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Associated and intermediate factors between genetic variants of the dopaminergic D2 receptor gene and harmful alcohol use in young adults

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Abstract

Dopamine receptor D2 (*DRD2*) and *ankyrin repeat and kinase domain-containing protein 1* (*ANKK1*) genes have received considerable attention for their involvement in alcohol use disorder (AUD), but many questions remain on their exact role. We conducted a population-based case–control and genetic association study in a large sample of young adults. Our aim was to assess the association between *DRD2* and *ANKK1* single nucleotide polymorphisms (SNPs) and harmful alcohol use, disentangling associated and possible intermediate factors. A total of 1841 college students from the French region Champagne-Ardennes, aged between 18 and 21 years and who reported at least one lifetime alcohol consumption, were included in this study. Allele frequencies were analysed according to harmful alcohol use (assessed through the Alcohol Use Disorder Identification Test [AUDIT] questionnaire). Different substance use disorders, including nicotine and cannabis dependences, were also assessed through questionnaires, as was a list of potential associated factors (e.g., major depressive episode, conduct disorder, attention-deficit/hyperactivity disorder [ADHD], school failure, sugar consumption, sexual trauma, parents' use of alcohol, tobacco or cannabis). We found that *DRD2* rs1800498 was associated with harmful alcohol use. Many factors were detected, but a global path analysis revealed that *DRD2* rs1800498 had a significant direct effect on harmful alcohol use and that early age at first alcohol consumption and depressive symptoms moderated this effect. This study suggests an interplay between harmful alcohol use, *DRD2* genotypes and other risk factors that, with a full understanding, could be useful for preventive purposes.

KEYWORDS
addiction, alcohol use disorder, *ANKK1* gene, depression, *DRD2* gene, harmful alcohol use

1 | INTRODUCTION

Alcohol use disorder (AUD), which encompasses the concepts of harmful alcohol use and alcohol dependence, is a common and complex disorder with a prevalence of 8.8% in Europe.[1] Environmental and genetic factors play a crucial role in its etiology.[2] Twin studies have indeed found that 50% to 60% of the variability of AUD liability are associated with genetic factors (heritability).[3]

Dopamine plays a major role in reward mechanisms and mediates its effects through dopamine receptors.[4] Over the last three decades, two genes involved in the dopaminergic system have received considerable attention for their involvement in AUD: the *dopamine receptor D2* (*DRD2*) gene, located on chromosome 11,[5] and the *Ankyrin repeat and kinase domain-containing protein 1* (*ANKK1*) gene that we located within 10 kb downstream the *DRD2* gene.[6]

In humans, the most frequently studied single nucleotide polymorphisms (SNPs) of the *DRD2* gene are rs6277 and rs1800498, which are synonymous and intronic variants, respectively, whereas in the *ANKK1* gene, they are intronic variant rs4938015 and missense variant rs1800497 (historically called *DRD2* Taq1A).[7] Several genetic association studies and meta-analyses have reported positive associations between either *DRD2* rs6277 or *ANKK1* rs1800497 A alleles and AUD.[8–10] These SNPs have also been involved in other psychiatric diseases and drug use disorders.[11,12] Similarly, other studies reported a role of *ANKK1* rs4938015 C allele and *DRD2* rs1800498 A allele in drug use disorders such as cannabis and opiates,[13–15] thereby highlighting the role of these two genes in the broader concept of addiction.

Many questions remain on the exact role of the *DRD2* and *ANKK1* genes on AUD because of a relatively long list of potential problems, limiting the possibilities to use such a genetic risk factor, for example, in prevention approaches. First, meta-analyses assessing the association of the *DRD2/ANKK1* genes with AUD[16] demonstrated strong evidence ($p < 0.001$), but in favour of a weak association (OR [95% CI] = 1.23 [1.14–1.31]). Large sample sizes are therefore a prerequisite to detect an association. Second, large phenotype heterogeneity is another potential issue, as the concepts of alcohol 'use disorder', 'dependence' or 'addiction' encompass overlapping but not identical phenotypes. This problem pushes for larger phenotype analyses, including tobacco and drug use, which are highly comorbid.[17,18] This might be needed not only for each patient but also for parents, because parental substance use is associated with a significantly increased risk of substance use in offspring.[19,20] Third, a lack of specificity might constitute another issue, as in a group of patients with AUD, level of severity and modality of uses are variable. Focusing on patients in the early process of AUD (e.g., at the harmful alcohol use stage) might offer interesting advantages, such as capturing all patients whatever their latter trajectories,[21] and facilitating possible early interventions. Fourth, because the majority of studies on AUD relies on treated patients, the risk of stratification biases exists. Basing such studies on the general population should facilitate the reliability of associations. Fifth, the presence of many possible hidden intermediate factors might be a major problem as well, because they may vary from one sample to the other, impacting the detected association. Attention-deficit/hyperactivity disorder (ADHD), conduct disorder and past trauma are interesting examples as they all are associated with AUD and with each other.[22,23] Moreover, ADHD is associated with *DRD2/ANKK1* polymorphisms,[24] whereas conduct disorder may have a mediating role in the association between ADHD and AUD.[25] This complex interplay suggests that, when studying the relationship between *DRD2/ANKK1* polymorphisms and AUD, it is essential to consider other factors. Lastly, because so many associated and intermediate factors might impact the association between the *DRD2/ANKK1* genes and AUD, it is important to use more global analyses, such as path analyses, to assess whether *DRD2/ANKK1* polymorphisms impact AUD directly or through interactions with other factors.[26]

