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 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.

INTRODUCTION

Substance use disorders (SUDs) constitute one of the major public health issues around the world,1,2 and are major contributors to burden of disease3 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.\(^\text{10}\) 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.\(^\text{11}\) 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,\(^\text{12}\) has been related to the increased risk of a range of mental health outcomes or disorders\(^\text{13−16}\) and, specifically, to alcohol, heroin and cocaine dependence.\(^\text{11}\) 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 polymorphism with SUDs. The second meta-analysis detected a potential publication bias.\(^\text{18}\) The third showed a significant association of SUDs (including alcohol, heroin and cocaine dependence) with the polymorphism.\(^\text{11}\) Finally, the most recent meta-analysis did not find an overall association with alcohol dependence, but highlighted several methodological limitations in published studies\(^\text{19}\) 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 (\(L_{\text{c}}\))\(^\text{20}\) with an equivalence in expression to the S allele\(^\text{15}\) allowed triallelic genotyping or functional reclassification on the basis of lower and higher levels of expression\(^\text{21−22}\) with \(L_{\text{c}}\) and S classified as \(S'\), and \(L_{\text{c}}\) as \(L\). Few studies have been published with this functional classification suggesting either a positive and significant\(^\text{23}\) or a non-significant effect\(^\text{24}\) associated with \(S'S'\). Moreover, possible GxE interactions between traumatic life events and the polymorphism have been described.\(^\text{25−27}\) Childhood adversities (CAs) seem to be a good candidate for GxE interactions.\(^\text{28}\) The adversities analysed included: childhood neglect,\(^\text{29}\) maltreatment,\(^\text{30}\) poor mother–child relations and family functioning.\(^\text{31}\) Results suggested positive GxE interactions in predicting early onset or adolescent alcohol use,\(^\text{31−32}\) increased susceptibility to experiment with illicit drugs\(^\text{33}\) or a significant moderating effect on cannabis use, but not on alcohol use problems.\(^\text{30}\)

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 GxE 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.\(^\text{33}\) 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.\(^\text{34}\) 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.\(^\text{35}\) 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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\textsuperscript{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\textsuperscript{40} has been used in a number of international general population epidemiological studies.\textsuperscript{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 QIAaamp 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 1kb 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\textsuperscript{42}:

\texttt{sense-ATCGCTCCGTGATCCCATTTAT} and \texttt{antisense-GAGGTCAGGGGATGCTGGA}A. Briefly, 25\,\mu\text{L} reaction included 50ng genomic DNA, 1x amplification buffer, 0.2 mM dNTPs (deoxynucleotide triphosphates), 1.5 mM MgSO\textsubscript{4}, 0.2\,\mu\text{M} of each primer, 1 unit Platinum Taq PCRx polymerase (Invitrogen) and 1x 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 (30s), 60°C (30s) and 68°C (30s) and a final elongation step of 72°C (5\,min). To distinguish between S (103bp) and L (146bp) 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 classified as S/S (103 bp), S/L (103 bp and 146 bp) or L/L (146 bp) allele. The product sizes after digestion are: S (103bp) and L (146bp) allele were suggested to be similar to the S allele\textsuperscript{30} the triallelic classification (S, L\textsubscript{S}, L\textsubscript{G}) arranged S/S, S/L\textsubscript{G}, L\textsubscript{S}/L\textsubscript{G}, L\textsubscript{S}/L\textsubscript{L} and L\textsubscript{G}/L\textsubscript{L}. Given that the expression of L\textsubscript{S} allele was suggested to be similar to the S allele\textsuperscript{30} the triallelic classification (S, L\textsubscript{S}, L\textsubscript{G}) arranged S/S, S/L\textsubscript{G}, L\textsubscript{S}/L\textsubscript{G}, L\textsubscript{S}/L\textsubscript{L} and L\textsubscript{G}/L\textsubscript{L}. The product sizes after digestion are: S (103bp), L\textsubscript{S} (146bp) and L\textsubscript{G} (83bp, 65bp). 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 $\chi^2$ 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 $\chi^2$ tests of goodness of fit for biallelic and triallelic 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-HTTLPR polymorphism classification, two genetic approaches were used with the number of S' or S alleles (triaallelic 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 (SS' 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 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 not strictly required since they may increase the likelihood that actual effects would be missed (type II error rates).

