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Childhood adversities and 5-HTTLPR polymorphism as risk factors of substance use disorders: retrospective case-control study in Murcia (Spain)

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ABSTRACT

Objective To explore the separate and joint associations of childhood adversities and 5-HTTLPR polymorphism as risk factors for substance use disorders among adults.

Design Retrospective case-control study.

Setting Cases from the substance unit and controls from a representative sample of the adult general population in the metropolitan area of Murcia (Spain).

Participants Cases were defined as outpatients 18 years old or older currently in the treatment for alcohol, opioids or cocaine use disorders in the clinical unit. Controls were randomly selected among individuals without substance use disorders who participated in the Psychiatric Enquiry to General Population in Southeast Spain-Murcia (PEGASUS-Murcia) project, a cross-sectional study of a representative sample of the adult general population. In all, 142 cases and 531 controls were interviewed and a subsample of 114 cases (80.3%) and 329 controls (62%) provided a biological sample.

Exposure A history of 12 childhood adversities, lifetime mental disorders and sociodemographic variables was assessed with the Composite International Diagnostic Interview (CIDI)version 3.0). Buccal swabs were obtained to genotype the 5-HTTLPR polymorphism with the biallelic and the triallelic classification.

Main outcome and measure Multivariable logistic regression models were performed to estimate adjusted ORs and 95% CI.

Results Childhood adversities were associated with an elevated risk of substance use disorders (OR=5.77, 95% CI 3.46 to 9.61). Homozygotes for the short allele of the 5-HTTLPR polymorphism also showed the elevated risk of substance use disorders for the biallelic and triallelic classification: (1.97 (1.10 to 3.55) and 2.01 (1.11 to 3.64), respectively). No evidence for gene × environment interactions was found.

Conclusions Childhood adversities and the 5-HTTLPR polymorphism are involved in the aetiology of substance use disorders though findings exploring the existence of a gene–environment interaction were inconclusive.

Strengths and limitations of this study

- In this case-control study, controls were randomly selected among a representative sample of the general population without any substance use disorders from the same metropolitan area of Murcia (Spain).
- A careful screening for other mental disorders, performed in both cases and controls using a structured clinical interview, the Composite International Diagnostic Interview 3.0, and a comprehensive number of variables was controlled in the multivariate analyses.
- Quality genetic controls and the biallelic and triallelic approaches to the 5-HTTLPR polymorphism were tested.
- Statistical power may be insufficient to detect gene–environment interactions of the modest effect.
- Only a subgroup of participants provided biological samples, but no major differences with regard to sociodemographic variables, number of lifetime mental disorders, principal substance of abuse and exposure to childhood adversities were found when they were compared with those who provided them.

INTRODUCTION

Substance use disorders (SUD) constitute one of the major public health issues around the world,[1 2] and are major contributors to burden of disease[3] with greater risk of disability and mortality.[4 5] SUDs are considered a highly multifactorial syndrome with a wide diversity of biological, psychological and sociocultural risk factors acting and interacting throughout their development.[6] SUDs have been described as moderately to highly heritable.[7–9] One of the studied genes is the polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) and its interest is related to its potential role

in drug consumption dependent on exposure to stress (see Goldman *et al*[10] for a comprehensive review).

The serotonin transporter protein is the presynaptic neuronal reuptake site for serotonin and has been linked to the mechanism of action of several drugs.[11] The promoter activity of the *SLC6A4* gene, located at *17q11.1–q12*, could be modified by sequential elements within the proximal five regulatory region, designated as the serotonin transporter gene-linked polymorphic region (5-HTTLPR). The less frequent short (*S*) allele, associated with lower transcriptional efficiency compared with the more frequent long (*L*) allele,[12] has been related to the increased risk of a range of mental health outcomes or disorders[13–16] and, specifically to alcohol, heroin and cocaine dependence.[11] However, evidence of the relation between the 5-HTTLPR polymorphism and SUDs is conflicting. Four meta-analyses have examined the association between the 5-HTTLPR polymorphism and SUDs. The first meta-analysis found a modest association of the S allele with individuals diagnosed with alcohol dependence, and a greater association with individuals with a comorbid psychiatric condition.[17] These results highlight the importance of measuring comorbid psychiatric conditions to control for their potential moderating effect in the association of the polymorphism with SUDs. The second meta-analysis detected a potential publication bias.[18] The third showed a significant association of SUDs (including alcohol, heroin and cocaine dependence) with the polymorphism.[11] Finally, the most recent meta-analysis did not find an overall association with alcohol dependence, but highlighted several methodological limitations in published studies[19] including: inconsistencies in the screening of highly comorbid psychiatric disorders, lack of an adequate control group as many of the studies relied on convenience samples rather than population-based controls, insufficient description of genotyping methods and heterogeneity in case definition.

