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ORIGINAL RESEARCH

Association of Variants of Arginine Vasopressin and Arginine Vasopressin Receptor 1A With Severe Acetaminophen Liver Injury

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SUMMARY

Acetaminophen (APAP)-related acute liver injury/liver failure (ALF) often appears to occur in the setting of substance abuse. We identified two single nucleotide polymorphisms previously associated with drug use disorder in a carefully adjudicated group of APAP ALF patients.

BACKGROUND & AIMS: Acetaminophen-related acute liver injury and liver failure (ALF) result from ingestion of supratherapeutic quantities of this analgesic, frequently in association with other forms of substance abuse including alcohol, opioids, and cocaine. Thus, overdosing represents a unique high-risk behavior associated with other forms of drug use disorder.

METHODS: We examined a series of 21 single nucleotide polymorphisms (SNPs) in 9 genes related to impulsivity and/or stress responsivity that may modify response to stress. Study subjects were 229 white patients admitted to tertiary care liver centers for ALF that was determined to be due to acetaminophen toxicity after careful review of historical and biochemical data. Identification of relevant SNPs used Sanger sequencing, TaqMan, or custom microarray. Association tests were carried out to compare genotype frequencies between patients and healthy white controls.

RESULTS: The mean age was 37 years, and 75.6% were female, with similar numbers classified as intentional overdose or unintentional (without suicidal intent, occurring for a period of several days, usually due to pain). There was concomitant alcohol abuse in 30%, opioid use in 33.6%, and use of other drugs of abuse in 30.6%. The genotype frequencies of 2 SNPs were found to be significantly different between the cases and controls, specifically SNP rs2282018 in the arginine vasopressin gene (*AVP*, odds ratio 1.64) and SNP rs11174811 in the AVP receptor 1A gene (*AVPR1A*, odds ratio 1.89), both of which have been previously linked to a drug use disorder diagnosis.

CONCLUSIONS: Patients who develop acetaminophen-related ALF have increased frequency of gene variants that may cause altered stress responsivity, which has been shown to be associated with other unrelated substance use disorders. *(Cell Mol Gastroenterol Hepatol 2017;3:500–505; http://dx.doi.org/10.1016/j.jcmgh.2017.01.008)*

Keywords: Impulsivity; Stress Responsivity; Pituitary-Adrenal Axis; Overdose.

Acetaminophen (APAP)-induced liver injury is the most common cause of acute liver failure (ALF) in American adults,[1] with nearly 500 deaths annually attributed to excessive dosing of this ubiquitous pain reliever. The safe upper limit for daily dosing has variably been thought to be 4000 mg/day or possibly less.[2] Excessive APAP dosing can occur with intent of self-harm (suicide attempt) or unintentionally while seeking pain relief, typically for a period of several days by using increased amounts each day.[3,4] Early on, an association was made between alcohol abuse and APAP unintentional overdoses, referred to initially as therapeutic misadventure.[5] Both intentional and unintentional APAP overdoses have been shown to be associated with substance abuse of both alcohol and opioids, particularly the opioid (hydrocodone/acetaminophen) combination products.[6] In 1 study of 275 APAP-related ALF patients, 55% had a history of alcohol use and 35% a history of alcohol abuse, and 53% had taken an opioid-APAP combination product.[4] In a follow-up study of a larger cohort of 306 APAP toxicity survivors, 55% had a history of psychiatric disease compared with only 27% of non-APAP survivors, 46% of APAP patients had a history of prior substance abuse vs 15% of non-APAP patients, and 14% had a specific history of injection drug use vs 8% for the non-APAP group.[7] Understanding what determines these behaviors might lead to better identification of high-risk patients and development of prevention strategies.

With either intentional or unintentional etiologies, critical behavioral differences could lead to high-risk behaviors.