In this paper, we examined the role of *DRD2/ANKK1* SNPs on harmful alcohol use, which is defined by the World Health Organization as 'drinking that causes detrimental health and social consequences for the drinker, the people around the drinker and society at large, as well as patterns of drinking that are associated with increased risk of adverse health outcomes'.[27] The dual goal of the present study was to (1) measure the association between four *DRD2/ANKK1* SNPs (risk alleles rs1800498A and rs6277A on *DRD2* gene, and risk alleles rs4938015C and rs1800497A on *ANKK1* gene) and harmful alcohol use and (2) include other addictive disorders and a list of potential risk factors in order to distinguish their direct from indirect roles. Importantly, we evaluated this association early in the process of AUD (i.e., at harmful alcohol use stage), in a large population of young adults. Knowing the genes involved in this initial phase of the process of AUD is crucial to facilitate prevention approaches in order to reduce the risk of later development of AUD in at-risk individuals.

2 | METHODS

2.1 | Design

We conducted a case–control and genetic association study aiming to evaluate the role of *ANKK1* and *DRD2* genes in harmful alcohol use in a population of young adults recruited from the SAGE (*Susceptibility Addiction Gene Environment*) survey. This survey was designed to assess the link between different addictive disorders (alcohol, tobacco and cannabis), psychiatric comorbidities and risk factors, with four variants of interest within the *DRD2/ANKK1* genes. Details on this population and assessment methods have previously been published.[28] Briefly, in the SAGE survey, all college students from the French academic region Champagne Ardennes were eligible. Each student who signed an informed consent form completed a French-written self-questionnaire during a school day and had a saliva sample collected. No data were collected on subjects who refused the interview or were not present the day of interview. This survey was promoted by the French national research institute (Institut National de la Santé et de la Recherche Médicale [INSERM]) and received ethical approval by the national council for ethic regulation (Commission Nationale de l'Informatique et des Libertés [CNIL], #907003).

2.2 | Participants

The SAGE survey initially included 3056 young adults (including 1834 men; mean age 20.4 years, standard deviation 1.4; median age 20). In the present study, we included those aged between 18 and 21, who had consumed alcohol at least once and had at least three Caucasian grandparents (to limit genomic background heterogeneity). Subjects who were adopted were excluded, as well as subjects with no information available for gender, or with non-exploitable DNA. As a result, the studied subsample counted 1841 young adults (see Supporting information, Figure S1). For each subject, incoherent data between questions were censored (7842 values out of a total of 984 032 values, 0.80%). All participants signed a written consent to participate after a detailed explanation of the protocol.

2.3 | Measures

2.3.1 | Genotyping, allele carrier frequencies and heterozygous frequencies

Genomic DNA was extracted from saliva samples and by using the SNPlex Genotyping System,[29] four SNPs were screened in the dopamine system: rs4938015(C>T) and rs1800497(A>G) in the *ANKK1* gene, and rs1800498(A>G) and rs6277(A>G) in the *DRD2* gene. Allele carrier frequencies were then calculated in different subgroups. These frequencies were calculated for *ANKK1* rs4938015 C allele and for *ANKK1* rs1800497, *DRD2* rs1800498 and *DRD2* rs6277 A allele. The four SNPs are located on chromosome 11 and were selected based on their location on a set of close candidate genes (*DRD2* and *ANKK1*) for addictive disorders in adults.

2.3.2 | Harmful alcohol use

Harmful alcohol use was ascertained with a French version[30] of the Alcohol Use Disorder Identification Test (AUDIT),[31] which is a 10-item questionnaire developed by the World Health Organization (WHO) screening subjects for harmful alcohol use or alcohol dependence. Following the WHO guidelines, males were considered cases (with either harmful alcohol use or alcohol dependence) if they had an AUDIT score of eight or more, whereas females were considered cases if they had an AUDIT score of seven or more. Among the 1841 young adults included in our study, 641 qualified as cases (mean AUDIT score 11.51 ± 3.8). All other participants of the subsample were considered controls ($N = 1200$, mean AUDIT score 3.21 ± 2.1).

2.3.3 | Risk factors

General information including gender, age, ethnic origins, family structure, body height and weight was collected. Height and weight were used to calculate the body mass index (BMI).

School failure was assessed through four items. The first three items were based on the question 'Over the past 12 months, have you ever … ?', including skipped classes, been late for class and been absent for at least a day. A fourth item indicated whether participants had ever repeated a year. School failure score corresponded to the number of positive answers, which ranged from zero to four.

Parents' education was assessed by asking the highest level of education completed by each parent.

Conduct disorder during childhood was evaluated using the 12 criteria from the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Score ranged from zero to 12. A score of three or above indicates a diagnosis of conduct disorder during childhood.

History of ADHD was assessed with the Wender Utah Rating Scale, a 25-item questionnaire designed to retrospectively assess, in adults, childhood ADHD symptoms. Score ranged from zero to 100. A score of 46 or above has been shown to differentiate patients suffering with ADHD from those without.[32]

Current depression score was measured with a French version of the Adolescent Depression Rating Scale (ADRS), a 10-item questionnaire leading to a score of depression between zero and 10.[33] A score of four or above indicates a diagnosis of mild to severe depression.

Assessment of lifetime suicide attempt was realized through a single question: 'Have you ever attempted suicide?'

Participants completed a questionnaire assessing lifetime exposure to sexual abuse (involving either rape, attempted rape or other sexual abuse), as well as the age at first sexual abuse.

Sugar consumption was estimated from the daily consumption of sweet drinks, candies (Mars®, M&Ms®, KitKat®, etc.) and the quantity of sugar or sugar lumps added to food.

