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Association of Alcohol Use Disorder Risk With *ADH1B*, *DRD2*, *FAAH*, *SLC39A8*, *GCKR*, and *PDYN* Genetic Polymorphisms

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Abstract. *Background/Aim: Alcohol use disorder (AUD) is a chronic, multifactorial psychiatric condition with an enormous impact on public health and social cost. Genetic studies suggest a heritability, and genome-wide association studies (GWAS) have revealed genetic polymorphisms influencing AUD development. Our study aimed to investigate known variants located in ADH1B, DRD2, FAAH, SLC39A8, GCKR, and PDYN genes (rs1229984, rs7121986, rs324420, rs13107325, rs1260326, rs2281285 respectively) in an AUD Greek cohort in order to shed more light on the genetic predisposition to AUD. Materials and Methods: Alcohol-dependent individuals (n=251) meeting both the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) and the ICD-10 guidelines for alcohol abuse and dependence, and control individuals (n=280) were recruited. DNA was extracted from whole blood and PCR-restriction fragment length polymorphism (RFLP-PCR) or allele-specific PCR method was used for genotyping. Results: Individuals carrying the FAAH rs324420 A allele were significantly associated with increased risk of AUD ($p<0.0001$). SLC39A8 rs13107325 T allele and ADH1B rs1229984 T allele are overrepresented in control subjects ($p<0.0001$ and $p<0.0001$, respectively). The associations are maintained following an adjustment for age and sex and Bonferroni correction. GCKR rs13107325, DRD2 rs7121986, and PDYN rs2281285 polymorphisms did not show a significant association with AUD in the studied population after Bonferroni correction. Conclusion: Susceptibility to AUD is related to variations in FAAH, ADH1B, and SLC39A8 genes. These polymorphisms could serve as potential biomarkers for AUD risk.*

Alcohol use disorders (AUDs) are characterized by uncontrolled alcohol consumption, compulsive drinking, and negative feelings during alcohol withdrawal that can lead to a chronic and relapsing course (1). AUD is described as a single spectrum of problematic use and clinically significant impairment based on endorsement of at least two of the 11 criteria that assess behavioral and physical manifestations of heavy alcohol consumption by the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) (2). Current estimates suggest that 5.6% of individuals met the AUD criteria during the previous year, leading to important socioeconomical issues and public health losses (3, 4). Recently, data showed that the total quantity of lifetime alcohol consumption and the combination of drinking frequency combined with the amount consumed per incident augment the risk of alcohol-related harm, in a dose-

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dependent manner (5). For instance, a causal and dose-related link between AUD and various types of cancer has been proposed mostly in the gastrointestinal system, breast, and larynx (6).

The prevalence of AUD is increased among high/upper middle-income countries in both sexes. Approximately 3 million deaths annually (5.3% of all deaths) are due to alcohol abuse, along with more than 5% of the disease burden globally as WHO data suggests (1). Nevertheless, alcohol use and its effects vary remarkably across countries. The European Union (EU) represents the region with the most increased alcohol consumption globally; 87% of the adolescent's drink at least once in their life. This percentage is higher than that of the American adolescents which is 70% (7). According to WHO, in 2016, the prevalence of alcohol abuse in the Greek population was 9.4% for males and 2.9% for females, while alcohol dependence was 4.2% and 1.3%, respectively (8), indicating that AUDs in Greece are not as common as in North Europe. During the past two decades, nationwide trends present a decline in alcohol use; however, the frequency of alcohol intake by Greek teenagers remains one of the highest in Europe (9). Although AUDs present a warning prevalence and an economic burden (that affects the individuals, their families, and the society) no more than 20% of individuals seek or receive any treatment (10). Even worse, only a small fraction of the affected individuals is prescribed medication with demonstrated efficacy in heavy drinking reduction or abstinence promotion (11).

The pathophysiology of AUDs, as other chronic multifactorial diseases, is attributed to the combination of genetic risk and environmental factors (12). Strong predictors of AUD initiation could be the endophenotypes that are to an extent heritable and include an individual's response to alcohol and neurobiological susceptibilities (13). A key for developing an effective treatment for AUD could be the deep understanding of its pathophysiological mechanisms (14). The substantial genetic component of alcohol consumption guides the efforts to recognize specific variants across the genome related to AUD, since the heritability of the disease is estimated as high as 50% (2). A plethora of variants have been identified by genome-wide association studies (GWAS) (15-19), with most studies using alcohol consumption (*e.g.*, drinks/week), since it represents an easily assessable measurement.

A twin study found a single-nucleotide polymorphism (SNP)-based heritability of AUD in more than a third of patients (15). Accumulated data from GWAS showed various chromosomal loci to be associated with a greater AUD risk, with some of them presenting significant reproducibility among studies. The most profound connections found in GWAS were the functional polymorphisms in two enzymes affecting alcohol metabolism: alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*) (19-24).

Among variants, *ADH1B* (rs1229984) has been unequivocally linked to the etiology of alcohol addiction (17, 25, 26). A significant conclusion was that missense polymorphisms in *ADH1B* were associated with AUD in two independent populations (15). An additional implicated gene is the glucokinase receptor gene (*GCKR*) that regulates cellular trafficking in liver cells. The SNP rs1260326 in *GCKR* was robustly correlated to alcohol intake (*i.e.*, drinks/week) in large-scale GWAS; this coding missense SNP has been linked with over 25 metabolic traits including type II diabetes. (15, 17-19, 26). To date, another gene locus, (rs7121986) in *DRD2* gene has been linked to AUD and other addiction phenotypes, but not to alcohol consumption (17). *DRD2* represents a biologically plausible candidate for alcohol dependence susceptibility as shown by *in vivo* and *in vitro* experiments (27); since affected DRD2 expression results in heterogenous responses to substances (28) bearing a high addiction risk (26). A few GWAS have linked the rs13107325 polymorphism, located in the Zn transporter gene *SLC39A8*, to AUD in European populations (17, 26). *SLC39A8*, one of the most pleiotropic genes, is involved in several biological processes [blood pressure (29), BMI (30), Crohn's disease (31), serum levels of Mn (32), HDL-cholesterol (33), and schizophrenia (34)]; however, its role in AUD is not sufficiently studied. The *FAAH* rs324420 variant has been linked to substance use disorders, such as cannabis dependence and evidence suggests that altered FAAH activity could influence alcohol use (35, 36), even though the findings are heterogeneous and complex (37-40). The above-mentioned variant has ancestry-specific effects, suggesting variable results between different populations (36). Likewise, various polymorphisms in *PDYN* are implicated in the risk for alcohol (41) opioid and cocaine dependence (42, 43). *PDYN* could be a biologically plausible candidate for substance use disorders, as this gene encodes dynorphins (DYNs), which belong to the opioid peptide family and are crucial regulators in a plethora of brain pathways. Specifically, *PDYN* rs2281825 has been correlated with depression symptoms in heroin addicted (44) and alcohol negative craving (45) but the findings remain inconsistent (46).

Most of the aforementioned gene polymorphisms have been linked to AUD susceptibility *via* the available GWAS. Although some of these SNPs have been already studied in multiple genetic studies, the results are inconsistent in different populations and therefore, their role remains to be elucidated. In an attempt to validate previous genetic associations with AUD, our study aimed to investigate known gene polymorphisms located in *ADH1B, DRD2, FAAH, SLC39A8* and *GCKR* genes (rs1229984, rs7121986, rs324420, rs13107325, rs1260326, respectively) in an AUD Greek cohort in order to shed more light on AUD genetic risk.

Materials and Methods

Study population. This case-control study included a total of 531 participants of Greek origin, 251 were alcohol-dependent individuals (cases) and 280 healthy individuals (controls), recruited from the Psychiatric Hospital of Attica (PHA). Cases attended a treatment inpatient program for alcohol dependence during the current study. Diagnostic assessments were determined using the Greek version of the International Neuropsychiatric Interview (M.I.N.I.) clinical inventory (47). Eligible participants met the DSM-IV as well as the ICD-10 criteria for alcohol abuse and dependence, whereas cases with comorbid major mental disorders, such as major depression, bipolar disorder, and schizophrenia were excluded. Furthermore, a family history of alcoholism was recorded. Alcohol dependence severity was estimated through the co-assessment of factors such as the total duration of alcohol use in years, as well as the amount of daily use/abuse of alcohol in years. The non-alcoholic control group was recruited from local communities. All healthy controls were exposed to alcohol but did not report any harmful use or alcohol dependence. A history of drug abuse (except nicotine) and major psychiatric disorders were excluded following the completion of self-report questionnaires. All participants provided written informed consent. The study was conformed to the Declaration of Helsinki.

Genotyping. Genomic DNA was extracted from peripheral whole blood using the NucleoSpin Blood Kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. The quality and concentration of purified DNA was estimated using the NanoDrop 8000 Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA).

Genotypes for the rs324420 (*FAAH* gene), rs1260326 (*GCKR* gene) rs1229984 (*ADH1B*), and rs2281285 (*PDYN*) were determined using the PCR-restriction fragment length polymorphism (RFLP-PCR) method. The primer sequences for rs324420 were forward primer: 5'-GGA AGT GAA CAA AGG GAC CA-3' and reverse primer: 5'-AAT GAC CCA AGA TGC AGA GC-3 (size 204 bp). The PCR products were digested by the restriction enzyme StyI (New England BioLabs, Ipswich, MA, USA) resulting in two fragments of 133 and 71 bp in CC genotype, whereas in the presence of the A allele, products remained uncut as a single fragment of 204 bp (48).

For the rs1260326, PCR was conducted using the forward primer: 5'-TGC AGA CTA TAG TGG AGC CG-3' and reverse: 5'-CAT CAC ATG GCC ACT GCT TT-3' followed by digestion with HpaII restriction enzyme (Fermentas, Burlington, ON, Canada). When the C allele was present, digestion resulted in 18, 63, and 150 bp fragments; whereas the TT genotype gave two fragments of 18 and 213 bp (49).