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 ($\chi^2$=2.43, p=0.119) and the biallelic classification ($\chi^2$=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 (SS'+SL' vs SS') and biallelic (SL+IL 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 SS' 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 | Controls | | Cases | | | P value | | | Subgroup with genetic data | Controls | | Cases | | | P value |
|--------------|----------|--------|--------|--------|--------|--------|--------|--------|--------------|----------|--------|--------|--------|--------|--------|
|              | n=531 %  | n=142 % | P value | n=329 % | n=114 % | P value |
| Sex          |          |        |        |        |        |        |
| Female       | 341 64.2 | 30 21.1 | <0.001 | 206 62.6 | 24 21.1 | <0.001 |
| Male         | 190 35.8 | 112 78.9 | <0.001 | 123 37.4 | 90 78.9 | <0.001 |
| Age (mean; SD) | 48.42 (16.14) | 42.30 (10.27) | <0.001 | 49.47 (16.39) | 42.36 (10.45) | <0.001 |
| Race         |          |        |        |        |        |        |
| White/Caucasic | 506 95.3 | 133 93.7 | <0.001 | 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 | <0.001 | 201 61.1 | 33 28.9 |        |
| Not living with a partner | 207 39.0 | 98 69.0 | <0.001 | 128 38.9 | 81 71.1 | <0.001 |
| Education† |          |        |        |        |        |        |
| Secondary/college | 250 48.1 | 65 45.8 | 0.626 | 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) | <0.001 | 0.34 (0.54) | 1.02 (1.17) | <0.001 |
| Principal substance |          |        |        |        |        |        |
| Alcohol       | – –     | 81 57.0 | <0.001 | – –     | 66 57.9 |        |
| Opioid        | – –     | 9 6.3  | – –     | 8 7.0  |        |        |
| Cocaine       | – –     | 52 36.6 | – –     | 40 35.1 | – –     |        |
| Triallelic genotype frequencies (number of S’) |          |        |        |        |        |        |
| LL’           | – –     | – –     | 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 |          |        |        |        |        |        |
| LL’           | – –     | – –     | 45 13.7 | 19 16.7 |        |        |
| S’S+S’L’      | – –     | – –     | 284 86.3 | 95 83.3 | 0.434  |        |
| Recessive triallelic heritage |          |        |        |        |        |        |
| S’L+LL’       | – –     | – –     | 216 65.7 | 55 48.2 | 0.001  |        |
| S’S’          | – –     | – –     | 113 34.3 | 59 51.8 |        |        |

Continued
Table 1  Continued

|                      | Total sample |                      | Subgroup with genetic data |                      |
|----------------------|--------------|----------------------|----------------------------|----------------------|
|                      | Controls     | Cases                | P value                    | Controls             | Cases                | P value                    |
|                      | n=531        | %                    |                            | n=329                | %                    |                            | n=142                | %                    |                            | n=114                | %                    |                            |
| 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                 | 0.002*                |                            |                       |                      |                            |
| SS                   | –            | –                    | –                          | 105                   | 31.9                 | 55                        | 48.2                 |                      |                            |                       |                      |                            |