Alcohol-related behaviours were assessed using the AUDIT, as described above, and additional questions including age at first alcohol consumption.

Lifetime nicotine dependence was evaluated using the Fagerström Test for Nicotine Dependence, a six-item questionnaire.[34] Subjects received a diagnosis of nicotine dependence if they had a score of three or above ($N = 205$; mean nicotine dependence score 4.61 ± 1.5).

Lifetime cannabis dependence was assessed according to the seven DSM-IV criteria. Subjects received a diagnostic of cannabis dependence if they met three or more DSM-IV criteria ($N = 97$, mean score 3.62 ± 0.93).

Exposure to other drugs was evaluated with 12 items based on the question 'Have you ever used … ?', including unprescribed tranquillizers, cocaine, amphetamine, opiates/heroin, solvents, ecstasy, phencyclidine (PCP), hallucinogenic drug, lysergic acid diethylamide (LSD), gamma-hydroxybutyrate (GHB), sniffed drug and injected drug. Exposure to other drugs ranged from zero to 12. Subjects were considered to be exposed if they had consumed at least one drug ($N = 248$, mean number of drugs = 6.44 ± 1.85).

Paternal and maternal drinking problems were assessed with questionnaires derived from the Michigan Alcoholism Screening Test (MAST). These questionnaires allow for the screening of alcohol abuse

symptoms in both parents according to their child.[35] Parents' alcohol use was considered problematic if at least one of the parents had a score of three or above.

Parents' use of tobacco was assessed with two items for each parent: 'does your father/mother currently smoke tobacco every day?' and 'has your father/mother smoked tobacco regularly at least once?'. Parents' use of tobacco was quoted as positive if at least one of the parents had one or more positive answers.

Similarly, parents' use of cannabis was assessed with three items for each parent: 'has your father/mother ever smoked hashish at least once in his/her life?', 'has he/she smoked hashish regularly at least once in his/her life (for at least one year)?' and 'does he/she currently smoke hashish every day?'. Parents' use of cannabis was considered present if at least one of the parents had one or more positive answers.

2.4 | Analyses

Statistical analyses were performed using R version 4.0.4 and R Studio software version 1.4.

Qualitative variables were described as numbers (*N*) and percentages (%). Quantitative variables were described as mean ± standard deviation (SD). To account for multiple comparisons, we applied Holm–Bonferroni correction.

Allele carrier frequencies were compared between subjects with and without addictive disorders (i.e., harmful alcohol use, nicotine dependence or cannabis dependence), as well as between subjects with or without potential risk factors of harmful alcohol use (i.e., gender and school absenteeism), by using chi-squared tests and odds ratios (95% confidence intervals). To test allele frequencies in nicotine dependence or cannabis dependence, the subsamples were limited to the 1354 or 928 subjects who had ever consumed nicotine or cannabis, respectively (nicotine subsample mean age = 19.90 ± 0.8; cannabis subsample mean age = 19.95 ± 0.8). Furthermore, as quantitative risk factors did not systematically have normal distributions, means were compared between allele carriers and non-carriers by using Mann–Whitney *U* tests.

Haplotypic analyses were performed using Haploview software version 4.2 for the SNPs that showed a significant association with harmful alcohol use (i.e., *DRD2* rs6277 and *DRD2* rs1800498). Haplotype frequencies were compared between subjects with and without harmful alcohol use, nicotine dependence or cannabis dependence. Linkage disequilibrium was also computed to assess the non-random association of *DRD2* rs6277 and *DRD2* rs1800498 on chromosome 11. A D′ value close to 1 indicated a strong linkage disequilibrium.

Socio-economic and clinical characteristics (potential risk factors) in subjects with and without harmful alcohol use were compared using either chi-squared tests for qualitative variables or logistic regressions for quantitative ones. Interactions between significant risk factors and the *DRD2* SNPs were tested with a logistic regression.

Finally, structural equation modelling (SEM) was used to model relationships between the dependent variable (harmful alcohol use) and independent variables. This multivariate statistical analysis technique, which includes simultaneous regressions, enables to test the direct and indirect effects on pre-supposed causal relationships.[36] In the present study, we performed a path analysis (which is a specific SEM) using the R package 'Lavaan' to model the effects of *DRD2* gene on harmful alcohol use. We included in the model the dependent variable (harmful alcohol use) and the independent variables that were significantly associated with harmful alcohol use and with the *DRD2* gene variants. These variables were normalized before being included in the model. The 'Lavaan' package uses the maximum likelihood estimation method to get maximum likelihood parameter estimates, handle missing data and provide goodness-of-fit indices. A series of measures were used to evaluate model fit: comparative fit index (CFI) > 0.95, Tucker Lewis index (TLI) > 0.95, root mean square error of approximation (RMSEA) < 0.05 and standardized root mean square residual (SRMR) < 0.08.

3 | RESULTS

3.1 | Characterizing the sample

The rate of harmful alcohol use at the time of interview was 35%, whereas the rates of other addictive disorders such as nicotine dependence and cannabis dependence were 11% and 5%, respectively. In addition, the rates of psychiatric morbidities such as depression, childhood ADHD and childhood conduct disorder were 21%, 3% and 11%, respectively.

3.2 | Association between *ANKK1* and *DRD2* SNPs and harmful alcohol use, other addictive disorders or risk factors

The two *DRD2* SNPs (rs6277 and rs1800498) were significantly associated with harmful alcohol use, with higher frequencies of allele A carriers among those with harmful alcohol use than those without for both SNPs (Table 1). On the other hand, there was no significant association between *ANKK1* SNPs (rs4938015 and rs1800497) and harmful alcohol use. After Holm–Bonferroni correction, significance remained only for the *DRD2* rs1800498A variant. Therefore, the following statistical analyses focus on the *DRD2* SNPs only (rs1800498 and rs6277).