Primers used for the rs1229984 genotypes were forward: 5' ACA ATC TTT TCT GAA TCT GAA CAG CTT CTC and reverse: 5' TTG CCA CTA ACC ACG TGG TCA TCT GCG. The PCR product (97 bp) was digested by Hin6I restriction enzyme (Fermentas International Inc.). The PCR product was cut in 70 bp and 27 bp restriction fragments where the common C allele was present. The rs2281285 was genotyped as described previously by Hashemi *et al*. (46).

Genotyping of the rs13107325 (*SLC39A8*) and rs7121986 (*DRD2*) was performed by allele specific PCR. The sequences of the primers were as follows: Common: 5' ACTTTGTGATCCTACT 3', C allele: 5' TATAATATTTGGAGC 3', T allele: 5' TATAATATTTGGAGC 3' for the rs13107325 and common 5' GTAGAAGAAAACATGAATGC 3' allele-specific C: 5' TGGCCTGCCTTCTCCAC 3', T: 5' GGCCTGCCTTCTCCAT 3' for the rs7121986.

Briefly, PCR for all SNPs was carried out in a total volume of 25 μl, containing 100 ng of genomic DNA, 2.5 μl of 10×PCR buffer, 0.5 μmol/l of each primer, 0.15 mmol/l of dNTP, 1.5 mmol/l of MgCl2, and 1 U of Taq DNA polymerase (Kappa Biosystems, Cape Town, South Africa).

Statistical analysis. The association between SNP genotypes and alcoholic status was evaluated using the SNPStats tool applying a chi-squared test (50). A Bonferroni corrected *p*-value was applied to the multifactorial analysis *p*-values to account for the multiple testing of six different SNPs in the same samples (corrected α=0.05/6=0.008). Hardy-Weinberg equilibrium was tested separately in patients and healthy controls using the Fischer's exact test. $p \leq 0.05$ (two-sided) was considered significant.

Table I. *Characteristics of alcohol-dependent individuals and nondependent controls*.

Characteristics	Alcohol-dependent individuals (n=251)	Non-alcohol dependent controls (n=280)	*p*-Value
Sex, N (%)			
Males	191 (76.1)	188 (67.14)	0.39
Females	60 (23.9)	92 (32.86)	0.11
Age, years			
Mean±SD	43.5±11.5	42.8±14.3	0.54
Range	26-62	21-68	
Familial history (n)	88 (35.06%)		
Alcohol consumable, years (mean±SD)	20.46±8.1		
Alcohol abuse, years (mean±SD)	11.93±6.67		
Drug use history (n)	15		

Results

Two hundred fifty-one (251) alcohol-dependent individuals, 191 males (76%) and 60 females (24%) with a mean age of 43.5±11.5 years (range=26-62 years) were recruited. The healthy control group consisted of two hundred eighty (280) non-alcohol dependent subjects with an average age of 42.8±14.3 years (range=21-68 years), 188 males (67%) and 92 females (33%). A positive family history of alcoholism in first degree relatives was reported by 88 cases (35%). Within cases, the average duration of alcohol use was 20.46±8.1 years, while the average daily alcohol use and abuse was 11.93±6.67 years. The demographic and clinical characteristics of participants are presented in Table I. The allele frequencies were in Hardy-Weinberg equilibrium in both patients and control groups (p>0.05). Table II depicts genotype distributions for each SNP in alcohol-dependent

Table II. *Genotype frequencies and allele distribution between alcohol-dependent individuals and controls.*

Gene, SNP	Alcohol-dependent individuals (%)	Non-alcohol dependent Controls (%)	OR (95%CI)	*p*-Value*
FAAH, rs324420				
CC	128 (50.99)	189 (67.5)	1	
AC	108 (43.03)	86 (30.71)	0.54 (0.37 - 0.77)	0.001*
AA	15 (5.98)	5 (1.79)	0.23 (0.08-0.64)	0.004*
C allele	364 (72.51)	464 (82.86)	1	
A allele (effect allele)	138 (27.49)	96 (17.14)	0.55 (0.41-0.73)	<0.0001*
GCKR, rs1260326				
CC	60 (23.90)	84 (30)	1	
CT	113 (45.01)	130 (46.43)	0.82 (0.54-1.25)	0.39
TT	78 (31.07)	66 (23.57)	0.60 (0.38-0.96)	0.04
C allele	233 (46.41)	298 (53.21)	1	
T allele (effect allele)	269 (53.59)	262 (46.79)	0.76 (0.59-0.97)	0.03
SLC39A8, rs13107325				
CC	185 (73.70)	135 (48.21)	1	
CT	61 (24.30)	123 (43.93)	2.76 (1.89-4.04)	<0.0001*
TT	5 (1.99)	22 (7.86)	6.03 (2.23-16.33)	<0.0001*
C allele (effect allele)	431 (85.86)	393 (70.18)	1	
T allele	71 (14.14)	167 (29.82)	2.58 (1.89-3.52)	<0.0001*
ADH1B, rs1229984				
CC	165 (65.74)	143 (51.07)	1	
CT	73 (29.08)	105 (37.50)	1.66 (1.14-2.41)	0.008
TT	13 (5.18)	32 (11.43)	2.84 (1.43-5.62)	0.002*
C allele (effect allele)	403 (80.28)	391 (69.82)	1	
T allele	99 (19.72)	169 (30.18)	1.76 (1.32-2.34)	<0.0001*
DRD2, rs7121986				
CC	141 (56.17)	167 (59.64)	1	
CT	89 (35.46)	102 (36.43)	0.97 (0.67-1.39)	0.93
TT	21 (8.37)	11 (3.93)	0.44 (0.21-0.95)	0.04
C allele	371 (73.90)	436 (77.86)	1	
T allele (effect allele)	131 (26.10)	124 (22.14)	0.80 (0.61-1.07)	0.15
DPYN, rs2281285				
AA	150 (59.76)	192 (68.57)	1	
AG	96 (38.25)	85 (30.36)	0.69 (0.48-0.99)	0.05
GG	5 (1.99)	3 (1.07)	0.47 (0.11-1.99)	0.47
A allele	396 (78.88)	469 (83.75)	1	
G allele (effect allele)	106 (21.12)	91 (16.25)	0.72 (0.53-0.99)	0.04

*Significant after Bonferroni correction ($p<0.008$).

and non-dependent healthy individuals. *FAAH* rs324420 A allele was found to be significantly associated with increased risk of AUD ($p<0.0001$). This association remained significant and after Bonferroni correction. A marginal association was observed between *GCKR*, rs1260326 TT genotype and AUD ($p=0.04$); however, this association did not remain significant after Bonferroni correction. *SLC39A8* rs13107325 T allele, and *ADH1B* rs1229984 T allele were over-represented in non-alcohol dependent controls ($p<0.0001$, and $p<0.0001$ respectively), and remained significant after Bonferroni correction, suggesting that *SLC39A8* rs13107325 C allele and *ADH1B* rs1229984 C allele are risk alleles for AUD. Regarding *DRD2* rs7121986 polymorphism, even if there is a marginal association before Bonferroni correction of TT genotype and AUD ($p=0.04$), the presence of the T allele was not found to be associated with AUD risk. For *DPYN* rs2281285, there is a marginal association before Bonferroni correction of the G allele and AUD ($p=0.04$); however, this association was not significant after Bonferroni correction.

It is important to note that, the same trends were maintained following an adjustment for age and sex (Table III) and after Bonferroni correction.

Discussion

Alcohol use disorder is a chronic, complex, multifactorial psychiatric condition with an enormous impact on public health and social cost. The likelihood of AUD is possibly attributed to environmental and genetic risk factors (12), yet

Table III. *Genotype distribution between alcohol-dependent individuals and controls adjusted for sex and age.*

Model	Genotype	Alcohol-dependent individuals (n=251)	Non-alcohol dependent controls (n=280)	OR (95%CI)	*p*-Value*
	FAAH, rs324420				
Codominant	C/C	128	189	1.00	
	A/C	108	86	0.55 (0.38-0.79)	3×10^{-4}*
	A/A	15	5	0.24 (0.08-0.68)	
Dominant	C/C	128	189	1.00	
	A/C – A/A	123	91	0.51 (0.36-0.73)	2×10^{-4}*
Recessive	C/C – A/C	236	275	1.00	
	A/A	15	5	0.30 (0.10-0.85)	0.015
Overdominant	C/C – A/A	143	194	1.00	
	A/C	108	86	0.60 (0.41-0.86)	0.005*
	GCKR, rs1260326				
Codominant	C/C	60	84	1.00	
	C/T	113	130	0.91 (0.59-1.39)	0.091
	T/T	78	66	0.61 (0.38-0.99)	
Dominant	C/C	60	84	1.00	
	C/T – T/T	191	196	0.78 (0.53-1.16)	0.22
Recessive	C/C – C/T	173	214	1.00	
	T/T	78	66	0.65 (0.44-0.97)	0.032
Overdominant	C/C – T/T	138	150	1.00	
	C/T	113	130	1.16 (0.82-1.65)	0.4
	SLC39A8, rs13107325				
Codominant	C/C	185	135	1.00	
	C/T	61	123	2.58 (1.76-3.80)	<0.0001*
	T/T	5	22	5.46 (1.99-14.96)	
Dominant	C/C	185	135	1.00	
	C/T – T/T	66	145	2.80 (1.93-4.07)	<0.0001*
Recessive	C/C – C/T	246	258	1.00	
	T/T	5	22	3.87 (1.42-10.53)	0.003*
Overdominant	C/C – T/T	190	157	1.00	
	C/T	161	123	2.29 (1.57-3.35)	<0.0001*
	ADH1B, rs1229984				
Codominant	C/C	165	143	1.00	
	C/T	73	105	1.60 (1.09-2.34)	0.0016*
	T/T	13	32	2.88 (1.43-5.77)	
Dominant	C/C	165	143	1.00	
	C/T – T/T	86	137	1.79 (1.25-2.56)	0.0014*
Recessive	C/C – C/T	238	248	1.00	
	T/T	13	32	2.43 (1.23-4.82)	0.008
Overdominant	C/C – T/T	178	175	1.00	
	C/T	73	105	1.41 (0.97-2.04)	0.071
	DRD2, rs7121986				
Codominant	C/C	141	167	1.00	
	C/T	89	102	0.90 (0.62-1.30)	0.048
	T/T	21	11	0.39 (0.18-0.84)	
Dominant	C/C	141	167	1.00	
	C/T – T/T	110	113	0.80 (0.56-1.14)	0.21
Recessive	C/C – C/T	230	269	1.00	
	T/T	21	11	0.40 (0.19-0.87)	0.017
Overdominant	C/C – T/T	162	178	1.00	
	C/T	89	102	0.98 (0.68-1.41)	0.92
	DPYN, rs2281285				
Codominant	A/A	150	192	1.00	
	A/G	96	85	0.72 (0.50-1.04)	0.13
	G/G	5	3	0.43 (0.10-1.85)	
Dominant	A/A	150	192	1.00	
	A/G – G/G	101	88	0.70 (0.49-1.01)	0.058
Recessive	A/A – A/G	246	277	1.00	
	G/G	5	3	0.48 (0.11-2.05)	0.31
Overdominant	A/A – G/G	155	195	1.00	
	A/G	96	85	0.73 (0.51-1.06)	0.098