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Abbreviations used in this paper: **ALF, acute liver failure; ALFSG, Acute Liver Failure Study Group; APAP, acetaminophen; SNP, single nucleotide polymorphism.**

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Impulsivity is a character trait defined as "acting suddenly in an unplanned manner to satisfy a desire," ie, not thinking things through to understand the potential impact of a decision. More recently, impulsivity has been recognized as a measurable trait that is correlated with a variety of addictive behaviors.[8] We have recently shown that higher impulsivity scores on the Barratt impulsivity scale are detected in both intentional and unintentional APAP overdoses when compared with control populations (Sanders C, Lee WM, unpublished data, submitted for publication).

During the last 15 years numerous single nucleotide polymorphisms (SNPs) have been found to be associated with addictive behavior, including impulsivity.[8–10] Many of these SNPs are in genes associated with the stress response, including the genes involved in hormonal systems of the hypothalamic/pituitary/adrenal axis.[11–18] For example, the μ-opioid receptor gene, *OPRM1*, has polymorphisms, specifically A118G, that have been linked to increased vulnerability/susceptibility to alcohol and opioid addiction.[11,14,15,19] The μ-opioid receptor is used by both exogenous and endogenous opiates and can exert control over stress responsivity, addiction, and withdrawal. We hypothesized that this polymorphism or others that are related may have an impact in patients with APAP overdoses, especially in patients who have multiple substance dependencies. Furthermore, we speculated that the genetic association may be stronger or more apparent in subjects with intentional APAP overdose compared with those with nonintentional overdose. Thus, variations in several of these genes that alter the host's ability to respond to stressful situations may indeed correlate with the observed tendency of addictive behavior.

The Acute Liver Failure Study Group (ALFSG) (ClinicalTrials.gov: NCT00518440) has been prospectively identifying and studying the etiologies, presenting features, and clinical outcomes of adults with ALF during the past 17 years while collecting biosamples—serum, plasma, and DNA. During this time period, ALFSG has enrolled more than 3000 subjects in its registry, 46% of whom suffered from severe or fatal APAP liver injury. Because stress and the response to stress are closely linked to addictive behavior and APAP toxicity patients have frequently been identified as substance abusers, we sought to determine whether specific variants in stress-related genes are over-represented in an APAP overdose cohort compared with population controls. By using DNA from 229 of these APAP subjects, we sought to identify whether selective variants in genes related to stress/impulsivity and addiction are associated with APAP overdose cases, because these findings might shed light on the patients' tendency to abuse APAP products.

Methods

Patients

Among the 1669 subjects who were enrolled in the ALFSG registry database between January 1, 2002 and December 31, 2013 according to principles established in the initial ALFSG report,[1] we selected a consecutive group of patients with unequivocal APAP in whom DNA had also been collected as part of enrollment. Subjects in the overall adult ALFSG registry were enrolled from 33 academic centers in the United States and met criteria for ALF, namely coagulopathy (international normalized ratio $\geq$ 1.5) and grade 1–4 of hepatic encephalopathy, within 26 weeks of the first symptoms, without overt underlying liver disease. Because patients enrolled are by definition encephalopathic, written informed consent was obtained from the legal next of kin in each case. Demographic, clinical, laboratory, radiologic, and outcomes data were recorded prospectively. For the purpose of the present study, we selected only patients who self-reported as white.

Etiologic diagnoses were made by each study site's primary investigator on the basis of the history and clinical presentation and laboratory, radiographic, and, when available, liver biopsy results. Further adjudication was provided by using an algorithm for confirmation of APAP overdoses developed by the ALFSG causality subcommittee. The algorithm includes the following specific criteria: a history of APAP ingestion, detection of APAP in plasma, biochemical pattern that is consistent with APAP toxicity with high serum aminotransferase levels ($\geq$1000 IU/L) along with total bilirubin $<$10.0 mg/dL at presentation, and, if available, presence of APAP-Cys adducts. These criteria have been validated previously in the ALFSG cohort.[4] All subjects were considered to fit the patient phenotype as highly likely/definite APAP toxicity if they met the criteria for history of ingestion and/or parent compound detectable, with appropriate biochemistries as outlined.