Regarding other addictive disorders, we found no association between *DRD2* SNPs and nicotine or cannabis dependence (Table 1).

Finally, regarding associations between *DRD2* SNPs and risk factors, none was significant after correction for multiple comparisons (Table 1).

3.3 | Association between *DRD2* haplotypes and harmful alcohol use

To assess whether the two SNPs were co-inherited and associated with harmful alcohol use, linkage disequilibrium and haplotype analyses were performed, respectively. We found that *DRD2* rs6277 and *DRD2* rs1800498 variants were in strong linkage disequilibrium

TABLE 1 Association between *dopamine receptor D2 (DRD2)/ankyrin repeat and kinase domain-containing protein 1 (ANKK1)* genetic variants and harmful alcohol use, other addictive disorders and known risk factors, versus the rest of the sample (without the studied disorder or risk factor)

		Allele-wise *N* (%)					
Disorder or risk factor	**SNP**	**A+**	**A−**	**OR [95% CI]**	**Raw *p*[a]**	**Corrected *p***	***N***
Harmful alcohol use	DRD2 rs1800498	559 (88%)	74 (12%)	1.45 [1.09–1.94]	**0.011**	**0.044**	1841
	DRD2 rs6277	522 (83%)	106 (17%)	1.29 [1.00–1.66]	**0.049**	0.196	1841
	ANKK1 rs4938015(C)	571 (91%)	59 (9%)	1.31 [0.95–1.82]	0.097	0.388	1841
	ANKK1 s1800497	211 (33%)	420 (67%)	1.00 [0.81–1.22]	0.979	1.000	1841
Addictive disorders							
Nicotine dependence	DRD2 rs1800498	170 (83%)	26 (13%)	1.07 [0.70–1.71]	0.750	1.000	1353
	DRD2 rs6277	160 (78%)	38 (19%)	0.98 [0.67–1.45]	0.919	1.000	1353
Cannabis dependence	DRD2 rs1800498	78 (81%)	16 (17%)	0.77 [0.44–1.41]	0.379	1.000	971
	DRD2 rs6277	76 (79%)	19 (20%)	0.92 [0.55–1.61]	0.772	1.000	971
Risk factors							
Gender male	DRD2 rs1800498	937 (85%)	155 (14%)	1.07 [0.82–1.40]	0.579	1.000	1841
	DRD2 rs6277	876 (79%)	210 (19%)	1.01 [0.79–1.28]	0.942	0.942	1841
School failure yes	DRD2 rs1800498	1494 (84%)	258 (15%)	0.70 [0.29–1.45]	0.351	1.000	1841
	DRD2 rs6277	1401 (79%)	342 (19%)	0.66 [0.30–1.28]	0.227	1.000	1841

		Allele-wise mean (SD)				
	SNP	**A+**	**A−**	**Raw *p*[b]**	**Corrected *p***	***N***
Age at first alcohol consumption	DRD2 rs1800498	13.50 (2.90)	13.38 (3.05)	0.772	1.000	1841
	DRD2 rs6277	13.51 (2.91)	13.34 (2.98)	0.326	1.000	1841
BMI	DRD2 rs1800498	22.09 (3.12)	21.94 (2.94)	0.482	1.000	1841
	DRD2 rs6277	22.11 (3.16)	21.98 (2.85)	0.635	1.000	1841
Sugar consumption	DRD2 rs1800498	21.93 (30.34)	20.88 (26.87)	0.802	0.802	1841
	DRD2 rs6277	21.76 (29.82)	22.78 (31.00)	0.347	1.000	1841
Depression score	DRD2 rs1800498	1.44 (1.94)	1.10 (1.53)	0.055	0.495	1841
	DRD2 rs6277	1.44 (1.95)	1.19 (1.63)	0.165	1.000	1841
Conduct disorder score	DRD2 rs1800498	0.93 (1.25)	0.85 (1.14)	0.480	1.000	1841
	DRD2 rs6277	0.94 (1.25)	0.85 (1.18)	0.202	1.000	1841
ADHD score	DRD2 rs1800498	17.64 (12.25)	19.30 (12.71)	**0.037**	0.370	1841
	DRD2 rs6277	17.60 (12.30)	19.08 (12.38)	**0.024**	0.240	1841

Note: A+: carriers of the risk allele (allele A for DRD2 rs1800498, DRD2 rs6277 and ANKK1 rs1800497, and allele C for ANKK1 rs4938015); A−: non-carriers of the risk allele. Bold numbers indicate significant *p*-values ($p < 0.05$).
Abbreviations: ADHD, attention deficit/hyperactivity disorder; BMI, body mass index; CI, confidence interval, OR, odds ratio; *p*, *p*-value, raw and with Holm–Bonferroni correction; SD, standard deviation; SNP, single nucleotide polymorphism.
[a]Chi-squared tests.
[b]Mann–Whitney *U* tests.

($D' = 0.97$, Table 2 and Figure 1), with no increased informativity, compared with each SNP, for the association with harmful alcohol use.