*Significant after Bonferroni correction ($p<0.008$).

the underlying pathophysiology of AUD remains poorly understood. Genetic components seem to be crucial in AUD pathogenesis with heritability estimated to be approximately 50% (51, 52). However, identifying genetic risk variants remains a challenge, mostly because of the large genetic and clinical heterogeneity, and the vast number of the implicated variants, which are only partially responsible for the total disease risk (53).

Linkage studies failed to identify specific risk alleles associated with AUD due to its complex, polygenetic pathobiology; thus, GWAS represent the most efficient approach to reveal associated SNPs (52). GWAS of AUD or excessive drinking using various assessment methods have successfully uncovered contingent risk genes (52). Recent large-scale GWAS and meta-analyses including many thousands of participants have revealed associations between AUD susceptibility and common genetic variants in *GCKR* (15-19, 26), *ADH1B* (15-19, 26), *DRD2* (16, 18, 26), and *SLC39A8* (16-19) gene loci.

In the current study, we aimed to clarify the associations between known DNA polymorphisms located in *ADH1B, DRD2, FAAH, SLC39A8, GCKR,* and *PDYN* genes and disease risk in a Greek cohort of AUD cases. The findings showed a robust association between *FAAH* rs324420, SLC39A8 rs13107325 and *ADH1B* rs1229984 polymorphisms and AUD. A recent systematic review concluded that FAAH protein product (fatty acid amide hydrolase) contributes to the biology and clinical features of AUD; the pharmaceutical targeting of this molecule could be effective for alcohol withdrawal as it may reduce anxiety and alcohol intake reinstatement (54). Fatty acid amide hydrolase metabolizes the endogenous cannabinoid anandamide (AEA), which regulates the brain reward signaling, thus possibly leading to increased addiction susceptibility (55). *FAAH* variant Pro129Thr (rs324420), reduces FAAH catalytic activity and influences the addictive properties of several substances (36). The association of rs324420 with substance use disorders (56) is in accordance with earlier studies reporting genetic associations with the use of methamphetamine (48), marijuana (57), cannabis (35), and cocaine (58). Regarding alcohol dependence, similarly with our results, Sloan *et al*. concluded that American European adults (mean age: 39 years) carrying the A allele had a significantly increased frequency of compulsive drinking episodes and increased AUDIT scores as opposed to individuals with the CC genotype (36). Similarly, Best *et al*. suggested that both AC and AA genotypes of the *FAAH* rs324420 polymorphism are responsible for abnormal drinking behaviors and increased AUDIT scores in youths (59). Animals carrying the polymorphism have displayed higher alcohol intake and alcohol dependence severity (60), while pharmacological inhibition of *FAAH* increased anandamide levels and ethanol intake (61).

Regarding, *GCKR* rs1260326 polymorphism even if this locus has consistently been linked to AUD through GWAS, in contrast to previous studies, in our population, *GCKR* rs1260326 T allele was found to be slightly over-presented in AUD cases compared to controls; however, this association was not significant (15, 17, 18). This discrepancy, maybe due to the differences between different ethnic groups or/and, the limitation of our study regarding the number of participants. *GCKR* regulates cellular trafficking in liver cells. Its polymorphism rs1260326 has also been implicated in diverse metabolic diseases (62, 63). Since alcohol intake is connected to metabolic and lipid profiles alike, it remains to be elucidated whether *GCKR* rs1260326 may have a functional impact on metabolic diseases or alcohol influences glucose and lipid metabolism depending on *GCKR* genotype.

SLC39A8, one of the most pleiotropic genes, is implicated in various pathophysiological pathways and has been linked to schizophrenia (64, 65), inflammatory bowel disease (31), cardiovascular (29, 66), and metabolic phenotypes (30). A functional study by Evangelou *et al*. indicated the pivotal role of *SLC39A8* in alcohol consumption in Drosophila. Furthermore, the same group suggested a significant association of SNP rs13107325 in the *SLC39A8* gene and putamen volume differences (16). Interestingly, evidence from animal studies has linked putamen with alcohol consumption (67). In accordance with our results, Thompson *et al*. (26) reported the SNP rs13107325 C allele as risk allele for alcohol consumption in European ancestry populations. Other member of the SLC39 family have also been associated to mental disorders, as in the case of *SLC39A3* that is associated with bipolar disorder and *SLC39A11* with depressive disorder, implying that disrupted transport of metal ions could disturb brain homeostasis (65).

Another genetic locus widely studied in conjunction with alcohol addiction is *DRD2*, coding for dopamine D2 receptor. The central dopaminergic system is believed to have a vital role in the development of addiction to a plethora of psychoactive substances such as opiates, cocaine, nicotine, and alcohol. *DRD2* encodes a receptor of the post-synaptic dopaminergic neurons; its down-regulation has been implicated with alcohol craving stimulating the medial prefrontal cortex (27). Earlier studies of the dopamine receptors have indicated that common *DRD2* polymorphisms (-141C Ins/Del, TaqI B, and TaqI A) are associated with alcohol dependence risk (68-73). Subsequent meta-analyses have robustly showed a link between polymorphisms near *DRD2* and alcohol dependence (18, 74); however, this gene only exhibits a mild effect that could be partially explained *via* publication bias influenced by racial ancestry (74). A recent GWAS meta-analysis suggested another intronic variant, the rs7121986, located in *DRD2*, to be associated with alcohol intake (16). Our study did not find an association with AUD risk in the Greek population. To the best of our

knowledge, no other genetic association study has been performed to investigate rs7121986 in alcohol dependence.

The most extensively investigated and well-replicated risk alleles for AUD are located in the *ADH* gene locus. There are several isoforms of *ADH* involved in liver alcohol metabolism (*ADH1A*, *ADH1B*, *ADH1C*, and *ADH4-7*). Alcohol dehydrogenase isoform 1B (*ADH1B*) is an important ethanol-oxidizing enzyme but is also involved in multiple molecular mechanisms and metabolic processes of several molecules such as fatty acids, acetone, epinephrine, glucose, and neurotransmitters (for instance serotonin and noradrenaline) (75, 76). The *ADH1B* rs1229984 is associated with alcohol-flush reaction; individuals with the rs1229984 X-allele present a higher ADH activity leading to an increased rate of alcohol metabolism (77). Consistent with previous studies that characterize the T allele as protective (21, 23, 78), our results confirm the decreased risk of developing AUD among those carrying the minor rs1229984 T-allele. Of note, this association is also obvious across different populations (17), such as individuals of European (15, 16, 26), European American (24), African American (23), and Asian origin (78-80).

The last gene examined in our study was *PDYN*. Either animal experimental studies or postmortem brain human studies have indicated that the dynorphin system has a noteworthy contribution in alcohol and substance addiction. DYNs are enriched in brain circuits affecting mood, motivation, and stimulus-response (habit) and have been associated to drug-seeking behavior (81, 82). The dynorphin (DYN)/k-opioid receptor (KOR) system could be responsible for unpleasant feelings and emotions and affect the motivational parameters of stress by inducing anhedonia, dysphoria, pain, and aversion in humans and animals (45, 83). Various polymorphisms in *PDYN* have been studied for their correlation to substance addiction (41, 84), but most recently, the rs2281285 has been further discussed for its role in alcohol dependence and negative craving (45, 83, 85). Our results showed no statistical association between this SNP and AUD and are in accordance with the results of Xuei *et al*. Although Karpyak *et al*. detected an association between alcohol dependence and rs2281285 (85), results could not reach statistical significance after correction. However, the role of this SNP in negative craving is well established (45) and may present a sex-specific effect (83). Of note, haplotypes including rs2281285 SNP are linked to both alcohol dependence and negative craving, suggesting its involvement with these disorders (85); thus, further studies are needed to elucidate the exact role of this SNP.

In conclusion, our study demonstrated that rs324420 in *FAAH*, rs13107325 in *SLC39A8*, and rs1229984 in *ADH1B* are linked with AUD susceptibility in a Greek population. These polymorphisms could serve as potential biomarkers for AUD risk; however, taking into consideration the complexity of AUD pathogenesis and the variety of the genetic and environmental factors involved, further case-control studies including increased population size and of different origin are needed to confirm these findings.

Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

Authors' Contributions

EL, AH, MG analyzed the data and wrote the paper. DT, ES, MP, GM, VM, LL collected the samples and clinical data. DT, MG, NS designed the study. MG, AH and NS reviewed and revised the paper. All Authors contributed to the article and approved the submitted version.