Other explanations of the heterogeneity of published results might stem from the existence of different classifications of the 5-HTTLPR polymorphism and gene–environment (GxE) interactions. The description of a third functional allele (L_G)[20] with an equivalence in expression to the *S* allele[13] allowed triallelic genotyping or functional reclassification on the basis of lower and higher levels of expression[21 22] with L_G and *S* classified as *S'*, and L_A as *L'*. Few studies have been published with this functional classification suggesting either a positive and significant[23] or a non-significant effect[24] associated with *S'S'*. Moreover, possible G×E interactions between traumatic life events and the polymorphism have been described.[25–27] Childhood adversities (CAs) seem to be a good candidate for G×E interactions.[28] The adversities analysed included: childhood neglect,[29 30] maltreatment,[31] poor mother–child relations and family functioning.[32] Results suggested positive G×E interactions in predicting early onset or adolescent alcohol use,[31 32] increased susceptibility to experiment with illicit drugs[29] or a significant moderating effect on cannabis use, but not on alcohol use problems.[30]

The heterogeneity of findings underscores the necessity of new studies to clarify the implications of the 5-HTTLPR polymorphism in SUDs.

The aims of the current research are to replicate, in a case-control study, the association between CAs and the 5-HTTLPR polymorphism as determinants of SUDs in adults, and to explore potential G×E interactions on SUD risk, addressing previous limitations in the existing literature.

METHODS

Study design

The current case-control study described in accordance with the Strengthening The Reporting of Genetic Association Studies (STREGA) guidelines, an extension of the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines for a candidate gene study.[33] Two signed informed consents, one for the interview and the other for the collection of biological samples, were obtained from all participants.

Patient and public involvement

There was no patient or public involvement in the design or planning of this study.

Selection of participants

Inclusion criteria for cases were being at least 18 years old currently receiving outpatient treatment for an alcohol, heroin or cocaine use disorder in the Substance Abuse Center, the main substance use treatment facility in the Murcia metropolitan area (775 000 inhabitants). Exclusion criteria were being unable to understand Spanish or having a physical or mental condition that precluded them from being interviewed. Cases were selected and interviewed between February 2014 and March 2014. A control:case ratio of approximately 3:1 was previously determined to achieve a sufficient number of controls to allow for powerful calculations while balancing the cost of genetic analyses. Controls were selected from the participants of the Psychiatric Enquiry to General Population in Southeast Spain (PEGASUS)-Murcia project, a cross-sectional study that was part of WHO World Mental Health survey initiative.[34] It was designed to carry out face-to-face interviews with a representative sample of non-institutionalised adults in the general population of the Region of Murcia. Inclusion criteria for controls were being 18 years old or older, residing in the same metropolitan area of Murcia and exclusively having no lifetime SUD. Details of the PEGASUS-Murcia project protocol, sampling frame, selection and weighting procedures have been described elsewhere.[35] Briefly, the eligible population was all non-institutionalised people aged 18 or older and were interviewed between June 2010 and May 2012. A stratified multistage clustered probability random sample design was used. Overall, PEGASUS comprised a total of 2621 participants (overall response rate of 67.4%) interviewed

by trained lay interviewers using a structured diagnostic interview.[36]

The final sample included 142 cases receiving the treatment for SUDs associated with use of either alcohol (n=81, 57.0%), opioids (n=9, 6.3%) or cocaine (n=52, 36.6%). They were compared with a sample of 531 controls randomly selected among those participants of PEGASUS-Murcia project with no lifetime SUDs (~35% of 1456 eligible controls). Overall, a subsample of 114 cases (80.3%) and 329 controls (62%) provided buccal swabs for DNA isolation and posterior SLC6A4 genotype analysis.

Sociodemographic variables

Sociodemographic variables evaluated in this study were: age at interview; sex; declared race (white/Caucasian or non-white/non-Caucasian); completed years of education (none, primary or basic: 0–11 years; secondary or college: 12 or more years of education) and marital status (married–cohabitating or separated–widowed–divorced–never married).

Diagnostic assessment

A revised version of the Composite International Diagnostic Interview (CIDI 3.0, hereafter referred to as the CIDI) adapted for use in Spain was used.[37] Briefly, the CIDI is a structured interview designed by WHO for the purpose of ascertaining diagnoses of mental disorders for international comparative epidemiological research.[38] The number of lifetime mental disorders according to Diagnostic and Statistical Manual of Mental Disorders) DSM-IV diagnostic criteria (ie, major depression, mania, hypomania, bipolar I and II, dysthymia, post-traumatic stress disorder (PTSD) and other disorders such as attention deficit, conduct and oppositional defiant disorders) was determined. The evaluation of SUDs, including alcohol and drug abuse and/or dependence disorders, was used to determine eligibility of controls in the present study.