3.4 | Association between harmful alcohol use and other addictive disorders or risk factors

Frequencies of nicotine dependence (OR [95% CI] = 3.22 [2.39–4.36]), cannabis dependence (4.40 [2.62–7.83]) and exposition to other drugs (5.02 [3.74–6.78]) were higher in those with harmful alcohol use than those without (Table 3). As regards risk factors, there were higher frequencies of males (OR [95% CI] = 2.59 [2.10–3.20]), school failure (2.43 [1.07–6.56], 3.93 [1.80–10.35], 7.60 [3.51–19.92] and 9.37 [4.25–24.85] for the four different items, respectively), family history of tobacco use (1.35 [1.08–1.69]) and family history of cannabis use (2.83 [2.25–3.55]) in participants with harmful alcohol use. There were also significant associations between harmful alcohol use and a younger age at first alcohol consumption (OR [95% CI] = 0.95 [0.92–0.98]), a higher BMI (1.05 [1.02–1.08]), sugar consumption

TABLE 2 Haplotype distribution based on the two *dopamine receptor D2* single nucleotide polymorphisms (*DRD2* SNPs) (rs1800498 and rs6277) in subjects with harmful alcohol use (cases) and those without (controls)

Disorder	Haplotype	Frequency	Case, control ratio counts	Case, control frequencies	X^2	*p*
Harmful alcohol use	AA	0.554	713.5:564.5, 1321.6:1074.4	0.558, 0.552	0.151	0.697
	CC	0.370	454.4:823.6, 904.9:1491.1	0.356, 0.378	1.750	0.186
	CA	0.072	98.6:1179.4, 164.6:2231.4	0.077, 0.069	0.897	0.344

Note: Haplotypes correspond to the combination of DRD2 rs1800498 and DRD2 rs6277 alleles along a single chromosome; AA, CC and CA indicate the combinations of two A alleles (rs1800498A and rs6277A), two C alleles (rs1800498C and rs6277C), or one A and one C alleles (rs1800498A and rs6277C, or rs1800498C and rs6277A), respectively. X^2: chi-squared test; *p*: *p*-value.

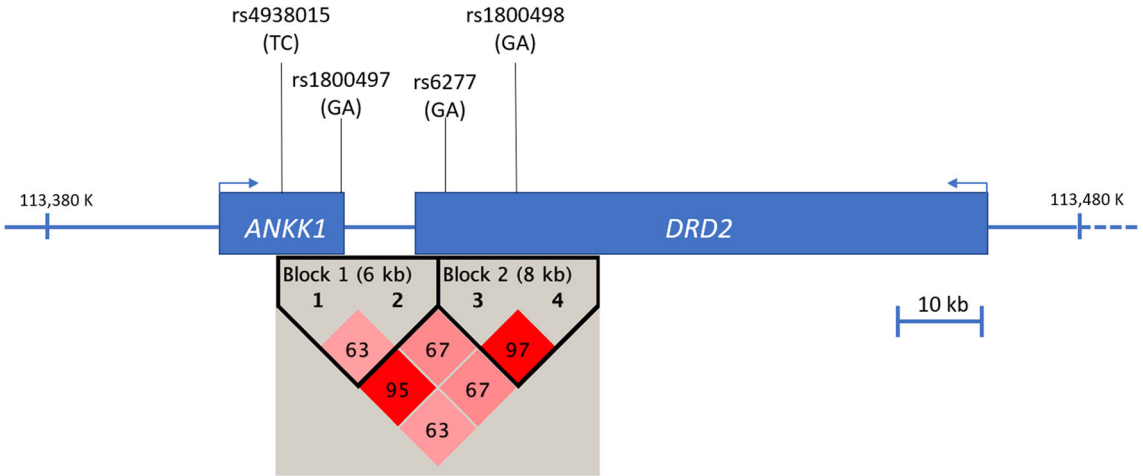

FIGURE 1 Pairwise linkage disequilibrium between single nucleotide polymorphisms (SNPs) of the *ankyrin repeat and kinase domain-containing protein 1* (*ANKK1*) and *dopamine receptor D2* (*DRD2*) genes. TC and GA refer to the nucleotides thymine, cytosine, guanine and adenine. Colour-coding represents D′ values, and values in cells are D′ * 100. Block 1: *ANKK1* gene; 1: rs4938015; 2: rs1800497; Block 2: *DRD2* gene; 3: rs6277; 4: rs1800498. Arrows indicate gene directionality.

(1.01 [1.01–1.01]), childhood ADHD score (1.02 [1.01–1.02]) and childhood conduct disorder score (1.67 [1.53–1.82]; Table 3).

To better explain harmful alcohol use, we assessed whether these significant risk factors interact with the *DRD2* SNPs, using logistic regressions (Table 4). We found significant interactions that involved *DRD2* rs1800498 and either early age at first alcohol consumption ($p = 0.022$) or depression score ($p = 0.044$). We also found significant interactions between *DRD2* rs6277 and either age at first alcohol consumption ($p = 0.030$), depression score ($p = 0.044$) or childhood conduct disorder ($p = 0.016$). As for interactions between *DRD2* SNPs and addictive disorders, the only significant interaction involved *DRD2* rs6277 and nicotine dependence ($p = 0.0495$; Table 4).

3.5 | Path analysis

We further characterized the relationship between the *DRD2* gene and harmful alcohol use with a path analysis focused on *DRD2* rs1800498 integrating mediation and moderation (Figure 2). The dependent variable was harmful alcohol use, and we included as independent variables those significantly associated with harmful alcohol use and the *DRD2* gene, that is, nicotine dependence, *DRD2* rs6277, age at first alcohol consumption and depression score. The model fitted well the data set (*p*-value model test baseline model < 0.001, CFI = 0.988, TLI = 0.976, RMSEA = 0.038 and SRMR = 0.027).