References

1 Carvalho AF, Heilig M, Perez A, Probst C and Rehm J: Alcohol use disorders. Lancet *394(10200)*: 781-792, 2019. PMID: 31478502. DOI: 10.1016/S0140-6736(19)31775-1

2 Deak JD, Miller AP and Gizer IR: Genetics of alcohol use disorder: a review. Curr Opin Psychol *27*: 56-61, 2019. PMID: 30170251. DOI: 10.1016/j.copsyc.2018.07.012

3 Bouchery EE, Harwood HJ, Sacks JJ, Simon CJ and Brewer RD: Economic costs of excessive alcohol consumption in the U.S., 2006. Am J Prev Med *41(5)*: 516-524, 2011. PMID: 22011424. DOI: 10.1016/j.amepre.2011.06.045

4 Rehm J, Gmel GE Sr, Gmel G, Hasan OSM, Imtiaz S, Popova S, Probst C, Roerecke M, Room R, Samokhvalov AV, Shield KD and Shuper PA: The relationship between different dimensions of alcohol use and the burden of disease-an update. Addiction *112(6)*: 968-1001, 2017. PMID: 28220587. DOI: 10.1111/add.13757

5 Rehm J, Baliunas D, Borges GL, Graham K, Irving H, Kehoe T, Parry CD, Patra J, Popova S, Poznyak V, Roerecke M, Room R, Samokhvalov AV and Taylor B: The relation between different dimensions of alcohol consumption and burden of disease: an overview. Addiction *105(5)*: 817-843, 2010. PMID: 20331573. DOI: 10.1111/j.1360-0443.2010.02899.x

6 LoConte NK, Brewster AM, Kaur JS, Merrill JK and Alberg AJ: Alcohol and Cancer: A statement of the American Society of Clinical Oncology. J Clin Oncol *36(1)*: 83-93, 2018. PMID: 29112463. DOI: 10.1200/JCO.2017.76.1155

7 World Health Organization (WHO), Moeller, Lars, Galea, Gauden. Regional Office for Europe. (2012). Alcohol in the European Union: consumption, harm and policy approaches/edited by Peter Anderson, Lars Moeller and Gauden Galea. Copenhagen: WHO Regional Office for Europe. Available at: https://apps.who.int/iris/handle/10665/107301 [Last accessed on October 10, 2021]

8 World Health Organization (WHO) (2016) Alcohol Consumption Greece. Available at: https://www.euro.who.int/__data/assets/pdf_file/0004/402187/ACHP_FS_Greece.pdf [Last accessed on October 10, 2021]

9 Tsoumakas K, Tanaka M, Petsios K, Fildisis G, Gkoutzivelakis A and Pavlopoulou I: Alcohol drinking habits and negative

experiences among adolescents in Greece. Open Journal of Pediatrics *04(03)*: 222-230, 2017. DOI: 10.4236/ojped.2014.43029

10 Grant BF, Goldstein RB, Saha TD, Chou SP, Jung J, Zhang H, Pickering RP, Ruan WJ, Smith SM, Huang B and Hasin DS: Epidemiology of DSM-5 alcohol use disorder: Results from the National Epidemiologic Survey on Alcohol and Related Conditions III. JAMA Psychiatry *72(8)*: 757-766, 2015. PMID: 26039070. DOI: 10.1001/jamapsychiatry.2015.0584

11 Hartwell EE and Kranzler HR: Pharmacogenetics of alcohol use disorder treatments: an update. Expert Opin Drug Metab Toxicol *15(7)*: 553-564, 2019. PMID: 31162983. DOI: 10.1080/17425255.2019.1628218

12 Fairbanks J, Umbreit A, Kolla BP, Karpyak VM, Schneekloth TD, Loukianova LL and Sinha S: Evidence-based pharmacotherapies for alcohol use disorder: Clinical pearls. Mayo Clin Proc *95(9)*: 1964-1977, 2020. PMID: 32446635. DOI: 10.1016/j.mayocp.2020.01.030

13 Salvatore JE, Gottesman II and Dick DM: Endophenotypes for alcohol use disorder: an update on the field. Curr Addict Rep *2(1)*: 76-90, 2015. PMID: 26236574. DOI: 10.1007/s40429-015-0046-y

14 Jones JD, Comer SD and Kranzler HR: The pharmacogenetics of alcohol use disorder. Alcohol Clin Exp Res *39(3)*: 391-402, 2015. PMID: 25703505. DOI: 10.1111/acer.12643

15 Clarke TK, Adams MJ, Davies G, Howard DM, Hall LS, Padmanabhan S, Murray AD, Smith BH, Campbell A, Hayward C, Porteous DJ, Deary IJ and McIntosh AM: Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112 117). Mol Psychiatry *22(10)*: 1376-1384, 2017. PMID: 28937693. DOI: 10.1038/mp.2017.153

16 Evangelou E, Gao H, Chu C, Ntritsos G, Blakeley P, Butts AR, Pazoki R, Suzuki H, Koskeridis F, Yiorkas AM, Karaman I, Elliott J, Luo Q, Aeschbacher S, Bartz TM, Baumeister SE, Braund PS, Brown MR, Brody JA, Clarke TK, Dimou N, Faul JD, Homuth G, Jackson AU, Kentistou KA, Joshi PK, Lemaitre RN, Lind PA, Lyytikäinen LP, Mangino M, Milaneschi Y, Nelson CP, Nolte IM, Perälä MM, Polasek O, Porteous D, Ratliff SM, Smith JA, Stančáková A, Teumer A, Tuominen S, Thériault S, Vangipurapu J, Whitfield JB, Wood A, Yao J, Yu B, Zhao W, Arking DE, Auvinen J, Liu C, Männikkö M, Risch L, Rotter JI, Snieder H, Veijola J, Blakemore AI, Boehnke M, Campbell H, Conen D, Eriksson JG, Grabe HJ, Guo X, van der Harst P, Hartman CA, Hayward C, Heath AC, Jarvelin MR, Kähönen M, Kardia SLR, Kühne M, Kuusisto J, Laakso M, Lahti J, Lehtimäki T, McIntosh AM, Mohlke KL, Morrison AC, Martin NG, Oldehinkel AJ, Penninx BWJH, Psaty BM, Raitakari OT, Rudan I, Samani NJ, Scott LJ, Spector TD, Verweij N, Weir DR, Wilson JF, Levy D, Tzoulaki I, Bell JD, Matthews PM, Rothenfluh A, Desrivières S, Schumann G and Elliott P: New alcohol-related genes suggest shared genetic mechanisms with neuropsychiatric disorders. Nat Hum Behav *3(9)*: 950-961, 2019. PMID: 31358974. DOI: 10.1038/s41562-019-0653-z

17 Kranzler HR, Zhou H, Kember RL, Vickers Smith R, Justice AC, Damrauer S, Tsao PS, Klarin D, Baras A, Reid J, Overton J, Rader DJ, Cheng Z, Tate JP, Becker WC, Concato J, Xu K, Polimanti R, Zhao H and Gelernter J: Genome-wide association study of alcohol consumption and use disorder in 274,424 individuals from multiple populations. Nat Commun *10(1)*: 1499, 2019. PMID: 30940813. DOI: 10.1038/s41467-019-09480-8

18 Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, Datta G, Davila-Velderrain J, McGuire D, Tian C, Zhan X, 23andMe Research Team, HUNT All-In Psychiatry, Choquet H, Docherty AR, Faul JD, Foerster JR, Fritsche LG, Gabrielsen ME, Gordon SD, Haessler J, Hottenga JJ, Huang H, Jang SK, Jansen PR, Ling Y, Mägi R, Matoba N, McMahon G, Mulas A, Orrù V, Palviainen T, Pandit A, Reginsson GW, Skogholt AH, Smith JA, Taylor AE, Turman C, Willemsen G, Young H, Young KA, Zajac GJM, Zhao W, Zhou W, Bjornsdottir G, Boardman JD, Boehnke M, Boomsma DI, Chen C, Cucca F, Davies GE, Eaton CB, Ehringer MA, Esko T, Fiorillo E, Gillespie NA, Gudbjartsson DF, Haller T, Harris KM, Heath AC, Hewitt JK, Hickie IB, Hokanson JE, Hopfer CJ, Hunter DJ, Iacono WG, Johnson EO, Kamatani Y, Kardia SLR, Keller MC, Kellis M, Kooperberg C, Kraft P, Krauter KS, Laakso M, Lind PA, Loukola A, Lutz SM, Madden PAF, Martin NG, McGue M, McQueen MB, Medland SE, Metspalu A, Mohlke KL, Nielsen JB, Okada Y, Peters U, Polderman TJC, Posthuma D, Reiner AP, Rice JP, Rimm E, Rose RJ, Runarsdottir V, Stallings MC, Stančáková A, Stefansson H, Thai KK, Tindle HA, Tyrfingsson T, Wall TL, Weir DR, Weisner C, Whitfield JB, Winsvold BS, Yin J, Zuccolo L, Bierut LJ, Hveem K, Lee JJ, Munafò MR, Saccone NL, Willer CJ, Cornelis MC, David SP, Hinds DA, Jorgenson E, Kaprio J, Stitzel JA, Stefansson K, Thorgeirsson TE, Abecasis G, Liu DJ and Vrieze S: Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. Nat Genet *51(2)*: 237-244, 2019. PMID: 30643251. DOI: 10.1038/s41588-018-0307-5

19 Sanchez-Roige S, Palmer AA, Fontanillas P, Elson SL, 23andMe Research Team, the Substance Use Disorder Working Group of the Psychiatric Genomics Consortium, Adams MJ, Howard DM, Edenberg HJ, Davies G, Crist RC, Deary IJ, McIntosh AM and Clarke TK: Genome-Wide association study meta-analysis of the alcohol use disorders identification test (AUDIT) in two population-based cohorts. Am J Psychiatry *176(2)*: 107-118, 2019. PMID: 30336701. DOI: 10.1176/appi.ajp.2018.18040369

20 Zintzaras E, Stefanidis I, Santos M and Vidal F: Do alcohol-metabolizing enzyme gene polymorphisms increase the risk of alcoholism and alcoholic liver disease? Hepatology *43(2)*: 352-361, 2006. PMID: 16440362. DOI: 10.1002/hep.21023