Intentionality (intentional/suicidal/single time point vs unintentional [typically for pain relief, suicide denied]) was determined by the site investigator on the basis of findings outlined in Schiødt et al.[3] The control subjects (n = 208) were healthy volunteers ascertained by the Laboratory of the Biology of Addictive Diseases at the Rockefeller University who were previously genotyped on a custom Illumina addiction array for other studies.[13,17]

Genotyping

Genes/single nucleotide polymorphism selection. Nine genes were selected on the basis of their known involvement with impulsivity and/or stress responsivity. A total of 21 SNPs from these 9 genes were selected on the basis of previous reports of potential functionality and/or association with impulsivity and stress responsivity (Table 1). These SNPs were chosen because they were previously found to be associated with heroin and/or cocaine dependence or show changes in their gene expression when exposed to the drug. The μ-opioid receptor (*OPRM1)* variants, rs1799971 (A118G) and rs1799972 (C17T), have previously been linked to both alcohol and heroin addiction.[14,15,19,20] We have previously shown the catechol-O-methyltransferase (*COMT*) variants, rs4680 and rs4818, to be associated with opioid dependence.[21] The variants in FK506 binding protein 5 (*FKBP5*), galanin (*GAL*), arginine vasopressin (*AVP*), arginine vasopressin receptor 1A (*AVPR1A*), corticotropin releasing factor (*CRH*), and CRH receptors 1 and 2 (*CRHR1* and *CRHR2*) were chosen for study because they have been found to be associated with a greater vulnerability for dependence, or

Table 1. List of SNPs Evaluated

Gene	SNP	CHR	Position	Location	Alleles	MAF	Model	p_0	EMP1	EMP2	OR (95% CI)
AVP	rs2282018	20	3064949	Intronic	T/C	0.42	Dom	**.028**	0.030	0.258	1.89 (1.07–3.34)
	rs2740204	20	3062467	Intronic	C/A	0.40	Dom	.104	0.099	0.832	
AVPR1A	rs11174811	12	63540476	3′ UTR	C/A	0.18	Dom	**.018**	0.015	0.252	1.64 (1.08–2.50)
	rs3021529	12	63545680	5′ UTR	C/T	0.15	Dom	.053	0.044	0.589	
COMT	rs4818	22	19951207	Intronic	C/G	0.41	Rec	.383	0.357	0.999	
	rs4680	22	19951271	Intronic	G/A	0.49	Rec	.829	0.821	1	
CRH	rs6996265	8	67086350	3′UTR	G/A	0.11	Rec	.181	0.141	0.948	
	rs3176921	8	67091379	Promoter	T/C	0.11	Rec	.115	0.102	0.727	
	rs6472257	8	67092180	Promoter	G/A	0.10	Rec	.181	0.141	0.948	
CRHR1	rs81189	17	43894798	Intronic	G/C	0.22	Dom	.554	0.566	1	
	rs242939	17	43895579	Intronic	T/C	0.49	Dom	.989	0.999	1	
	rs8072451	17	44282654	Intronic	C/T	0.07	Dom	.543	0.522	1	
CRHR2	rs2284217	7	30713608	Intronic	C/T	0.22	Dom	.106	0.112	0.842	
FKBP5	rs3800373	6	35542476	3′ UTR	A/C	0.31	Dom	.366	0.364	0.999	
	rs7757037	6	35548236	Intronic	G/A	0.45	Dom	.580	0.595	1	
	rs1360780	6	35607571	Intronic	C/T	0.32	Dom	.358	0.356	0.999	
	rs9470080	6	35646435	Intronic	C/T	0.33	Dom	.265	0.293	0.993	
GAL	rs694066	11	68452985	Intronic	C/T	0.06	Dom	.405	0.390	1.000	
	rs3136541	11	68457943	Intronic	T/C	0.33	Dom	.138	0.119	0.909	
OPRM1	rs1799971	6	154360797	Intronic	A/G	0.13	Rec	.418	0.479	1.000	