Exposure

Childhood adversities

The CIDI includes a specific section on CAs that assesses 12 dichotomously scored CAs experienced prior the age of 18 with retrospective self-reports (for a more comprehensive description, see Kessler *et al*[39]). Briefly, CAs have been described to be highly interrelated and, based on a published factor analysis, were categorised in two meaningful groups: (1) 'maladaptive family functioning' (MFF) (that included four types of parental maladjustment—mental illness, substance misuse, criminality and violence and three types of maltreatment—physical abuse, sexual abuse and neglect), and (2) 'other CAs' (covering five types of adversities: parental death, parental divorce, other parental loss, serious physical illness and family economic adversity). This instrument[39] has been used in a number of international general population epidemiological studies.[40 41]

Genotyping

Biological samples of oral mucosal epithelium were provided by participants on completion of the interview. Samples were collected in sterile 1.5 mL tubes, registered, processed and stored at Plataforma Biobanco de Murcia (BIOBANC-MUR) (Instituto Murciano de Investigación Biosanitaria Virgen de la Arrixaca (IMIB-Arrixaca) Biobank; Spanish Biobanks Registry number: B.0000859, partner of Spanish Biobanks Platform Instituto de Salud Carlos III (ISCIII): PT17/0015/0038; http://www.biobanco.imib.es). Genomic DNA was isolated from buccal swabs using QIAamp DNA Blood Mini Kit (QIAGEN), according to the manufacturer's instructions, and was performed automatically in a QIAcube system (QIAGEN) to minimise the variability associated with manual handling. *SLC6A4* gene keeps a variable number of tandem repeat polymorphisms in the transcription control region of the gene, which is located approximately 1 kb upstream from the transcription start site. Three polymorphisms of the *SLC6A4* promoter (5-HTTLPR) were genotyped in two steps: first, PCR was carried out; second, the restriction fragment length polymorphism method was developed. The primers used to perform the PCR have been previously described[42]: *sense-ATCGCTCCTGCATCCCCCATTAT* and *antisense-GAGGTGCAGGGGGATGCTGGAA*. Briefly, 25 µl reaction included 50 ng genomic DNA, 1× amplification buffer, 0.2 mM dNTPs (deoxynucleotide tryphosphates) , 1.5 mM $MgSO_4$, 0.2 µM of each primer, 1 unit Platinum Taq PCRx polymerase (Invitrogen) and 1× PCR enhancer owing to the high GC content in the polymorphism region. The reaction was initially heated to 95°C (5 min), followed by 35 cycles of 95°C (35 s), 60°C (30 s) and 68°C (30 s) and a final elongation step of 72°C (5 min). To distinguish between *S* (103 bp) and *L* (146 bp) alleles, PCR product reactions were analysed by size determination on a QIAxcel Advanced System (QIAGEN) by high-resolution capillary electrophoresis. As a result of biallelic genotyping, individuals were genotyped as *S/S*, *S/L* or *L/L*.

Afterwards, fast HpaII restriction enzyme digestion (Thermoscientific) was carried out for genotyping SNP rs25531 according to the manufacturer's instructions. This SNP consists in the presence of adenine (A) or guanine (G), being digested in the last case. Final digested products were visualised on a QIAexcel, and individuals were genotyped as *S/S*, S/L_A, S/L_G, L_A/L_A, L_A/L_G and L_G/L_G. Given that the expression of L_G allele was suggested to be similar to the *S* allele,[20] the triallelic classification (*S*, L_G, L_A) arranged *S/S*, S/L_G and L_G/L_G individuals as *S'S'*, and S/L_A and L_A/L_G individuals as *S'L'* and L_A/L_A individuals as *L' L'*. The product sizes after digestion are: *S* (103 bp), L_A (146 bp) and L_G (83 bp, 63 bp). As a quality control of the genetic procedures, all genetic analyses were performed similarly, blinded to the case-control status of the participants and, finally, 37 cases (32.5%) also provided blood samples, so that it was possible to isolate DNA and to genotype the 5-HTTLPR polymorphisms from both origins with 100% of concordance.

Statistical methods

Risk associated to the exposure to CAs was estimated in the whole sample, whereas the effect associated to the 5-HTTLPR polymorphism and potential G×E interactions were evaluated only in the subgroup of participants with available genetic information. Student's t-tests or χ^2 tests for continuous or categorical variables, respectively, were used to explore differences in sociodemographic characteristics, number of lifetime mental disorders and CAs between those participants with and without genetic data. Calculations for deviation from the Hardy-Weinberg equilibrium were performed using the χ^2 tests of goodness of fit for biallelic and triallegic genotype frequencies in controls. A series of simple and multiple logistic regression models with case/control status as the dependent variable were built to estimate the associated risk. To explore the association with the type of *5-HHTLPR* polymorphism classification, two genetic approaches were used with the number of *S'* or *S* alleles (triallelic or biallelic frequency model, respectively). As the type of inheritance of the 5-HTTLPR polymorphism is not yet known, the exploratory analyses were repeated assuming a triallelic or biallelic dominant heritage for the short allele (at least one S' or S allele), or a recessive heritage (*S'S'* vs *L'_*, or *SS* vs *L_*). Crude and adjusted ORs and their 95% CIs were computed.