21 Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, Agrawal A, Bucholz KK, Grucza R, Hesselbrock V, Kramer J, Kuperman S, Nurnberger J, Porjesz B, Saccone NL, Schuckit M, Tischfield J, Wang JC, Foroud T, Rice JP and Edenberg HJ: ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry *17(4)*: 445-450, 2012. PMID: 21968928. DOI: 10.1038/mp.2011.124

22 Edenberg HJ and McClintick JN: Alcohol dehydrogenases, aldehyde dehydrogenases, and alcohol use disorders: a critical review. Alcohol Clin Exp Res *42(12)*: 2281-2297, 2018. PMID: 30320893. DOI: 10.1111/acer.13904

23 Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H and Farrer LA: Genome-wide association study of alcohol dependence:significant findings in African- and European-Americans including novel risk loci. Mol Psychiatry *19(1)*: 41-49, 2014. PMID: 24166409. DOI: 10.1038/mp.2013.145

24 Gelernter J, Zhou H, Nuñez YZ, Mutirangura A, Malison RT and Kalayasiri R: Genomewide association study of alcohol dependence and related traits in a Thai population. Alcohol Clin Exp Res *42(5)*: 861-868, 2018. PMID: 29460428. DOI: 10.1111/acer.13614

25 Edenberg HJ, Gelernter J and Agrawal A: Genetics of alcoholism. Curr Psychiatry Rep *21(4)*: 26, 2019. PMID: 30852706. DOI: 10.1007/s11920-019-1008-1

26 Thompson A, Cook J, Choquet H, Jorgenson E, Yin J, Kinnunen T, Barclay J, Morris AP and Pirmohamed M: Functional validity, role, and implications of heavy alcohol consumption genetic loci. Sci Adv *6(3)*: eaay5034, 2020. PMID: 31998841. DOI: 10.1126/sciadv.aay5034

27 Kienast T and Heinz A: Dopamine and the diseased brain. CNS Neurol Disord Drug Targets *5(1)*: 109-131, 2006. PMID: 16613557. DOI: 10.2174/187152706784111560

28 Huang W, Payne TJ, Ma JZ, Beuten J, Dupont RT, Inohara N and Li MD: Significant association of ANKK1 and detection of a functional polymorphism with nicotine dependence in an African-American sample. Neuropsychopharmacology *34(2)*: 319-330, 2009. PMID: 18354387. DOI: 10.1038/npp.2008.37

29 International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang SJ, Pihur V, Vollenweider P, O'Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Zhao JH, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Go MJ, van der Harst P, Kao WH, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Nguyen KD, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Onland-Moret NC, Cooper MN, Platou CG, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CS, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, CARDIoGRAM consortium, CKDGen Consortium, KidneyGen Consortium, EchoGen consortium, CHARGE-HF consortium, Aspelund T, Garcia M, Chang YP, O'Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day IN, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Young JH, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen MH, Olden M, Pattaro C, Bolton JA, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw KT, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand SM, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Corsi AM, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann HE, Cho YS, Kim HL, Lee JY, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Smith GD, Wong A, Narisu N, Stančáková A, Raffel LJ, Yao J, Kathiresan S, O'Donnell CJ, Schwartz SM, Ikram MA, Longstreth WT Jr, Mosley TH, Seshadri S, Shrine NR, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJ, van Gilst WH, Janipalli CS, Mani KR, Yajnik CS, Hofman A, Mattace-Raso FU, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen LP, Soininen P, Tukiainen T, Würtz P, Ong RT, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Fowkes FG, Charchar FJ, Schwarz PE, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Tai ES, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han BG, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJ, Altshuler D, Loos RJ, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JC, Hartikainen AL, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Järvelin MR, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ and Johnson T: Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. Nature *478(7367)*: 103-109, 2011. PMID: 21909115. DOI: 10.1038/nature10405

30 Locke AE, Kahali B, Berndt SI, Justice AE, Pers TH, Day FR, Powell C, Vedantam S, Buchkovich ML, Yang J, Croteau-Chonka DC, Esko T, Fall T, Ferreira T, Gustafsson S, Kutalik Z, Luan J, Mägi R, Randall JC, Winkler TW, Wood AR, Workalemahu T, Faul JD, Smith JA, Zhao JH, Zhao W, Chen J, Fehrmann R, Hedman ÅK, Karjalainen J, Schmidt EM, Absher D, Amin N, Anderson D, Beekman M, Bolton JL, Bragg-Gresham JL, Buyske S, Demirkan A, Deng G, Ehret GB, Feenstra B, Feitosa MF, Fischer K, Goel A, Gong J, Jackson AU, Kanoni S, Kleber ME, Kristiansson K, Lim U, Lotay V, Mangino M, Leach IM, Medina-Gomez C, Medland SE, Nalls MA, Palmer CD, Pasko D, Pechlivanis S, Peters MJ, Prokopenko I, Shungin D, Stančáková A, Strawbridge RJ, Sung YJ, Tanaka T, Teumer A, Trompet S, van der Laan SW, van Setten J, Van Vliet-Ostaptchouk JV, Wang Z, Yengo L, Zhang W, Isaacs A, Albrecht E, Ärnlöv J, Arscott GM, Attwood AP, Bandinelli S, Barrett A, Bas IN, Bellis C, Bennett AJ, Berne C, Blagieva R, Blüher M, Böhringer S, Bonnycastle LL, Böttcher Y, Boyd HA, Bruinenberg M, Caspersen IH, Chen YI, Clarke R, Daw EW, de Craen AJM, Delgado G, Dimitriou M, Doney ASF, Eklund N, Estrada K, Eury E, Folkersen L, Fraser RM, Garcia ME, Geller F, Giedraitis V, Gigante B, Go AS, Golay A, Goodall AH, Gordon SD, Gorski M, Grabe HJ, Grallert H, Grammer TB, Gräßler J, Grönberg H, Groves CJ, Gusto G, Haessler J, Hall P, Haller T, Hallmans G, Hartman CA, Hassinen M, Hayward C, Heard-Costa NL, Helmer Q, Hengstenberg C, Holmen O,

Hottenga JJ, James AL, Jeff JM, Johansson Å, Jolley J, Juliusdottir T, Kinnunen L, Koenig W, Koskenvuo M, Kratzer W, Laitinen J, Lamina C, Leander K, Lee NR, Lichtner P, Lind L, Lindström J, Lo KS, Lobbens S, Lorbeer R, Lu Y, Mach F, Magnusson PKE, Mahajan A, McArdle WL, McLachlan S, Menni C, Merger S, Mihailov E, Milani L, Moayyeri A, Monda KL, Morken MA, Mulas A, Müller G, Müller-Nurasyid M, Musk AW, Nagaraja R, Nöthen MM, Nolte IM, Pilz S, Rayner NW, Renstrom F, Rettig R, Ried JS, Ripke S, Robertson NR, Rose LM, Sanna S, Scharnagl H, Scholtens S, Schumacher FR, Scott WR, Seufferlein T, Shi J, Smith AV, Smolonska J, Stanton AV, Steinthorsdottir V, Stirrups K, Stringham HM, Sundström J, Swertz MA, Swift AJ, Syvänen AC, Tan ST, Tayo BO, Thorand B, Thorleifsson G, Tyrer JP, Uh HW, Vandenput L, Verhulst FC, Vermeulen SH, Verweij N, Vonk JM, Waite LL, Warren HR, Waterworth D, Weedon MN, Wilkens LR, Willenborg C, Wilsgaard T, Wojczynski MK, Wong A, Wright AF, Zhang Q, LifeLines Cohort Study, Brennan EP, Choi M, Dastani Z, Drong AW, Eriksson P, Franco-Cereceda A, Gådin JR, Gharavi AG, Goddard ME, Handsaker RE, Huang J, Karpe F, Kathiresan S, Keildson S, Kiryluk K, Kubo M, Lee JY, Liang L, Lifton RP, Ma B, McCarroll SA, McKnight AJ, Min JL, Moffatt MF, Montgomery GW, Murabito JM, Nicholson G, Nyholt DR, Okada Y, Perry JRB, Dorajoo R, Reinmaa E, Salem RM, Sandholm N, Scott RA, Stolk L, Takahashi A, Tanaka T, van 't Hooft FM, Vinkhuyzen AAE, Westra HJ, Zheng W, Zondervan KT, ADIPOGen Consortium, AGEN-BMI Working Group, CARDIOGRAMplusC4D Consortium, CKDGen Consortium, GLGC, ICBP, MAGIC Investigators, MuTHER Consortium, MIGen Consortium, PAGE Consortium, ReproGen Consortium, GENIE Consortium, International Endogene Consortium, Heath AC, Arveiler D, Bakker SJL, Beilby J, Bergman RN, Blangero J, Bovet P, Campbell H, Caulfield MJ, Cesana G, Chakravarti A, Chasman DI, Chines PS, Collins FS, Crawford DC, Cupples LA, Cusi D, Danesh J, de Faire U, den Ruijter HM, Dominiczak AF, Erbel R, Erdmann J, Eriksson JG, Farrall M, Felix SB, Ferrannini E, Ferrières J, Ford I, Forouhi NG, Forrester T, Franco OH, Gansevoort RT, Gejman PV, Gieger C, Gottesman O, Gudnason V, Gyllensten U, Hall AS, Harris TB, Hattersley AT, Hicks AA, Hindorff LA, Hingorani AD, Hofman A, Homuth G, Hovingh GK, Humphries SE, Hunt SC, Hyppönen E, Illig T, Jacobs KB, Jarvelin MR, Jöckel KH, Johansen B, Jousilahti P, Jukema JW, Jula AM, Kaprio J, Kastelein JJP, Keinanen-Kiukaanniemi SM, Kiemeney LA, Knekt P, Kooner JS, Kooperberg C, Kovacs P, Kraja AT, Kumari M, Kuusisto J, Lakka TA, Langenberg C, Marchand LL, Lehtimäki T, Lyssenko V, Männistö S, Marette A, Matise TC, McKenzie CA, McKnight B, Moll FL, Morris AD, Morris AP, Murray JC, Nelis M, Ohlsson C, Oldehinkel AJ, Ong KK, Madden PAF, Pasterkamp G, Peden JF, Peters A, Postma DS, Pramstaller PP, Price JF, Qi L, Raitakari OT, Rankinen T, Rao DC, Rice TK, Ridker PM, Rioux JD, Ritchie MD, Rudan I, Salomaa V, Samani NJ, Saramies J, Sarzynski MA, Schunkert H, Schwarz PEH, Sever P, Shuldiner AR, Sinisalo J, Stolk RP, Strauch K, Tönjes A, Trégouët DA, Tremblay A, Tremoli E, Virtamo J, Vohl MC, Völker U, Waeber G, Willemsen G, Witteman JC, Zillikens MC, Adair LS, Amouyel P, Asselbergs FW, Assimes TL, Bochud M, Boehm BO, Boerwinkle E, Bornstein SR, Bottinger EP, Bouchard C, Cauchi S, Chambers JC, Chanock SJ, Cooper RS, de Bakker PIW, Dedoussis G, Ferrucci L, Franks PW, Froguel P, Groop LC, Haiman CA, Hamsten A, Hui J, Hunter DJ, Hveem K, Kaplan RC, Kivimaki M, Kuh D, Laakso M, Liu Y, Martin NG, März W, Melbye M, Metspalu A, Moebus S, Munroe PB, Njølstad I, Oostra BA, Palmer CNA, Pedersen NL, Perola M, Pérusse L, Peters U, Power C, Quertermous T, Rauramaa R, Rivadeneira F, Saaristo TE, Saleheen D, Sattar N, Schadt EE, Schlessinger D, Slagboom PE, Snieder H, Spector TD, Thorsteinsdottir U, Stumvoll M, Tuomilehto J, Uitterlinden AG, Uusitupa M, van der Harst P, Walker M, Wallaschofski H, Wareham NJ, Watkins H, Weir DR, Wichmann HE, Wilson JF, Zanen P, Borecki IB, Deloukas P, Fox CS, Heid IM, O'Connell JR, Strachan DP, Stefansson K, van Duijn CM, Abecasis GR, Franke L, Frayling TM, McCarthy MI, Visscher PM, Scherag A, Willer CJ, Boehnke M, Mohlke KL, Lindgren CM, Beckmann JS, Barroso I, North KE, Ingelsson E, Hirschhorn JN, Loos RJF and Speliotes EK: Genetic studies of body mass index yield new insights for obesity biology. Nature *518(7538)*: 197-206, 2015. PMID: 25673413. DOI: 10.1038/nature14177