NOTE. The two p_o values in bold are the only associations found to be nominally significant. OR above 1 represents risk effect of the major allele. Alleles are listed as major/minor.
Chr, chromosome; CI, confidence interval; DOM, dominant; EMP1, point-wise empirical *P* value obtained by permutation; EMP2, empirical *P* value that controls for multiple SNPs; MAF, minor allele frequency; OR, odds ratio; p_o, technical *P* value obtained from logistic regression; REC, recessive.

they are in high-linkage disequilibrium with an SNP that was found to be associated with addiction.[22]

Genotyping

The *OPRM1* SNPs, both located in exon 1, were genotyped by Sanger sequencing a 300–base pair region of the DNA that was first amplified by polymerase chain reaction. Each sequence was manually evaluated for determination of the genotype at the 2 *OPRM1* variants. Genotyping of the remaining variants for the case samples was performed by using TaqMan SNP genotyping assays (Life Technologies, Carlsbad, CA) following the manufacturer's recommended protocol. The end-point reactions were scanned and then analyzed on an ABI Prism 7900HT Sequence Detection System (Life Technologies). All TaqMan genotype data were visually inspected for quality. The control samples were genotyped by using a custom Illumina array as described.[17]

Statistical Analysis

Association tests were carried out between the following groups: intentional vs unintentional overdoses, unintentional vs controls, and all overdose cases (intentional, unintentional, and unknown) vs controls with PLINK v 1.9. Deviations from Hardy-Weinberg equilibrium were checked by χ^2 test with a critical limit of significance of *P* values $<.001$ (taking into account Bonferroni correction for multiple SNPs). In addition, SNPs with a minor allele frequency of less than 0.05 were excluded from further analysis. Association analysis was carried out by logistic regression that considered 3 models of inheritance: recessive, dominant, and additive. The nominal significance level was computed according to 2 different methods within each association model: first, from logistic regression (*p0*) based on the asymptotic distribution of the test statistic (*t* from Wald test); second, by permutation analysis (EMP1) that compares the observed statistic with 100,000 statistics obtained in permutations in the correspondent SNP. To control the family-wise error rate when testing multiple SNPs, the observed statistic was compared with the maximum of permuted statistics over all SNPs (EMP2).

Results

The study group met stringent criteria for APAP diagnosis to be included (Table 2). In addition to clinical criteria, all 43 that were tested were positive for the presence of APAP-Cys adducts, confirming a toxic ingestion.[23,24] Selected clinical data are shown in Tables 2 and 3, divided by intentional, unintentional, and unknown overdose type as described above. The proportion giving a history of alcohol and/or drug abuse or having positive toxicology screening was similar to prior studies. Overall, recorded evidence for abuse of alcohol, opiates, or other drugs of

Table 2. Age, Gender, and History of Opioid Use, Other Drug Abuse, and Alcohol Use or Abuse for the Overall APAP Group and Subgroups

	All (N = 229)		Intentional (N = 99)		Unintentional (N = 98)		Unknown (N = 32)	
Age, *y* (median)	37		34		38		50	
	N	%	N	%	N	%	N	%
Gender (female)	173	75.6	73	73.7	77	78.6	23	71.8
Opioid use	77	33.6	19	19.2	45	45.9	13	59.4
Other drug abuse	70	30.6	33	33.3	28	28.6	9	28.1
EtOH use	84	36.7	33	33.3	40	40.8	11	34.4
EtOH abuse	69	30.1	27	27.3	33	33.7	9	28.1

NOTE. Of the 229 APAP subjects, 197 were classifiable as intentional or unintentional, with 32 remaining as unknown type. EtOH abuse, >4 drinks daily or >14 drinks per week; EtOH (alcohol) use, 1–4 drinks daily or <14 per week (16 mg/drink); other drug abuse, history or toxicology screen positive for cocaine, benzodiazepines, other non-specified polysubstances.

abuse (principally cocaine) was greater than 30%. Among intentional cases, 34 of 99 were believed to have used only 1 of the 3 categories of drugs of abuse listed, 18 took 2, and 3 were positive in all 3 categories. Likewise, among the unintentional group, 45 were found to have used only 1 drug of abuse, 23 had taken 2 classes, and 5 were found to have used 3 types of abuse agents.

