use disorders. Philbert *et al*. [42] reported no effect of average DNA methylation in CpG island on 5-HTT mRNA expression in lymphoblasts of a clinical sample, and another study found similar results in alcohol dependent subjects [70]. However Philibert *et al*. reported effects for a few specific CpGs, suggesting a potential effect on functioning of *5-HTT* gene assessed by mRNA expression [42]. A study of macaques showed that animals with S 5-httlpr allele have higher CpG methylation and lower 5-htt expression in peripheral blood mononuclear cells [71]. The same study showed no effect of early life stress on average methylation of the 5-htt gene, but provided suggestive evidence that selected CpGs near to the transcription start site showed higher methylation resulting from early life stress [71]. It has been observed that mother reared macaques carrying the S allele show higher Histone H3 trimethyl Lys4 binding [72]. Importantly, the study by Philibert *et al.* [42] suggests that sex influences 5-HT mRNA expression and CpG methylation, thus emphasizing the importance of taking account of sex in GxE studies. Which factor, a specific allele, epigenetic changes, or both drive the outcome? Studies that include both molecular and GxE perspectives are needed to increase understanding of alcohol use [67]. This view is supported by a recent investigation showing that polymorphisms in the glucocorticoid regulator gene, *FKBP5,* alone cannot predict post-traumatic stress disorder. Instead, an interaction of the high risk allele with childhood trauma was associated with demethylation of the gene and increased risk for PTSD. This study also highlights the fact that stress produced changes in DNA methylation of *FKBP5* in undifferentiated but not in differentiated cells, implying that stress in early life has a more profound effect on epigenetic mechanisms than stress in later life [67]. Thus, as reviewed by Nordquist *et al.* [3] investigations of the neurotrophic role of serotonin during development are needed.

Guidelines

The present literature review summarizes knowledge of the association of alcohol use with the interaction between 5-HTLLPR and stress. This is a first step in assessing the generalizability of findings that may differ depending on the definition and measurement of the phenotype, specific type, duration, and timing of stress, statistical analyses, and sample characteristics. There has been a bias in the publication of studies within the field of psychiatric genetics, as pointed out recently by Duncan and Keller. Replications of findings in large independent samples are needed, as is a standardization of methods [73]. Dunn *et al.* 2011 made a series of recommendations for GxE studies on depression [74]. Similar recommendations could be adopted for future GxE studies of alcohol use: (1) rigorous reporting of environmental and genetic data including comparisons for all genotypes and reporting of all parameters included in the statistical analysis, as well as main and interaction effects; (2) improved validity of definitions and measures of alcohol use and validation with clinical diagnoses if possible; (3) use of rigorous study designs, longitudinal, experimental or quasi-experimental; (4) use of robust methods to assess environmental factors, taking into account variables such as the social environment, and covariates such as sex, age, and ethnicity; and (5) use of large samples in order to have sufficient power and testing genetic data for Hardy-Weinberg equilibrium. Finally, it is important to aggregate findings across studies of both animals and humans.

Conclusion

The present review summarized findings on the interaction of 5-HTLLPR and stress and alcohol use in human and non-human primates. Differences in results were identified as were differences in methods and measures. Recommendations for future studies of GxE interactions were presented, and the necessity for integrating measures of molecular mechanisms as well as of partial *5-htt* knock-out animal models into studies of the role of GxE interactions in alcohol use.

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