31 Li D, Achkar JP, Haritunians T, Jacobs JP, Hui KY, D'Amato M, Brand S, Radford-Smith G, Halfvarson J, Niess JH, Kugathasan S, Büning C, Schumm LP, Klei L, Ananthakrishnan A, Aumais G, Baidoo L, Dubinsky M, Fiocchi C, Glas J, Milgrom R, Proctor DD, Regueiro M, Simms LA, Stempak JM, Targan SR, Törkvist L, Sharma Y, Devlin B, Borneman J, Hakonarson H, Xavier RJ, Daly M, Brant SR, Rioux JD, Silverberg MS, Cho JH, Braun J, McGovern DP and Duerr RH: A pleiotropic missense variant in SLC39A8 is associated with Crohn's disease and human gut microbiome composition. Gastroenterology *151(4)*: 724-732, 2016. PMID: 27492617. DOI: 10.1053/j.gastro.2016.06.051

32 Ng E, Lind PM, Lindgren C, Ingelsson E, Mahajan A, Morris A and Lind L: Genome-wide association study of toxic metals and trace elements reveals novel associations. Hum Mol Genet *24(16)*: 4739-4745, 2015. PMID: 26025379. DOI: 10.1093/hmg/ddv190

33 Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Do R, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkilä K, Hyppönen E, Isaacs A, Jackson AU, Johansson Å, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikäinen LP, Magnusson PKE, Mangino M, Mihailov E, Montasser ME, Müller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney ASF, Döring A, Elliott P, Epstein SE, Ingi Eyjolfsson G, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJP, Khaw KT, Kim E, Klopp N, Komulainen P, Kumari M, Langenberg C, Lehtimäki T, Lin SY, Lindström J, Loos RJF, Mach F, McArdle WL, Meisinger C, Mitchell BD, Müller G, Nagaraja R, Narisu N, Nieminen TVM, Nsubuga RN, Olafsson I, Ong KK, Palotie A, Papamarkou T, Pomilla C, Pouta A, Rader DJ, Reilly MP, Ridker PM, Rivadeneira F, Rudan I, Ruokonen A,

Samani N, Scharnagl H, Seeley J, Silander K, Stančáková A, Stirrups K, Swift AJ, Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild SH, Willemsen G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL, Bandinelli S, Bennett F, Bochud M, Boehm BO, Boomsma DI, Borecki IB, Bornstein SR, Bovet P, Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YI, Collins FS, Cooper RS, Danesh J, Dedoussis G, de Faire U, Feranil AB, Ferrières J, Ferrucci L, Freimer NB, Gieger C, Groop LC, Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A, Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Järvelin MR, Jula A, Kähönen M, Kaprio J, Kesäniemi A, Kivimaki M, Kooner JS, Koudstaal PJ, Krauss RM, Kuh D, Kuusisto J, Kyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, März W, McCarthy MI, McKenzie CA, Meneton P, Metspalu A, Moilanen L, Morris AD, Munroe PB, Njølstad I, Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quertermous T, Rauramaa R, Saleheen D, Salomaa V, Sanghera DK, Saramies J, Schwarz PEH, Sheu WH, Shuldiner AR, Siegbahn A, Spector TD, Stefansson K, Strachan DP, Tayo BO, Tremoli E, Tuomilehto J, Uusitupa M, van Duijn CM, Vollenweider P, Wallentin L, Wareham NJ, Whitfield JB, Wolffenbuttel BHR, Ordovas JM, Boerwinkle E, Palmer CNA, Thorsteinsdottir U, Chasman DI, Rotter JI, Franks PW, Ripatti S, Cupples LA, Sandhu MS, Rich SS, Boehnke M, Deloukas P, Kathiresan S, Mohlke KL, Ingelsson E, Abecasis GR and Global Lipids Genetics Consortium: Discovery and refinement of loci associated with lipid levels. Nat Genet *45(11)*: 1274-1283, 2013. PMID: 24097068. DOI: 10.1038/ng.2797

34 Li Z, Chen J, Yu H, He L, Xu Y, Zhang D, Yi Q, Li C, Li X, Shen J, Song Z, Ji W, Wang M, Zhou J, Chen B, Liu Y, Wang J, Wang P, Yang P, Wang Q, Feng G, Liu B, Sun W, Li B, He G, Li W, Wan C, Xu Q, Li W, Wen Z, Liu K, Huang F, Ji J, Ripke S, Yue W, Sullivan PF, O'Donovan MC and Shi Y: Genome-wide association analysis identifies 30 new susceptibility loci for schizophrenia. Nat Genet *49(11)*: 1576-1583, 2017. PMID: 28991256. DOI: 10.1038/ng.3973

35 Arias Horcajadas F, Dávila Príz JR, Parra González A, Sánchez Romero S, Sánchez-Morla E, Ampuero Sánchez I and Ramos Atance JA: Cannabinoid receptor type 2 gene is associated with comorbidity of schizophrenia and cannabis dependence and fatty acid amide hydrolase gene is associated with cannabis dependence in the Spanish population. Adicciones *0(0)*: 1587, 2021. PMID: 34171108. DOI: 10.20882/adicciones.1587

36 Sloan ME, Gowin JL, Yan J, Schwandt ML, Spagnolo PA, Sun H, Hodgkinson CA, Goldman D and Ramchandani VA: Severity of alcohol dependence is associated with the fatty acid amide hydrolase Pro129Thr missense variant. Addict Biol *23(1)*: 474-484, 2018. PMID: 28150397. DOI: 10.1111/adb.12491

37 Morita Y, Ujike H, Tanaka Y, Uchida N, Nomura A, Ohtani K, Kishimoto M, Morio A, Imamura T, Sakai A, Inada T, Harano M, Komiyama T, Yamada M, Sekine Y, Iwata N, Iyo M, Sora I, Ozaki N and Kuroda S: A nonsynonymous polymorphism in the human fatty acid amide hydrolase gene did not associate with either methamphetamine dependence or schizophrenia. Neurosci Lett *376(3)*: 182-187, 2005. PMID: 15721218. DOI: 10.1016/j.neulet.2004.11.050

38 Tyndale RF, Payne JI, Gerber AL and Sipe JC: The fatty acid amide hydrolase C385A (P129T) missense variant in cannabis users: studies of drug use and dependence in Caucasians. Am J Med Genet B Neuropsychiatr Genet *144B(5)*: 660-666, 2007. PMID: 17290447. DOI: 10.1002/ajmg.b.30491

39 Iwasaki S, Ishiguro H, Higuchi S, Onaivi ES and Arinami T: Association study between alcoholism and endocannabinoid metabolic enzyme genes encoding fatty acid amide hydrolase and monoglyceride lipase in a Japanese population. Psychiatr Genet *17(4)*: 215-220, 2007. PMID: 17621164. DOI: 10.1097/YPG.0b013e32809913d8

40 Bühler KM, Huertas E, Echeverry-Alzate V, Giné E, Moltó E, Montoliu L and López-Moreno JA: Risky alcohol consumption in young people is associated with the fatty acid amide hydrolase gene polymorphism C385A and affective rating of drug pictures. Mol Genet Genomics *289(3)*: 279-289, 2014. PMID: 24407958. DOI: 10.1007/s00438-013-0809-x