We found that *DRD2* rs1800498 had a direct effect on harmful alcohol use ($\beta = 0.48$), which indicates that *DRD2* rs1800498A carriers have a higher risk for harmful alcohol use. We also observed that age at first alcohol consumption ($\beta = 0.15$) and depression score ($\beta = 0.06$) moderated the effect of *DRD2* rs1800498 on harmful alcohol use, which indicates that *DRD2* rs1800498A carriers with a lower age at first alcohol consumption or a higher depression score have a higher risk for harmful alcohol use. We did not find any indirect effect of *DRD2* rs1800498 on harmful alcohol use ($p > 0.05$; Figure 2).

4 | DISCUSSION

In the present study, we found in a large population-based sample of young adults that *DRD2* SNP rs1800498 was associated with harmful alcohol use. Carrying the A allele directly (or through other untested or unknown factors) increased the risk of harmful alcohol use, and this

TABLE 3 Association between harmful alcohol use and other addictive disorders or risk factors

		Harmful alcohol use				
		No ($N = 1200$)	Yes ($N = 641$)			
Addictive disorders		***N* (%)**		**OR [95% CI][a]**	**Raw *p*[a]**	**Corrected *p*[a]**
Nicotine dependence		80 (7%)	124 (20%)	**3.22 [2.39–4.36]**	**<0.001**	**<0.001**
Cannabis use disorder		17 (4%)	80 (16%)	**4.40 [2.62–7.83]**	**<0.001**	**<0.001**
Ever used other drugs		77 (9%)	171 (32%)	**5.02 [3.74–6.78]**	**<0.001**	**<0.001**
		Mean (SD)		**OR [95% CI][b]**		
Nicotine dependence score		0.6 (1.2)	1.4 (1.9)	**1.44 [1.34–1.54]**	**<0.001**	**<0.001**
Cannabis dependence score		0.3 (0.9)	0.9 (1.4)	**1.64 [1.42–1.90]**	**<0.001**	**<0.001**
Risk factors		***N* (%)**		**OR [95% CI][a,b]**		
Gender (male)		630 (52%)	475 (74%)	**2.59 [2.10–3.20]**	**<0.001**	**<0.001**
School failure	*1*	222 (19%)	54 (8%)	**2.43 [1.07–6.56]**	**0.05**	0.300
	2	394 (33%)	155 (24%)	**3.93 [1.80–10.35]**	**0.002**	**0.022**
	3	363 (30%)	276 (43%)	**7.60 [3.51–19.92]**	**<0.001**	**<0.001**
	4	160 (13%)	150 (23%)	**9.37 [4.25–24.85]**	**<0.001**	**<0.001**
Lifetime sexual abuse		26 (2%)	24 (4%)	1.75 [0.99–3.09]	0.048	0.336
Lifetime suicide attempt		55 (5%)	22 (3%)	0.75 [0.44–1.22]	0.250	0.750
Parents' education	*1*	511 (45%)	293 (47%)	1.42 [0.99–2.06]	0.059	0.295
	2	496 (44%)	278 (45%)	1.39 [0.97–2.02]	0.078	0.312
Parents' use of tobacco		818 (70%)	466 (76%)	**1.35 [1.08–1.69]**	**0.011**	0.088
Parents' use of cannabis		193 (17%)	217 (36%)	**2.83 [2.25–3.55]**	**<0.001**	**<0.001**
Parents' alcohol dependence		167 (14%)	94 (15%)	1.07 [0.81–1.41]	0.620	1.000
		Mean (SD)		**OR [95% CI][b]**		
Age at first alcohol consumption		13.6 (3.0)	13.2 (2.6)	**0.95 [0.92–0.98]**	**0.002**	**0.020**
BMI		21.9 (3.1)	22.4 (3.1)	**1.05 [1.02–1.08]**	**0.003**	**0.027**
Sugar consumption		19.0 (26.3)	27.2 (35.0)	**1.01 [1.01–1.01]**	**<0.001**	**<0.001**
Depression score		1.4 (1.9)	1.4 (1.9)	1.01 [0.96–1.06]	0.639	0.639
ADHD score		17.0 (12.1)	19.6 (12.6)	**1.02 [1.01–1.02]**	**<0.001**	**<0.001**
Conduct disorder score		0.7 (1.0)	1.4 (1.5)	**1.67 [1.53–1.82]**	**<0.001**	**<0.001**

Note: Bold numbers indicate significant odds ratios.
Abbreviations: ADHD, attention deficit/hyperactivity disorder; BMI, body mass index; CI, confidence interval; OR, odds ratio; *p*, *p*-value, raw and with Holm–Bonferroni correction; SD, standard deviation.
[a]Chi-squared tests.
[b]Logistic regression.

effect was moderated by earlier age at first alcohol consumption and higher depression score (Figure 2). In other words, a younger age at first consumption and a higher depression score may represent a higher risk for harmful alcohol use in subjects carrying the A allele of *DRD2* rs1800498. Furthermore, we identified different risk factors for harmful alcohol use, namely, male gender, scholar absenteeism, parents' use of cannabis, early age at first alcohol consumption, BMI, sugar consumption, childhood conduct disorder and ADHD—variables which are commonly reported to be associated with AUD.[20,22,37–40] Surprisingly, *DRD2* rs1800498 was not associated with nicotine or cannabis dependence.