Finally, to explore the presence of G×E interactions, independent sequential multivariable logistic regression models were built including, in a hierarchical manner, the previously defined interaction terms formed with the product of the 5-HTTLPR genotype with the exposure to CAs and adjusted by all variables previously mentioned. The relative excess risk due to interaction as a measure of G×E interactions on the additive scale with logistic regression[43] and bootstrapping to estimate 95% CI was performed using the R statistical software. All other analyses were conducted using SPSS (V.20.0). All statistics used two-sided tests with alpha level of 0.05. An a priori decision was made not to correct for multiple testing as conditions in which a correction for multiple testing is necessary are still a matter of controversy. It has been suggested that in exploratory analyses of a genetically complex trait in which the relationship between genotype and phenotype has not yet been established,[11 17–19 44] multiple test adjustments are not strictly required[45] since they may increase the likelihood that actual effects would be missed (type II error rates).[46]

RESULTS

Table 1 describes the sociodemographic characteristics of the total sample (n=673) and of the subsample with DNA (n=443). Genotype frequencies in controls did not deviate from those predicted by the Hardy-Weinberg equilibrium, both in the triallelic (χ^2=2.43, p=0.119) and the biallelic classification (χ^2=0.51, p=0.473). All CAs were significantly more frequent among cases, except for the exposure to a life-threatening physical illness (table 2). There were no differences in any variable between those cases and controls with or without DNA sample (see online supplementary file 1), except for a history of parental mental illness.

Childhood adversities

The exposure to CAs was associated with a higher risk of substance abuse disorders in the whole sample in both models (table 3). A similar pattern was obtained for each independent CA, except for a history of a parental loss and a life-threatening physical illness (table 4). Among those statistically significant, sexual abuse was independently associated with the highest risk (OR=11.58, 95% CI 2.23 to 60.04) and exposure to other parental loss associated with the lowest risk (2.56, 2.09 to 7.65). The risk associated with exposure to CAs when adjusted by the number of *S'* and *S* alleles was even higher than the unadjusted risk (table 3 and see online supplementary file 2, respectively) and a similar pattern was observed when the different CAs were analysed independently (table 4).

The 5-HTTLPR polymorphism

The number of *S'* and S alleles was significantly different between cases and controls (table 1), but the associated risk was non-significant in the multivariable logistic regression (table 3). When analyses focused on those without lifetime mental disorders, the associated risk remained significant for the number of S' and S alleles (1.95, 1.09 to 3.54 for the triallelic and 2.14, 1.22 to 3.75 for the biallelic classification). Different G×E interaction terms were entered in the multivariable but none of them reached significance, either with the triallelic or with the biallelic classification of the polymorphism.

Table 5 explores the association of the different forms of heritage of the 5-HTTLPR polymorphism in general and by the type of substance for which cases were being treated. When adjusted by all other variables, only the recessive triallelic (*S'S'*+*S'L'* vs *S'S'*) and biallelic (*SL+LL* vs *SS*) heritage remained significant. When the analyses were restricted to explore the type of substance they were in treatment for (alcohol, opioids or cocaine), only those in treatment for alcohol-related problems with two *S'S'* or *SS* alleles (recessive triallelic or biallelic heritage, respectively) and the frequency biallelic model (the number of the *S* alleles) were significantly associated. Again, none of the G×E interaction terms created with the different types of heritages was significant (neither in the multiplicative, nor in the additive scales, data not shown).

DISCUSSION

The aims of this study were to evaluate the association of CAs and the 5-HTTLPR polymorphism with SUD in adults and to explore whether the 5-HTTLPR moderates the risk of CAs in a G×E interaction model. First, exposure to CAs was associated with higher risk of SUDs. Second, our results suggest a main effect of the 5-HTTLPR polymorphism on SUDs. Though there were significant differences

Table 1 Demographics, prior lifetime history of other mental disorders, and 5-HTTLPR genotype in controls and cases of substance abuse disorder