*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, dysthymia, 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 | Controls | Cases | Subgroup with DNA data | Controls | Cases | P value | Controls | Cases | P value |
|-------------|----------|-------|------------------------|----------|-------|---------|----------|-------|---------|
|             | 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 | <0.0001* | 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

| Subgroup with DNA sample | Total sample | Model 1† | Model 2‡ | Model 3§ |
|--------------------------|--------------|----------|----------|----------|
|                          | OR           | 95% CI   | OR       | 95% CI   | OR       | 95% CI   | OR       | 95% CI   |
| **Childhood adversities**|              |          |          |          |          |          |          |          |
| Number of S' alleles (triallelic genotype) | 6.70 | 4.31 to 10.41                 | 5.77 | 3.64 to 9.31                 | 6.84 | 4.29 to 10.91                 |
| Male sex                 | 0.97 | 0.96 to 0.98*                 | 0.98 | 0.97 to 1.00                 | 0.98 | 0.95 to 0.99*                 |
| Age                      | 0.97 | 0.96 to 0.98*                 | 0.98 | 0.97 to 1.00                 | 0.98 | 0.95 to 0.99*                 |
| Non-white/non-caucasic   | 0.97 | 0.96 to 0.98*                 | 0.98 | 0.97 to 1.00                 | 0.98 | 0.95 to 0.99*                 |
| Not living with a partner| 0.97 | 0.96 to 0.98*                 | 0.98 | 0.97 to 1.00                 | 0.98 | 0.95 to 0.99*                 |
| None or primary basic educational level | 3.49 | 2.35 to 5.18                 | 3.36 | 2.35 to 5.18                 | 3.92 | 2.43 to 6.71                 |
| Number of any other lifetime mental disorder | 1.10 | 0.76 to 1.59                 | 1.06 | 0.69 to 1.63                 | 0.94 | 0.51 to 1.72                 |
| **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 variables and the number of any other lifetime mental disorders. | | | | | | | | |

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 variables and the number of any other lifetime mental disorders.

*P <0.05 (in bold text).

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 and the S allele to alcohol dependence. A meta-analysis including alcohol, heroin, cocaine, and methamphetamine dependence showed a significantly stronger association of the dominant biallelic model (SS+SL vs LL), although the most recent meta-analysis focused on alcohol dependence did not find an overall association. Only a few studies have analysed the triallelic functional classification with conflicting results. Alleles with low SLC6A4 promoter activity (SS') predicted comorbid alcohol, cocaine and heroin dependence but not alcohol dependence alone. 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. 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. The S allele interacted with physical, sexual and emotional abuse, neglect and exposure to domestic violence to predict early alcohol use in adolescents and with reduced perceived maternal care to increase the 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.
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* | 14.00  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 polymorphism with substance abuse disorders by type of heritage

| Triallelic genotype | 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 |
| Number of S’ alleles | 1.40 1.04 to 1.89* | 1.64 1.09 to 2.49* | 0.48 0.17 to 1.37 | 0.79 0.21 to 2.67 |
| Dominant triallelic heritage (S’S+S’L vs LL (ref)) | 0.79 0.44 to 1.42 | 0.94 0.42 to 1.86 | 0.47 0.09 to 2.43 | 0.75 0.31 to 1.79 |
| Recessive triallelic heritage (S’S vs S’L+LL (ref)) | 2.05 1.33 to 3.16* | 2.59 1.51 to 4.44* | 0.27 0.03 to 2.25 | 1.91 0.99 to 3.70 |
|  | 1.99 1.29 to 3.07* | 2.41 1.41 to 4.12* | 0.30 0.04 to 2.51 | 1.93 0.99 to 3.74 |

| Biallelic genotype | Alcohol | Opioid | Cocaine |
|---------------------|---------|--------|---------|
|                      | Model 1†      | Model 2‡ | Model 1†  | Model 2‡ |
|                     | OR 95% CI     | OR 95% CI | OR 95% CI | OR 95% CI |
| Number of S alleles | 1.35 0.90 to 2.02 | 1.53 1.05 to 2.42* | 0.63 0.23 to 1.69 | 1.45 0.90 to 2.31 |
| Dominant biallelic heritage (SS vs S+L (ref)) | 1.09 0.64 to 1.86 | 1.06 0.55 to 2.06 | 0.78 0.15 to 3.96 | 1.23 0.52 to 2.90 |
| Recessive biallelic heritage (SS vs S+LL (ref)) | 1.99 1.29 to 3.07* | 2.41 1.41 to 4.12* | 0.30 0.04 to 2.51 | 1.93 0.99 to 3.74 |

*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.
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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.