41 Xuei X, Dick D, Flury-Wetherill L, Tian HJ, Agrawal A, Bierut L, Goate A, Bucholz K, Schuckit M, Nurnberger J Jr, Tischfield J, Kuperman S, Porjesz B, Begleiter H, Foroud T and Edenberg HJ: Association of the kappa-opioid system with alcohol dependence. Mol Psychiatry *11(11)*: 1016-1024, 2006. PMID: 16924269. DOI: 10.1038/sj.mp.4001882

42 Yuferov V, Ji F, Nielsen DA, Levran O, Ho A, Morgello S, Shi R, Ott J and Kreek MJ: A functional haplotype implicated in vulnerability to develop cocaine dependence is associated with reduced PDYN expression in human brain. Neuropsychopharmacology *34(5)*: 1185-1197, 2009. PMID: 18923396. DOI: 10.1038/npp.2008.187

43 Wei SG, Zhu YS, Lai JH, Xue HX, Chai ZQ and Li SB: Association between heroin dependence and prodynorphin gene polymorphisms. Brain Res Bull *85(3-4)*: 238-242, 2011. PMID: 21382455. DOI: 10.1016/j.brainresbull.2011.02.010

44 Kaya-Akyüzlü D, Özkan-Kotiloğlu S, Yalçın-Şahiner S and Ayaz N: Effect of PDYN rs2281285, rs2235749 and rs910080 gene polymorphisms on the intensity of depression symptoms and negative craving in heroin addicts. Arch Biol Sci *73(4)*: 473-482, 2021. DOI: 10.2298/ABS210929041K

45 Preuss UW, Winham SJ, Biernacka JM, Geske JR, Bakalkin G, Koller G, Zill P, Soyka M and Karpyak VM: PDYN rs2281285 variant association with drinking to avoid emotional or somatic discomfort. PLoS One *8(11)*: e78688, 2013. PMID: 24223163. DOI: 10.1371/journal.pone.0078688

46 Hashemi M, Shakiba M, Sanaei S, Shahkar G, Rezaei M, Mojahed A and Bahari G: Evaluation of prodynorphin gene polymorphisms and their association with heroin addiction in a sample of the southeast Iranian population. Mol Biol Res Commun *7(1)*: 1-6, 2018. PMID: 29911117. DOI: 10.22099/mbrc.2017.27182.1294

47 Papadimitriou G, Berati S, Matsoukas T and Soldatos KP: Mini International Neuropsychiatric Interview. Greek Version 5.0.0, 2005.

48 Zhang W, Liu H, Deng XD, Ma Y and Liu Y: FAAH levels and its genetic polymorphism association with susceptibility to methamphetamine dependence. Ann Hum Genet *84(3)*: 259-270, 2020. PMID: 31789429. DOI: 10.1111/ahg.12368

49 Mohás M, Kisfali P, Járomi L, Maász A, Fehér E, Csöngei V, Polgár N, Sáfrány E, Cseh J, Sümegi K, Hetyésy K, Wittmann I and Melegh B: GCKR gene functional variants in type 2 diabetes and metabolic syndrome: do the rare variants associate with increased carotid intima-media thickness? Cardiovasc Diabetol *9*: 79, 2010. PMID: 21114848. DOI: 10.1186/1475-2840-9-79

50 Solé X, Guinó E, Valls J, Iniesta R and Moreno V: SNPStats: a web tool for the analysis of association studies. Bioinformatics *22(15)*: 1928-1929, 2006. PMID: 16720584. DOI: 10.1093/bioinformatics/btl268

51 Agrawal A and Lynskey MT: Are there genetic influences on addiction: evidence from family, adoption and twin studies. Addiction *103(7)*: 1069-1081, 2008. PMID: 18494843. DOI: 10.1111/j.1360-0443.2008.02213.x

52 Tawa EA, Hall SD and Lohoff FW: Overview of the genetics of alcohol use disorder. Alcohol Alcohol *51(5)*: 507-514, 2016. PMID: 27445363. DOI: 10.1093/alcalc/agw046

53 Lohoff FW, Roy A, Jung J, Longley M, Rosoff DB, Luo A, O'Connell E, Sorcher JL, Sun H, Schwandt M, Hodgkinson CA, Goldman D, Momenan R, McIntosh AM, Adams MJ, Walker RM, Evans KL, Porteous D, Smith AK, Lee J, Muench C, Charlet K, Clarke TK and Kaminsky ZA: Epigenome-wide association study and multi-tissue replication of individuals with alcohol use disorder: evidence for abnormal glucocorticoid signaling pathway gene regulation. Mol Psychiatry *26(6)*: 2224-2237, 2021. PMID: 32398718. DOI: 10.1038/s41380-020-0734-4

54 Niemela G and Terry GE: Contribution of fatty acid amide hydrolase to alcohol use disorder: a systematic review. Cannabis Cannabinoid Res *6(2)*: 105-118, 2021. PMID: 33989054. DOI: 10.1089/can.2020.0158

55 Parsons LH and Hurd YL: Endocannabinoid signalling in reward and addiction. Nat Rev Neurosci *16(10)*: 579-594, 2015. PMID: 26373473. DOI: 10.1038/nrn4004

56 Sipe JC, Chiang K, Gerber AL, Beutler E and Cravatt BF: A missense mutation in human fatty acid amide hydrolase associated with problem drug use. Proc Natl Acad Sci U.S.A. *99(12)*: 8394-8399, 2002. PMID: 12060782. DOI: 10.1073/pnas.082235799

57 Filbey FM, Schacht JP, Myers US, Chavez RS and Hutchison KE: Individual and additive effects of the CNR1 and FAAH genes on brain response to marijuana cues. Neuropsychopharmacology *35(4)*: 967-975, 2010. PMID: 20010552. DOI: 10.1038/npp.2009.200

58 Patel MM, Nielsen DA, Kosten TR, De La Garza R 2nd, Newton TF and Verrico CD: FAAH variant Pro129Thr modulates subjective effects produced by cocaine administration. Am J Addict *27(7)*: 567-573, 2018. PMID: 30126012. DOI: 10.1111/ajad.12788

59 Best LM, Wardell JD, Tyndale RF, McPhee MD, Le Foll B, Kish SJ, Boileau I and Hendershot CS: Association of the fatty acid amide hydrolase C385A polymorphism with alcohol use severity and coping motives in heavy-drinking youth. Alcohol Clin Exp Res *45(3)*: 507-517, 2021. PMID: 33460184. DOI: 10.1111/acer.14552

60 Zhou Y, Huang T, Lee F and Kreek MJ: Involvement of endocannabinoids in alcohol "binge" drinking: Studies of mice with human fatty acid amide hydrolase genetic variation and after CB1 receptor antagonists. Alcohol Clin Exp Res *40(3)*: 467-473, 2016. PMID: 26857901. DOI: 10.1111/acer.12989

61 Vinod KY, Sanguino E, Yalamanchili R, Manzanares J and Hungund BL: Manipulation of fatty acid amide hydrolase functional activity alters sensitivity and dependence to ethanol. J Neurochem *104(1)*: 233-243, 2008. PMID: 17944864. DOI: 10.1111/j.1471-4159.2007.04956.x

62 Raimondo A, Rees MG and Gloyn AL: Glucokinase regulatory protein: complexity at the crossroads of triglyceride and glucose metabolism. Curr Opin Lipidol *26(2)*: 88-95, 2015. PMID: 25692341. DOI: 10.1097/MOL.0000000000000155

63 Rasheed H, Stamp LK, Dalbeth N and Merriman TR: Interaction of the GCKR and A1CF loci with alcohol consumption to influence the risk of gout. Arthritis Res Ther *19(1)*: 161, 2017. PMID: 28679452. DOI: 10.1186/s13075-017-1369-y

64 Costas J: The highly pleiotropic gene SLC39A8 as an opportunity to gain insight into the molecular pathogenesis of schizophrenia. Am J Med Genet B Neuropsychiatr Genet *177(2)*: 274-283, 2018. PMID: 28557351. DOI: 10.1002/ajmg.b.32545

65 Jian X, Chen J, Li Z, Song Z, Zhou J, Xu W, Liu Y, Shen J, Wang Y, Yi Q and Shi Y: SLC39A8 is a risk factor for schizophrenia in Uygur Chinese: a case-control study. BMC Psychiatry *19(1)*: 293, 2019. PMID: 31533672. DOI: 10.1186/s12888-019-2240-2

66 Johansson Å, Eriksson N, Lindholm D, Varenhorst C, James S, Syvänen AC, Axelsson T, Siegbahn A, Barratt BJ, Becker RC, Himmelmann A, Katus HA, Steg PG, Storey RF, Wallentin L and PLATO Investigators: Genome-wide association and Mendelian randomization study of NT-proBNP in patients with acute coronary syndrome. Hum Mol Genet *25(7)*: 1447-1456, 2016. PMID: 26908625. DOI: 10.1093/hmg/ddw012

67 Chen G, Cuzon Carlson VC, Wang J, Beck A, Heinz A, Ron D, Lovinger DM and Buck KJ: Striatal involvement in human alcoholism and alcohol consumption, and withdrawal in animal models. Alcohol Clin Exp Res *35(10)*: 1739-1748, 2011. PMID: 21615425. DOI: 10.1111/j.1530-0277.2011.01520.x

68 Pato CN, Macciardi F, Pato MT, Verga M and Kennedy JL: Review of the putative association of dopamine D2 receptor and alcoholism: a meta-analysis. Am J Med Genet *48(2)*: 78-82, 1993. PMID: 8362930. DOI: 10.1002/ajmg.1320480204

69 Berggren U, Fahlke C, Aronsson E, Karanti A, Eriksson M, Blennow K, Thelle D, Zetterberg H and Balldin J: The taqI DRD2 A1 allele is associated with alcohol-dependence although its effect size is small. Alcohol Alcohol *41(5)*: 479-485, 2006. PMID: 16751215. DOI: 10.1093/alcalc/agl043