	Total sample					Subgroup with genetic data				
	Controls		Cases			Controls		Cases		
	n=531	%	n=142	%	P value	n=329	%	n=114	%	P value
Sex										
Female	341	64.2	30	21.1		206	62.6	24	21.1	
Male	190	35.8	112	78.9	**<0.0001***	123	37.4	90	78.9	**<0.0001***
Age (mean; SD)	48.42	(16.14)	42.30	(10.27)	**<0.0001***	49.47	(16.39)	42.36	(10.45)	**<0.0001***
Race										
White/Caucasic	506	95.3	133	93.7		316	96.0	106	93.0	
Non-white/Non-Caucasic	25	4.7	9	6.3	0.431	13	4.0	8	7.0	0.184
Marital status										
Married/cohabitating	324	61.0	44	31.0		201	61.1	33	28.9	
Not living with a partner	207	39.0	98	69.0	**<0.0001***	128	38.9	81	71.1	**<0.0001***
Education†										
Secondary/college	250	48.1	65	45.8		146	45.3	50	43.9	
None or primary/basic	270	51.9	77	54.2	0.626	176	54.7	64	56.1	0.785
Number of any lifetime mental disorders‡ (mean, SD)	0.36	(0.57)	1.06	(1.22)	**<0.0001***	0.34	(0.54)	1.02	(1.17)	**<0.0001***
Principal substance										
Alcohol	–	–	81	57.0		–	–	66	57.9	
Opioid	–	–	9	6.3		–	–	8	7.0	
Cocaine	–	–	52	36.6	–	–	–	40	35.1	–
Triallelic genotype frequencies (number of *S'*)										
L'L'	–	–	–	–		45	13.7	19	16.7	
S'L'	–	–	–	–		171	52.0	36	31.6	
S'S'	–	–	–	–	–	113	34.3	59	51.8	**0.001***
Dominant triallelic heritage										
L'L'	–	–	–	–		45	13.7	19	16.7	
S'S'+S'L'	–	–	–	–	–	284	86.3	95	83.3	0.434
Recessive triallelic heritage										
S'L'+L'L'	–	–	–	–		216	65.7	55	48.2	
S'S'	–	–	–	–	–	113	34.3	59	51.8	**0.001***

Continued

Table 1 Continued

	Total sample					Subgroup with genetic data				
	Controls		Cases			Controls		Cases		
	n=531	%	n=142	%	P value	n=329	%	n=114	%	P value
Biallelic genotype frequencies (number of *S*)										
LL	–	–	–	–		68	20.7	22	19.3	
SL	–	–	–	–		156	47.4	37	32.5	
SS	–	–	–	–	–	105	31.9	55	48.2	**0.005***
Dominant biallelic heritage										
LL	–	–	–	–		68	20.7	22	19.3	
SS+SL	–	–	–	–	–	261	79.3	92	80.7	0.754
Recessive biallelic heritage										
SL+LL	–	–	–	–		224	68.1	59	51.8	
SS	–	–	–	–	–	105	31.9	55	48.2	**0.002***

*P <0.05 (in bold text).
†Completed years of education (two categories: none , primary or basic: 0–11 years; secondary/college: 12 or more years of education).
‡Number of any lifetime mental disorders include the following DSM-IV lifetime diagnosis: mania, hypomania, major depressive disorder, bipolar I, bipolar II, disthymia, post-traumatic stress disorder, attention deficit disorder, conduct disorder and oppositional defiant disorder.

Table 2 Childhood adversities in cases of substance abuse disorder and controls

	Total sample					Subgroup with DNA data				
	Controls		Cases			Controls		Cases		
	n=531	%	n=142	%	P value	n=329	%	n=114	%	P value
I. Maladaptative family functioning										
Parental mental illness	27	5.1	30	21.1	**<0.0001***	10	3.0	26	22.8	**<0.0001***
Parental substance disorder	17	3.2	26	18.3	**<0.0001***	9	2.7	20	17.5	**<0.0001***
Parental criminal behaviour	6	1.1	10	7.0	**<0.0001*†**	2	0.6	8	7.0	**<0.0001*†**
Family violence	32	6.0	37	26.1	**<0.0001***	22	6.7	27	23.7	**<0.0001***
Physical abuse	28	5.3	40	28.2	**<0.0001***	14	4.2	29	25.4	**<0.0001***
Sexual abuse	3	0.6	5	3.5	**0.013*†**	0	0.0	3	2.6	**0.017*†**
Neglect	15	2.8	19	13.4	**<0.0001***	8	2.4	12	10.5	**<0.0001***
II. Other childhood adversities										
Parental death	17	3.2	10	7.0	**0.038***	11	3.3	10	8.8	**0.019***
Parental divorce	5	0.9	10	7.0	**<0.0001*†**	1	0.3	7	6.1	**<0.0001*†**
Other parental loss	12	2.3	11	7.7	**0.003***	10	3.0	10	8.8	**0.011***
Physical illness	10	1.9	6	4.2	0.120	8	2.4	5	4.4	0.334†
Economic adversities	6	1.1	13	9.2	**<0.0001*†**	4	1.2	11	9.6	**<0.0001*†**
III. Number of childhood adversities										
0	412	77.6	52	36.6		259	78.5	40	35.1	
1	80	15.1	28	19.7		52	15.8	25	21.9	
2+	39	7.3	62	43.7	**<0.0001***	19	5.8	49	43.0	**<0.0001***
IV. Childhood adversities										
No	412	77.6	52	36.6		259	78.5	40	35.1	
Yes	119	22.4	90	63.4	**<0.0001***	71	21.5	74	64.9	**<0.0001***

*P <0.05 (in bold text).
†Fisher's exact test.