Our study shows that *DRD2* is involved in harmful alcohol use in population-based young adults, a result that is in line with previous reports of a significant association between *DRD2* and AUD.[16] However, the significant association was observed more specifically for *DRD2* rs1800498, which has, to our knowledge, never been found to be associated with AUD before. Indeed, although several studies found an association between this SNP and drug use disorders such as cannabis and heroin dependence,[13,15] the only study assessing the role of *DRD2* rs1800498 in AUD reported a non-significant result.[41] Nevertheless, this study differed from ours because it was conducted in patients who met the diagnosis of alcohol dependence, whereas in our larger and population-based sample, subjects with either alcohol dependence or harmful alcohol use were considered cases. Our finding that *DRD2* rs1800498A increases the risk of harmful alcohol use is interesting

TABLE 4 Interactions between DRD2 genetic variants and each risk factor or addictive disorder to explain harmful alcohol use

Interaction	*p*-Value[a] DRD2 rs1800498(A)	DRD2 rs6277(A)	*N*
Addictive disorders			
Nicotine dependence	<0.001	<0.001	1841
SNP	0.001	0.008	
Nicotine dependence × SNP	0.056	**0.050**	
Cannabis dependence	0.006	0.013	1841
SNP	0.023	0.208	
Cannabis dependence × SNP	0.663	0.952	
Ever used other drugs	<0.001	<0.001	1841
SNP	0.009	0.053	
Ever used other drugs × SNP	0.842	0.993	
Risk factors			
Gender male	<0.001	<0.001	1841
SNP	0.034	0.121	
Gender male × SNP	0.319	0.577	
School failure_1	0.983	0.519	1841
School failure_2	0.609	0.443	
School failure_3	0.321	0.182	
School failure_4	0.151	0.065	
SNP	0.618	0.821	
School failure_1 × SNP	0.441	0.857	
School failure_2 × SNP	0.436	0.582	
School failure_3 × SNP	0.376	0.554	
School failure_4 × SNP	0.549	0.843	
Age of alcohol first consumption	0.346	0.526	1841
SNP	0.006	0.012	
Age at first alcohol consumption × SNP	**0.022**	**0.030**	
BMI	0.097	0.301	1841
SNP	0.313	0.860	
BMI × SNP	0.487	0.946	
Sugar	0.094	0.007	1841
SNP	0.076	0.077	
Sugar × SNP	0.809	0.800	
Depression score	0.049	0.005	1841
SNP	0.002	0.001	
Depression score × SNP	**0.044**	**0.004**	
Conduct disorder score	0.004	0.002	1841
SNP	0.477	0.885	
Conduct disorder score × SNP	0.076	**0.016**	
ADHD score	0.020	0.001	1841
SNP	0.035	0.009	
ADHD score × SNP	0.432	0.075	

Note: For each potential association, we compared carriers to non-carriers of the A allele of either SNP DRD2 rs1800498 (first column) or SNP DRD2 rs6277 (second column). In bold, significant interactions

Abbreviations: ADHD, attention deficit/hyperactivity disorder; BMI, body mass index; SNP, single nucleotide polymorphism.

[a]Logistic regression.

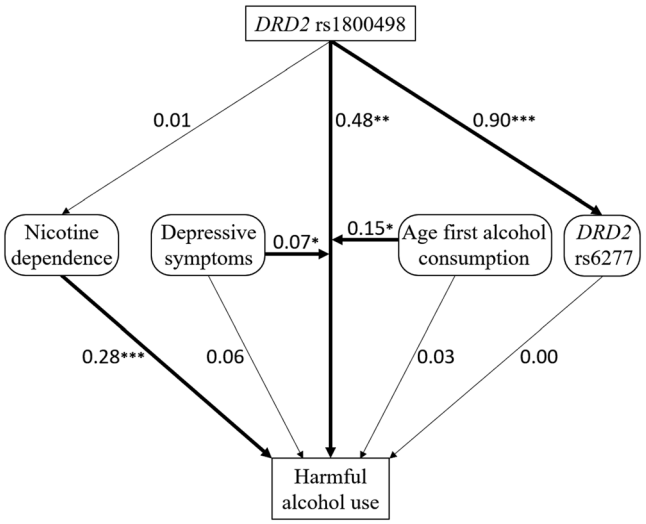

FIGURE 2 Structural equation model of the relationship between *dopamine receptor D2* (*DRD2*) rs1800498 and harmful alcohol use. *DRD2* rs1800498 has a significant direct effect on harmful alcohol use. This effect is significantly moderated by depressive symptoms and age at first alcohol consumption. Mediation of the effect of *DRD2* rs1800498 on harmful alcohol use by nicotine dependence and *DRD2* rs6277 is not significant. Numbers indicate standardized regression coefficients. Bold arrows represent significant effects. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

as this association could potentially be used for preventive purposes.

Age at first alcohol consumption is a strong risk factor for AUD, as a younger age at first drink is associated with higher rates of lifetime alcohol abuse and dependence.[40,42] The relationship between early age at first alcohol consumption and AUD has been examined, and both genetic and environmental factors have been shown to play a role.[42–44] Only few studies have assessed the influence of specific genes. The association between age at first drink and AUD symptoms was moderated by ethnicity and the alcohol-metabolizing gene *ALDH2*2* variant allele: Early age at drinking initiation was associated with an increased risk for AUD in Koreans without *ALDH2*2* allele, but not in Koreans with *ALDH2*2* allele. This protective effect of *ALDH2*2* was not observed in the two other studied ethnicities (Hens and Caucasians).[45] Two other studies focused on dopamine-related genes but did not find a significant association between AUD, genotype and age at first alcohol consumption. Alcohol-dependent patients carrying the A1 allele of the *DRD2* gene were characterized by greater severity of AUD and younger age of problem drinking onset than those without the A1 allele, but there was no significant difference in age at first drink between carriers and non-carriers.[46] Similarly, the association between dopamine transporter (*DAT1*) gene and alcohol consumption was moderated by age at first intoxication but not by age at first drink.[47] Therefore, the association we found between *DRD2* rs1800498, age at first alcohol consumption and harmful alcohol use is uncommon and calls for further research.