70 Ponce G, Hoenicka J, Jiménez-Arriero MA, Rodríguez-Jiménez R, Aragüés M, Martín-Suñé N, Huertas E and Palomo T: DRD2 and ANKK1 genotype in alcohol-dependent patients with psychopathic traits: association and interaction study. Br J Psychiatry *193(2)*: 121-125, 2008. PMID: 18669994. DOI: 10.1192/bjp.bp.107.041582

71 Le Foll B, Gallo A, Le Strat Y, Lu L and Gorwood P: Genetics of dopamine receptors and drug addiction: a comprehensive review. Behav Pharmacol *20(1)*: 1-17, 2009. PMID: 19179847. DOI: 10.1097/FBP.0b013e3283242f05

72 Swagell CD, Lawford BR, Hughes IP, Voisey J, Feeney GF, van Daal A, Connor JP, Noble EP, Morris CP and Young RM: DRD2 C957T and TaqIA genotyping reveals gender effects and unique low-risk and high-risk genotypes in alcohol dependence. Alcohol Alcohol *47(4)*: 397-403, 2012. PMID: 22582185. DOI: 10.1093/alcalc/ags047

73 Prasad P, Ambekar A and Vaswani M: Dopamine D2 receptor polymorphisms and susceptibility to alcohol dependence in Indian males: a preliminary study. BMC Med Genet *11*: 24, 2010. PMID: 20146828. DOI: 10.1186/1471-2350-11-24

74 Wang F, Simen A, Arias A, Lu QW and Zhang H: A large-scale meta-analysis of the association between the ANKK1/DRD2 Taq1A polymorphism and alcohol dependence. Hum Genet *132(3)*: 347-358, 2013. PMID: 23203481. DOI: 10.1007/s00439-012-1251-6

75 Li D, Zhao H and Gelernter J: Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. Biol Psychiatry *70(6)*: 504-512, 2011. PMID: 21497796. DOI: 10.1016/j.biopsych.2011.02.024

76 Polimanti R and Gelernter J: ADH1B: From alcoholism, natural selection, and cancer to the human phenome. Am J Med Genet B Neuropsychiatr Genet *177(2)*: 113-125, 2018. PMID: 28349588. DOI: 10.1002/ajmg.b.32523

77 Katsarou MS, Karakonstantis K, Demertzis N, Vourakis E, Skarpathioti A, Nosyrev AE, Tsatsakis A, Kalogridis T and Drakoulis N: Effect of single-nucleotide polymorphisms in ADH1B, ADH4, ADH1C, OPRM1, DRD2, BDNF, and ALDH2 genes on alcohol dependence in a Caucasian population. Pharmacol Res Perspect *5(4)*: e00326, 2017. PMID: 28805974. DOI: 10.1002/prp2.326

78 Sun Y, Chang S, Wang F, Sun H, Ni Z, Yue W, Zhou H, Gelernter J, Malison RT, Kalayasiri R, Wu P, Lu L and Shi J: Genome-wide association study of alcohol dependence in male Han Chinese and cross-ethnic polygenic risk score comparison. Transl Psychiatry *9(1)*: 249, 2019. PMID: 31591379. DOI: 10.1038/s41398-019-0586-3

79 Luczak SE, Glatt SJ and Wall TL: Meta-analyses of ALDH2 and ADH1B with alcohol dependence in Asians. Psychol Bull *132(4)*: 607-621, 2006. PMID: 16822169. DOI: 10.1037/0033-2909.132.4.607

80 Park BL, Kim JW, Cheong HS, Kim LH, Lee BC, Seo CH, Kang TC, Nam YW, Kim GB, Shin HD and Choi IG: Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: from GWAS to replication. Hum Genet *132(6)*: 657-668, 2013. PMID: 23456092. DOI: 10.1007/s00439-013-1281-8

81 Wee S and Koob GF: The role of the dynorphin-kappa opioid system in the reinforcing effects of drugs of abuse. Psychopharmacology (Berl) *210(2)*: 121-135, 2010. PMID: 20352414. DOI: 10.1007/s00213-010-1825-8

82 Sirohi S, Bakalkin G and Walker BM: Alcohol-induced plasticity in the dynorphin/kappa-opioid receptor system. Front Mol Neurosci *5*: 95, 2012. PMID: 23060746. DOI: 10.3389/fnmol.2012.00095

83 Willer CJ, Schmidt EM, Sengupta S, Peloso GM, Gustafsson S, Kanoni S, Ganna A, Chen J, Buchkovich ML, Mora S, Beckmann JS, Bragg-Gresham JL, Chang HY, Demirkan A, Den Hertog HM, Do R, Donnelly LA, Ehret GB, Esko T, Feitosa MF, Ferreira T, Fischer K, Fontanillas P, Fraser RM, Freitag DF, Gurdasani D, Heikkilä K, Hyppönen E, Isaacs A, Jackson AU, Johansson Å, Johnson T, Kaakinen M, Kettunen J, Kleber ME, Li X, Luan J, Lyytikäinen LP, Magnusson PKE, Mangino M, Mihailov E, Montasser ME, Müller-Nurasyid M, Nolte IM, O'Connell JR, Palmer CD, Perola M, Petersen AK, Sanna S, Saxena R, Service SK, Shah S, Shungin D, Sidore C, Song C, Strawbridge RJ, Surakka I, Tanaka T, Teslovich TM, Thorleifsson G, Van den Herik EG, Voight BF, Volcik KA, Waite LL, Wong A, Wu Y, Zhang W, Absher D, Asiki G, Barroso I, Been LF, Bolton JL, Bonnycastle LL, Brambilla P, Burnett MS, Cesana G, Dimitriou M, Doney ASF, Döring A, Elliott P, Epstein SE, Ingi Eyjolfsson G, Gigante B, Goodarzi MO, Grallert H, Gravito ML, Groves CJ, Hallmans G, Hartikainen AL, Hayward C, Hernandez D, Hicks AA, Holm H, Hung YJ, Illig T, Jones MR, Kaleebu P, Kastelein JJP, Khaw KT, Kim E, Klopp N, Komulainen P, Kumari M, Langenberg C, Lehtimäki T, Lin SY, Lindström J, Loos RJF, Mach F, McArdle WL, Meisinger C, Mitchell BD, Müller G, Nagaraja R, Narisu N, Nieminen TVM, Nsubuga RN, Olafsson I, Ong KK, Palotie A, Papamarkou T, Pomilla C, Pouta A, Rader DJ, Reilly MP, Ridker PM, Rivadeneira F, Rudan I, Ruokonen A, Samani N, Scharnagl H, Seeley J, Silander K, Stančáková A, Stirrups K, Swift AJ, Tiret L, Uitterlinden AG, van Pelt LJ, Vedantam S, Wainwright N, Wijmenga C, Wild SH, Willemsen G, Wilsgaard T, Wilson JF, Young EH, Zhao JH, Adair LS, Arveiler D, Assimes TL, Bandinelli S, Bennett F, Bochud M, Boehm BO, Boomsma DI, Borecki IB, Bornstein SR, Bovet P, Burnier M, Campbell H, Chakravarti A, Chambers JC, Chen YI, Collins FS, Cooper RS, Danesh J, Dedoussis G, de Faire U, Feranil AB, Ferrières J, Ferrucci L, Freimer NB, Gieger C, Groop LC, Gudnason V, Gyllensten U, Hamsten A, Harris TB, Hingorani A, Hirschhorn JN, Hofman A, Hovingh GK, Hsiung CA, Humphries SE, Hunt SC, Hveem K, Iribarren C, Järvelin MR, Jula A, Kähönen M, Kaprio J, Kesäniemi A, Kivimaki M, Kooner JS, Koudstaal PJ, Krauss RM, Kuh D, Kuusisto J, Kyvik KO, Laakso M, Lakka TA, Lind L, Lindgren CM, Martin NG, März W, McCarthy MI, McKenzie CA, Meneton P, Metspalu A, Moilanen L, Morris AD, Munroe PB, Njølstad I, Pedersen NL, Power C, Pramstaller PP, Price JF, Psaty BM, Quertermous T, Rauramaa R, Saleheen D, Salomaa V, Sanghera DK, Saramies J, Schwarz PEH, Sheu WH, Shuldiner AR, Siegbahn A, Spector TD, Stefansson K, Strachan DP, Tayo BO, Tremoli E, Tuomilehto J, Uusitupa M, van Duijn CM, Vollenweider P, Wallentin L, Wareham NJ, Whitfield JB, Wolffenbuttel BHR, Ordovas JM, Boerwinkle E, Palmer CNA, Thorsteinsdottir U, Chasman DI, Rotter JI, Franks PW, Ripatti S, Cupples LA, Sandhu MS, Rich SS, Boehnke M, Deloukas P, Kathiresan S, Mohlke KL, Ingelsson E, Abecasis GR and Global Lipids Genetics Consortium: Discovery and refinement of loci associated with lipid levels. Nat Genet *45(11)*: 1274-1283, 2013. PMID: 24097068. DOI: 10.1038/ng.2797

84 Clarke TK, Ambrose-Lanci L, Ferraro TN, Berrettini WH, Kampman KM, Dackis CA, Pettinati HM, O'Brien CP, Oslin DW and Lohoff FW: Genetic association analyses of PDYN polymorphisms with heroin and cocaine addiction. Genes Brain Behav *11(4)*: 415-423, 2012. PMID: 22443215. DOI: 10.1111/j.1601-183X.2012.00785.x

85 Karpyak VM, Winham SJ, Preuss UW, Zill P, Cunningham JM, Walker DL, Lewis KA, Geske JR, Colby CL, Abulseoud OA, Hall-Flavin DK, Loukianova LL, Schneekloth TD, Frye MA, Bazov I, Heit JA, Bakalkin G, Mrazek DA and Biernacka JM: Association of the PDYN gene with alcohol dependence and the propensity to drink in negative emotional states. Int J Neuropsychopharmacol *16(5)*: 975-985, 2013. PMID: 23101464. DOI: 10.1017/S1461145712001137

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