Table 3 Multivariable logistic regression models to analyse the association of childhood adversities and 5-HTTLPR triallelic genotype with substance abuse disorder

	Total sample				Subgroup with DNA sample			
	Model 1†		Model 2‡		Model 1†		Model 3§	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Childhood adversities	**5.99**	**4.03 to 8.92***	**5.77**	**3.46 to 9.61***	**6.84**	**4.29 to 10.91***	**6.74**	**3.71 to 12.23***
Number of *S'* alleles (triallelic genotype)	–	–	–	–	**1.4**	**1.04 to 1.89***	1.23	0.80 to 1.90
Male sex	**6.70**	**4.31 to 10.41***	**11.57**	**5.61 to 20.26***	**6.28**	**3.80 to 10.38***	**10.45**	**5.41 to 20.18***
Age	**0.97**	**0.96 to 0.98***	0.98	0.97 to 1.00	**0.97**	**0.95 to 0.98***	0.98	0.96 to 1.00
Non-white/non-caucasic	1.37	0.62 to 3.00	0.51	0.15 to 1.70	1.83	0.74 to 4.55	0.98	0.23 to 4.09
Not living with a partner	**3.49**	**2.35 to 5.18***	**4.24**	**2.52 to 7.11***	**3.85**	**2.43 to 6.11***	**3.79**	**2.04 to 7.04***
None or primary basic educational level	1.10	0.76 to 1.59	0.95	0.58 to 1.58	1.06	0.69 to 1.63	0.94	0.51 to 1.72
Number of any other lifetime mental disorder	**2.65**	**2.08 to 3.37***	**2.45**	**1.79 to 3.34***	**2.75**	**2.05 to 3.71***	**2.83**	**1.87 to 4.27***

*P <0.05 (in bold text).
†Model 1: ORs and 95% CI estimated by simple logistic regression analysis with case-control status as the dependent variable.
‡Model 2: multivariable logistic regression analyses of childhood adversities in the total sample adjusted by all sociodemographic and the number of any other lifetime mental disorders, with case-control status as the dependent variable.
§Model 3: multivariable logistic regression analyses of childhood adversities and the number of S' alleles in the subsample with DNA adjusted by all sociodemographic, the number of any other lifetime mental disorders with case-control status as the dependent variable.

in the triallelic and biallelic frequencies of both groups, the association disappeared when controlling for CAs, sociodemographic variables and the number of lifetime mental disorders. Lastly, our results do not support a G×E interaction between CAs and 5-HTTLPR. These results are discussed below.

Our finding that exposure to CAs increases the risk of SUDs in adults is consistent with the existing literature. Exposure to CAs has been associated with the increased risk of addictive behaviours, both in youth[47] and in adulthood.[48] This risk seems to be non-specific as the exposure to CAs also increases the risk of other mental disorders[40 49–54] as well as the risk of other non-psychiatric conditions, such as diabetes, asthma and cardiovascular disease.[55–59] CAs have been suggested to be more important from a public health point of view than all common mental disorders taken together.[60]

The recessive model of heritage increased the risk of SUDs, especially in the subgroup treated for an alcohol-related disorder. Nevertheless, this specificity should be interpreted with caution due to the smaller number of participants with opioid-related or cocaine-related disorders. Of note, the association of 5-HTTLPR with SUDs was significant in multivariable analyses restricted to participants without any lifetime mental disorders. This result suggests a mediating effect of previous mental disorders on the association between 5-HTTLPR and SUDs and highlights the importance of measuring other mental disorders related to 5-HTTLPR polymorphism when analysing its relation to SUDs. Attention should be paid to variables included in multivariable logistic regression models. Our results are in accordance with previous studies. The homozygote genotype *SS* has been shown to be significantly related to heroin dependence[61] and the *S* allele to alcohol dependence.[25 62 63] A meta-analysis including alcohol, heroin, cocaine, and methamphetamine dependence showed a significantly stronger association of the dominant biallelic model (*SS*+*SL* vs *LL*),[11] though the most recent meta-analysis focused on alcohol dependence did not find an overall association.[19] Only a few studies have analysed the triallelic functional classification with conflicting results. Alleles with low *SLC6A4* promoter activity (*S'S'*) predicted comorbid alcohol, cocaine and heroin dependence but not alcohol dependence alone.[23] The triallelic polymorphism was associated with early onset in men with alcohol dependence but a non-significant trend in the opposite direction was described in women.[24]

Contrary to expectations, the results of the exploratory analysis do not support a G×E interaction between CAs and 5-HTTLPR. Very few studies focused on this interaction have been published to date. Adolescents with the *LS* variant who came from families with conflicting relationships had an increased risk of problems with alcohol.[32] The *S* allele interacted with physical, sexual and emotional abuse, neglect and exposure to domestic violence to predict early alcohol use in adolescents[31] and with reduced perceived maternal care to increase the

Table 4 Association of the independent childhood adversities by case-control status in multivariable logistic regression models