The SNPs genotyped are listed in Table 1. Of these, one SNP, *OPRM1* rs1799972 (C17T), was excluded on the basis of minor allele frequency <.05. None of the SNPs in the study significantly violated Hardy-Weinberg equilibrium. Two SNPs, rs2282018 in *AVP* and rs11174811 in *AVPR1A*, showed significant association of genotype with APAP overdose when all overdose cases were compared with controls (Table 1, bold highlight). The SNPs are in an intron and the 3' untranslated region, respectively, on chromosome 12. The common alleles, T for *AVP* SNP rs2282018 (frequency, 0.58) and C for *AVPR1A* SNP rs11174811 (frequency, 0.72), are the risk alleles. The significant associations were detected under dominant model of inheritance with odds ratios of 1.89 and 1.64, respectively. No other significant associations were detected, and there were no differences observed when the unintentional group was considered separately and compared with the intentional overdose or control groups (data not shown).

Discussion

In the present study, we analyzed genotypes of carefully characterized APAP overdose subjects who had experienced severe liver injury, because both unintentional and intentional overdosing appears to represent high-risk behaviors. The 2 SNPs we identified in the *AVP* gene and the gene of one of its receptors, *AVPR1A,* have previously been associated with drug use disorders.[16,17] One of these studies[17] used the same control sample that was used in the current study but a different case sample. We did not observe differences in the gene frequency between the intentional and unintentional groups. This could relate to the relatively small sample sizes, or that both groups, as noted, are associated with drug use disorders and impulsivity.[4,6,7]

Analysis of a limited and select group of candidate systems of genes that are associated with drug use behavior allowed us to narrow our focus, providing a wider approach than specific targeted SNPs but a narrower one than conventional genome-wide association studies. *AVPR1A* receptors are located throughout the brain and may serve several functions, but they specifically are associated with stress responsivity, how the pituitary-adrenal axis responds to stress. *AVPR1A* variants have been studied extensively and show strong associations with several social behaviors including sibling conflict and autism.[16,25–29] The *AVPR1A*

Table 3. Laboratory Data for the Same APAP Groups

	All			Intentional			Unintentional			Unknown		
	N	Median	Mean	N	Median	Mean	N	Median	Mean	N	Median	Mean
AST (*IU/L*)	229	5734	6933	99	6333	7670	98	5423	6441	32	4123	6158
ALT (*IU/L*)	229	4867	5779	99	5864	6573	98	4538	5424	32	3810	4412
T Bili (*mg/dL*)	229	3.9	4.5	99	3.9	4.1	98	3.9	4.0	32	6.4	7.1
APAP level (*mg/L*)	217	32.0	79.6	96	69.3	119.4	91	24.0	44.1	30	42.0	59.7
Adduct[a] (*nmol/mL*)	43	9.9	13.0	21	11.0	15.9	19	7.4	9.7	3	17.1	14.5

NOTE. Values >1 nmol APAP-Cys/mL are considered significant and specific for APAP hepatotoxicity.[20]
ALT, alanine aminotransferase; AST, aspartate aminotransferase; T Bili, total bilirubin.
[a]Adduct levels are measured by high-pressure chromatography with electrochemical detection.