We also found a significant association between *DRD2* rs1800498, depression score and harmful alcohol use. AUD and depressive disorders often co-occur, increasing severity and worsening prognosis for both disorders.[48,49] A first hypothesis regarding the causal relationship between the two disorders suggests that increasing consumption of alcohol increases the risk of depression.[49] A second hypothesis suggests that depression causes AUD, for instance in individuals who drink to self-medicate negative mood states.[50] According to a third hypothesis, shared genetic factors predispose to both AUD and depressive disorders. Indeed, some genes such as *DRD2* have been reported to be associated with both AUD[4,9] and major depressive disorder.[51] However, in a genome-wide association study of comorbid depressive syndrome and AUD, no marker met significance criteria.[52] Similarly, a study focusing on SNPs in genes of interest, in a population-based sample where the majority reports consumption and distress far below diagnostic thresholds, found no modification by genotype on the relationship between alcohol consumption and mental distress.[53] With a different approach, Andersen et al.[54] found significant results: Pooling data sets from four independent genome-wide association studies, they calculated the polygenic risk score for major depressive disorder (PRS-MDD), a quantitative measure of the cumulative effects of common genetic variations across the genome on risk for major depressive disorder. After controlling that PRS-MDD accurately predicted MDD status, they reported that a higher PRS-MDD was associated with a significantly increased risk of alcohol dependence. While the results were highly significant, the proportion of variance in alcohol dependence explained by the PRS-MDD was small, suggesting that the contribution of shared genetic susceptibility to major depressive disorder and alcohol dependence comorbidity is significant but modest. Foo et al.[55] used the same approach in a larger discovery sample and replicated the findings from Andersen et al.,[54] confirming the contribution of a shared genetic risk for alcohol dependence and major depressive disorder. While research on this topic is still ongoing, there is therefore some evidence of an association between genotype, depression and AUD. Our study may be the first one to suggest a role of *DRD2* rs1800498, although with a modest weight, as none of the aforementioned studies have pinpointed a significant effect of this SNP.

Nicotine and cannabis dependences are highly comorbid with AUD.[17,18] We observe this high comorbidity in our sample. However, while we found that *DRD2* rs1800498 was associated with harmful alcohol use, we did not find associations between nicotine or cannabis dependence and *DRD2* or *ANKK1* SNPs. Both nicotine and cannabis dependences were previously reported to be associated with *DRD2*[4,56] and sometimes more specifically with *DRD2* rs1800498.[13] The absence of a significant association in our study may be due to the young age of our subjects for whom the exposure is therefore not as long-established and severe as it could be in older subjects.

When it comes to the other three studied SNPs, associations with harmful alcohol use failed to reach significance. One of the SNPs that is most commonly reported to be associated with AUD is *ANKK1* rs1800497, also known as *DRD2* Taq1A, for which the higher proportion of A1 allele in patients with AUD is modest but highly

significant.[4,16] A potential reason why we did not find an association is that, unlike the aforementioned studies which assess patients with AUD, our sample is population-based and we relied on harmful alcohol use, which is less severe than alcohol dependence. Another possible explanation is that, while our study shows a significant association between *DRD2* and harmful alcohol use, the four SNPs we examined are in strong linkage disequilibrium and the effects we observed may not be specific to *DRD2* rs1800498.

While our study has the advantages of being based on a large non-clinical sample and of including in genetic analyses many potential risk factors, it also presents a set of limitations. First, we only analysed four SNPs. This is explained by feasibility reasons: Buccal swabs are fast and easy to use in such a large sample, but they limit our capacities to test SNPs. They still allowed us to analyse SNPs from two different genes and assess linkage disequilibrium, but having data on more SNPs could have helped capture the impact of more genetic variants. Second, our data come from self-questionnaires. This is once again due to practical reasons, because data had to be collected in just a couple of days from a large number of participants. However, all of these questionnaires are validated and commonly used, which should reduce the risk of bias. Third, we had no data about the timeline of psychiatric and addictive disorders, preventing us from determining which disorder came first and whether harmful alcohol use caused or resulted from the associated disorders. Fourth, it would have been interesting to see the role of *DRD2* rs1800498 in genome-wide association study (GWAS). However, because of its moderate effect size in our study and especially as its effect involves other factors, we would not be surprised if this SNP had a very small odds ratio. Fifth, we could not eliminate potential rare subpopulations like we could have in a GWAS, so the risk of stratification biases still exists. Nevertheless, we tried to diminish this risk by including only subjects who had at least three Caucasian grandparents. Lastly, although our sample is large and population-based, it only includes young adults who are therefore on the early course of addictive disorders, which may provide limited informativity.

In conclusion, our study conducted in young adults suggests an interplay between *DRD2* genotype, factors such as age at first alcohol consumption or depression, and harmful alcohol use. Because there is no consensus on the SNPs or haplotypes involved, delimiting where the genetic vulnerability comes from is still challenging; the next logical steps would be the use of sequencing, at different steps in the addictive process. Eventually, understanding the interplay between AUD, genotype and other risk factors could help identify at-risk individuals for preventive purposes.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Julia Mattioni prepared the databank, performed the statistical analyses and wrote the first draft. Clément Vansteene completed the analyses and participated in the preparation of the manuscript. Daphnee Poupon finalized and submitted the manuscript. Philip Gorwood established the protocol, raised the grant and supervised the analyses. Nicolas Ramoz made the genetic analyses and supervised the protocol from initiation up to its submission.

DATA AVAILABILITY STATEMENT

Research data are not shared.

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