	Total sample				Subgroup with DNA sample			
	Model 1†		Model 2‡		Model 1†		Model 3§	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
I. Maladaptative family functioning								
Parental mental illness	**5.00**	**2.86 to 8.74***	**5.24**	**2.03 to 11.38***	**9.45**	**4.39 to 20.35***	**7.50**	**2.78 to 20.22***
Parental substance disorder	**6.78**	**3.56 to 12.90***	**6.22**	**2.56 to 15.08***	**7.59**	**3.34 to 17.22***	**6.98**	**2.26 to 21.55***
Parental criminal behaviour	**6.63**	**2.37 to 18.57***	**5.65**	**1.61 to 19.86***	**12.38**	**2.59 to 59.19***	**16.40**	**2.58 to 104.08***
Family violence	**5.49**	**3.27 to 9.22***	**7.73**	**3.67 to 16.26***	**4.34**	**2.36 to 8.01***	**5.05**	**2.13 to 11.98***
Physical abuse	**7.04**	**4.16 to 11.94***	**6.15**	**3.07 to 12.30***	**7.70**	**3.90 to 15.22***	**6.87**	**2.86 to 16.51***
Sexual abuse	**6.42**	**1.52 to 27.21***	**11.58**	**2.23 to 60.04***	–	–	–	–
Neglect	**5.31**	**2.63 to 10.75***	**2.80**	**1.06 to 7.44***	**4.73**	**1.88 to 11.90***	3.24	0.89 to 11.72
II. Other childhood adversities								
Parental death	**2.29**	**1.02 to 5.12***	1.64	0.56 to 4.83	**2.79**	**1.15 to 6.75***	2.81	0.82 to 9.68
Parental divorce	**7.97**	**2.68 to 23.71***	**6.37**	**1.65 to 24.56***	**21.52**	**2.62 to 176.93***	**30.41**	**2.99 to 309.14***
Other parental loss	**3.63**	**1.57 to 8.41***	**2.56**	**2.09 to 7.65***	**3.08**	**1.25 to 7.60***	2.84	0.86 to 9.45
Physical illness	2.30	0.82 to 6.44	1.54	0.44 to 5.35	1.85	0.59 to 5.76*	1.30	0.79 to 1.77
Economic adversities	**8.82**	**3.29 to 23.64***	**6.33**	**1.87 to 21.44***	**8.70**	**2.71 to 27.92***	**9.49**	**2.01 to 44.78***

*P <0.05 (in bold text).
‡Model 2: each row represents the ORs and 95% CI estimated by multivariable logistic regression analyses of childhood adversities in the total sample adjusted by all sociodemographic and the number of any other lifetime mental disorders with case-control status as the dependent variable.
†Model 1: each row represents the ORs and 95% CI of each independent childhood adversity estimated by simple logistic regression analysis with case-control status as the dependent variable.
§Model 3: each row represents the ORs and 95% CI of each independent childhood adversity estimated by multivariable logistic regression analyses of childhood adversities in the subsample with DNA adjusted by all sociodemographic, the number of any other lifetime mental disorders and the number of *S'* alleles with case-control status as the dependent variable.

Table 5 Association of the 5-HTTLPR poylymorphism with substance abuse disorders by type of heritage

	All substances				Alcohol				Opioid				Cocaine			
	Model 1†		Model 2‡		Model 1†		Model 2‡		Model 1†		Model 2‡		Model 1†		Model 2‡	
	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI	OR	95% CI
Triallelic genotype																
Number of *S* alleles	**1.40**	**1.04 to 1.89***	1.23	0.80 to 1.90	**1.64**	**1.09 to 2.49***	1.63	0.96 to 2.77	0.48	0.17 to 1.37	0.35	0.09 to 1.33	1.31	0.79 to 2.16	1.13	0.63 to 2.04
Dominant triallelic heritage (*S'S'+S'L'* vs *L'L'* (ref))	0.79	0.44 to 1.42	0.55	0.24 to 1.26	0.89	0.42 to 1.86	0.85	0.27 to 2.73	0.47	0.09 to 2.43	0.16	0.01 to 1.66	0.75	0.31 to 1.79	0.46	0.15 to 1.39
Recessive triallelic heritage (*S'S'* vs *S' L'+L'L'* (ref))	**2.05**	**1.33 to 3.16***	**1.97**	**1.10 to 3.55***	**2.59**	**1.51 to 4.44***	**2.85**	**1.32 to 6.15***	0.27	0.03 to 2.25	0.38	0.04 to 3.86	1.91	0.99 to 3.70	1.69	0.76 to 3.77
Biallelic genotype																
Number of S alleles	1.35	0.90 to 2.02	1.35	0.90 to 2.02	**1.53**	**1.05 to 2.42***	**1.66**	**1.03 to 2.73***	0.63	0.23 to 1.69	0.65	0.21 to 2.02	1.45	0.90 to 2.31	1.37	0.79 to 2.38
Dominant biallelic heritage (*SS+SL* vs *LL* (ref))	1.09	0.64 to 1.86	0.95	0.45 to 1.99	1.06	0.55 to 2.06	1.24	0.46 to 3.32	0.78	0.15 to 3.96	0.36	0.04 to 2.94	1.23	0.52 to 2.90	0.93	0.33 to 2.61
Recessive biallelic heritage (*SS* vs *SL+LL* (ref))	**1.99**	**1.29 to 3.07***	**2.01**	**1.11 to 3.64***	**2.41**	**1.41 to 4.12***	**2.90**	**1.34 to 6.31***	0.30	0.04 to 2.51	0.44	0.04 to 4.37	1.93	0.99 to 3.74	1.64	0.73 to 3.65