SNP rs11174811 identified in the current study is specifically thought to be associated with potential seed recognition sites for microRNAs miR-526b and miR-578 and has been shown to be functional in vitro.[28] Disruption of microRNA binding by the *AVPR1A* rs11174811 risk allele appears to confer poorer response to stress as shown in a variety of settings.[16] The functionality of *AVP* SNP rs2282018 is unknown; however, arginine vasopressin ligands and receptors compose the pituitary axis for at least some forms of stress responses.[29]

Although highly popular, APAP is a dose-related toxin with a relatively narrow therapeutic window, resulting in frequent overdosing in certain settings such as unrelieved pain and where the perception is of its great safety. The tendency to display impulsive behavior and to use 1 or more additional substances including alcohol and the combination opioid/acetaminophen products has been well-recognized, conferring increased risk associated with both suicidal and unintentional overdoses.[4,6,7] Subjects in the present study were selected for the specific phenotype of APAP ALF by using a detailed algorithm developed by the ALFSG, supplemented by the APAP adduct assay, to provide a high degree of certainty that APAP toxicity was present and caused the liver failure in the patients studied.[23,24] It is noteworthy that most of the SNPs examined encode for stress responsivity and the tendency to addictive behaviors across the drug use disorder spectrum: alcohol, opiates, and cocaine.[16]

Several unrelated SNPs have been identified that encode for increased sensitivity to APAP toxicity, involving polymorphisms in either the cytochrome P450 or UDP-glucuronosyltransferase genes.[30,31] These SNPs were not examined here. They might be important in certain patients who are susceptible to liver injury after ingesting less than 4 g/day, the maximum accepted level for safe APAP ingestion; however, this remains controversial. Such patients are infrequent and unlikely to account for the relatively common occurrence of ALF that is in association, for the most part, with documented overdoses of significant proportion.[5]

We conclude that APAP ALF patients, who are known to have a propensity for impulsivity and high-risk behavior, have higher frequency of certain genetic variants associated with drug use disorders. These variants have been previously shown to relate in part to altered stress responsivity. This clinical association in our population is largely confined to the *AVP* and *AVPR1A* gene loci.[16,17,29] These initial observations should now be confirmed in a larger study of carefully selected APAP overdose patients.

References

1. Ostapowicz G, Fontana RJ, Schiodt FV, et al. Results of a prospective study of acute liver failure at 17 tertiary care centers in the United States. Ann Intern Med 2002; 137:947–954.
2. Acetaminophen: background and overview. Meeting material from the June 29, 2009 joint meeting of the Drug Safety and Risk Management Advisory Committee with the Anesthetic and Life Support Advisory Committee and Nonprescription Drug Advisory Committee 2009. Silver Spring, MD: Food and Drug Administration, 2009, Available at: http://www.fda.gov/AdvisoryCommittees/Calendar/ucm143083.htm. Accessed January 19, 2016.
3. Schiødt FV, Rochling FJ, Casey DL, et al. Acetaminophen toxicity in an urban county hospital. N Engl J Med 1997;337:1112–1117.
4. Larson AM, Fontana RJ, Davern TJ, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatology 2005;42:1364–1372.
5. Seeff L, Cuccerini BA, Zimmerman HJ, et al. Acetaminophen hepatotoxicity in alcoholics: a therapeutic misadventure. Ann Intern Med 1986;104:399–404.
6. Lee WM. Acetaminophen hepatotoxicity: changing perceptions of a social/medical issue. Hepatology 2007; 46:966–970.
7. Fontana RJ, Ellerbe C, Durkalski VE, et al. Two-year outcomes in initial survivors with acute liver failure: results from a prospective, multicenter study. Liver Int 2015;35:370–380.
8. Kreek MJ, Nielsen DA, Butelman ER, et al. Genetic influences on impulsivity, risk-taking, stress responsivity, and vulnerability to drug abuse and addiction. Nat Neurosci 2006;8:1450–1457.
9. Kreek MJ, Levran O, Reed B, et al. Opiate addiction and cocaine addiction: underlying molecular neurobiology and genetics. J Clin Invest 2012;122:3387–3393.
10. Reed B, Butelman E, Yuferov V, et al. Genetics of opiate addiction. Curr Psychiatry Rep 2014;16:504.
11. Bond C, LaForge KS, Tian M, et al. Single-nucleotide polymorphism in the human Mu opioid receptor gene alters β-endorphin binding and activity: possible implications for opiate addiction. Proc Natl Acad Sci 1998; 95:9608–9613.
12. Kreek MJ. Role of a functional human gene polymorphism in stress responsivity and addictions. Clin Pharmacol Ther 2008;83:615–618.
13. Levran O, London O, O'Hara K, et al. Genetic susceptibility to heroin addiction: a candidate gene association study. Genes Brain Behavior 2008;7:720–729.
14. Bart G, Kreek MJ, LaForge S, et al. Increased attributable risk related to a functional m-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. Neuropsycho-pharmacology 2005;30: 417–422.
15. Bart G, Heilig M, LaForge S, et al. Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. Molecular Psychiatry 2004; 9:547–549.
16. Maher BS, Vladimirov VI, Latendresse SJ, et al. The AVPR1A gene and substance abuse disorders: association, replication and functional evidence. Biol Psychiatry 2011;70:519–527.
17. Levran O, Peles E, Randesi M, et al. Stress-related genes and heroin addiction: a role for a functional FKBP5 haplotype. Psychoneuroendocrinology 2014;45:67–76.
18. Levran O, Peles E, Randesi M, et al. Dopaminergic pathway polymorphisms and heroin addiction: further