*P <0.05 (in bold text).
†Model 1: each row represents the ORs and 95% CI of the type of heritage in a simple logistic regression analyses with case-control status as the dependent variable.
‡Model 2: each row represents the ORs and 95% CI of the type of heritage in a multivariable logistic regression analyses adjusted by all sociodemographic, the number of any other lifetime mental disorders and the childhood adversities with case-control status as the dependent variable.

susceptibility to use alcohol, cocaine and cannabis.[29] In contrast, there was no G×E interaction between the biallelic 5-HTTLPR polymorphisms with childhood neglect on alcohol use problems[30] and between the triallelic polymorphisms in a sample of alcohol-dependent adults with no current comorbid mental disorders in relation to smoking.[64] College students homozygous for the *S* allele who have experienced multiple negative life events are at greater risk for alcohol consumption and drug use.[25] The *S'* allele carriers were more susceptible to the effects of a history of family conflicts on alcohol misuse[26] and to the effects of greater residential instability on substance use across ages 10–24 years.[27] Nevertheless, our results should be interpreted with caution as many other and not yet well-known factors may contribute to the development of complex brain disorders such as SUDs. For example, the G×E interactions influencing SUDs may be more robust at specific periods, such as younger ages.[65] Other gene–gene interactions may obscure the potential role of specific genes in the aetiology of substance disorders.[66] Lastly, epigenetic mechanisms may modify gene expression and modulate the development of SUDs.[67] To the best of our knowledge, only one study has directly analysed the epigenetic changes of the 5-HTTLPR in a case-control study with no differences between alcohol-dependent and control participants.[68] However, additional research is needed on this topic as the latter study has important limitations, including a small sample size (only 27 patients and 15 controls) and analyses solely focused on one (the methylation patterns) among other known epigenetic mechanisms.[67]

Several limitations deserve consideration. First, and most importantly, it is reasonable to consider that statistical analyses may have been underpowered to detect a small G×E interaction. If this is the case, additional association studies with larger samples or the combination of similar studies in future meta-analyses of G×E interactions will contribute to clarify this point.[69] Second, only a subgroup of participants provided biological samples. However, those who provided the DNA sample did not differ from those who did not in terms of sociodemographic variables, number of lifetime mental disorders, principal substance of abuse and exposure to CAs. Third, a potential recall bias cannot be ruled out as CAs were assessed retrospectively. However, this bias is likely not to have affected the results presented as CAs that were evaluated through an identical retrospective structured questionnaire in both cases and controls.[70] Fourth, relatedness among participants has not been assessed, but it is highly unlikely that this possibility would have affected the results as case-control samples have been drawn from non-isolated populations. Fifth, the selection of cases from clinical treatment settings may have introduced a selection bias. As a result, caution is warranted in the generalisation of the results to other individuals who are not receiving formal treatment for their disorders. Finally, psychiatric diagnoses were determined based on fully structured interviews with the CIDI, but

moderate-to-excellent concordance has been described for most mental disorders in blind clinical reappraisal studies.[71 72]

A major strength of the study is related to measures introduced in the design to address some of the limitations described in previous studies.[19] First, an adequate control group was randomly selected among those without any SUDs from the PEGASUS-Murcia project and a comprehensive number of variables were controlled for in the multivariable analyses.[35 36] Second, a careful screening for other mental disorders related to the same polymorphism[15 73 74] was performed using a structured clinical interview.[37] Finally, biallelic and triallelic approaches to the 5-HTTLPR polymorphism were tested in the subsample of participants with DNA. Quality genetic controls were performed and the 5-HTTLPR polymorphism frequencies in controls were in accordance to the Hardy-Weinberg equilibrium.

In summary, the present study confirms the risk effect of CAs on SUDs and adds new evidence to support the role of the 5-HTTLPR polymorphism. Further studies and future meta-analyses focused on G×E interactions are needed to clarify these nature–nurture interactions as such findings might have important implications in the prevention and treatment of SUDs.[75]

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Competing interests RCK has served on advisory boards for Mensante Corporation, Plus One Health Management, Lake Nona Institute and US Preventive Medicine, is a co-owner of DataStat. In the past three years, RCK has been a consultant for Hoffman-La Roche, Johnson & Johnson Wellness and Prevention, and Sonofi-Aventis Groupe. There are no patents, products in development or marketed products to declare.

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