support for association of casein kinase 1ε variants. Pharmacogenomics 2014;16:2001–2009.
19. Kroslak T, LaForge S, Gianotti RJ, et al. The single nucleotide receptor polymorphism A118G alters functional properties of the mu opioid receptor. J Neurochem 2007;103:77–87.
20. Crystal HA, Hamon S, Randesi M, et al. A C17T polymorphism in the mu opiate receptor is associated with quantitative measures of drug use in African American women. Addict Biol 2012;17:181–191.
21. Levran O, Randesi M, da Rosa JC, et al. Overlapping dopaminergic pathway genetic susceptibility to heroin and cocaine addictions in African Americans. Ann Hum Genet 2015;79:188–198.
22. Reed B, Butelman HR, Yuferov V, et al. Genetics of opiate addiction. Curr Psychiatry Rep 2014;16:504–510.
23. Davern TJ II, James LP, Hinson JA, et al. Measurement of serum acetaminophen-protein adducts in patients with acute liver failure. Gastroenterology 2006;130:687–694.
24. Khandelwal N, James LP, Sanders C, et al. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. Hepatology 2011;53:567–576.
25. Meyer-Lindenberg A, Kolachana B, Gold B, et al. Genetic variants in AVPR1A linked to autism predict amygdala activation and personality traits in healthy humans. Mol Psychiatry 2009;14:968–975.
26. Bachner-Melman R, Zohar AH, Bacon-Shnoor N, et al. Link between vasopressin receptor (AVPR1A) promoter region microsatellites and measures of social behavior. J Individ Differ 2005;6:2–10.
27. Walum H, Westberg L, Henningsson S, et al. Genetic variation in the vasopressin receptor 1a gene (AVPR1A) associates with pair-bonding behavior in humans. Proc Natl Acad Sci U S A 2008;105:14153–14156.
28. Nossent AY, Hansen JL, Doggen C, et al. SNPs in microRNA binding sites in 3′-UTRs of RAAS genes influence arterial blood pressure and risk of myocardial infarction. Am J Hypertension 2011;24:999–1006.
29. Kreek MJ, Zhou Y, Levran O. Functions of arginine vasopressin and its receptors: importance of molecular genetic studies in bidirectional translational research. Biol Psychiatry 2011;70:502–503.
30. Court MH, Freytsis M, Wang X, et al. The UDP-glucuronosyltransferase (UGT) 1A polymorphism c.2042C>G (rs 8330) is associated with increased human liver acetaminophen glucuronidation, increased UGT1A Exon 5a/5b splice variant mRNA ratio, and decreased risk of unintentional acetaminophen-induced acute liver failure. J Pharmacol Exp Ther 2013;345:297–307.
31. Court MH, Peter I, Hazarika S, et al. Candidate gene polymorphisms in patients with acetaminophen-induced acute liver failure. Drug Metab Dispos 2014;42:28–32.

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