Patient consent for publication
Not required.

Ethics approval
The study was approved by the Clinical Research Ethics Committee of the University Hospital Virgen de la Arrixaca of Murcia in July 2012 (IDHUVA-A-6-2012).

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Data are available upon reasonable request.

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REFERENCES
1. Degenhardt L, Whiteford HA, Ferrari AJ, et al. Global burden of disease attributable to illicit drug use and dependence: findings from the Global Burden of Disease Study 2010. Lancet 2013;382:1564–74.
2. Whiteford HA, Ferrari AJ, Degenhardt L, et al. The global burden of mental, neurological and substance use disorders: an analysis from the Global Burden of Disease Study 2010. PLoS One 2015;10:e0116820.
3. Laremore P, Kusel J, Leonard S, et al. The economic burden of alcohol dependence in Europe. Alcohol Alcohol Oxf Oxfs 2013;48:259–69.
4. Laremore P, Leonard S, Buchanan-Hughes A, et al. Risk of all-cause mortality in alcohol-dependent individuals: a systematic literature review and meta-analysis. EBioMedicine 2015;2:1394–404.
5. Samokhvalov AV, Popova S, Room R, et al. Disability associated with alcohol abuse and dependence. Alcohol Clin Exp Res 2010;34:1871–8.
6. Kendler KS, Ohlsson H, Edwards AC, et al. A developmental etiological model for drug abuse in men. Drug Alcohol Depend 2017;179:220–6.
7. Enoch MA. Genetic influences on the development of alcoholism. Curr Psychiatry Rep 2013;15:412.
8. Jensen KP. A review of genome-wide association studies of stimulant and opioid use disorders. Mol Neuropsychiatry 2016;2:37–45.
9. Levran O, Yufnerov V, Kreek MJ. The genetics of the opioid system and specific drug addictions. Hum Genet 2012;131:823–42.
10. Goldman D, Orozzi G,ucci F. The genetics of addictions: uncovering the genes. Nat Rev Genet 2005;6:S21–32.
11. Cao J, Hu Xia, Li D. Multi-cultural association of the serotonin transporter gene (SLC6A4) with substance use disorder. Neuropsychopharmacology 2013;38:1737–47.
12. Lesch KP, Bengel D, Heils A, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;274:1527–31.
13. Hu X, Orozzi G, Chun J, et al. An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol Clin Exp Res 2005;29:8–16.
14. Li D, He L, Meta-analysis supports association between serotonin transporter (5-HTT) and suicidal behavior. *Mol Psychiatry* 2007;12:47–54.

15. Lottrich FE, Pollock BG. Meta-analysis of serotonin transporter polymorphisms and affective disorders. *Psychiatr Genet* 2004;14:121–9.

16. Takano A, Arakawa R, Hayashi M, et al. Relationship between neuroticism personality trait and serotonin transporter binding. *Biol Psychiatry* 2007;62:588–92.

17. Feinn R, Nellissey M, Kranzler HR. Meta-analysis of the association of a functional serotonin transporter promoter polymorphism with alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* 2005;138B:79–84.

18. McHugh RK, Hofmann SG, Asnaani A, et al. The serotonin transporter gene and risk for alcohol dependence: a meta-analytic review. *Drug Alcohol Depend* 2010;108:1–6.

19. Villalba K, Attonito J, Mendy A, et al. Meta-analysis of the associations between the SLC6A4 promoter polymorphism (HTTLLPR) and the risk for alcohol dependence. *Psychiatr Genet* 2015;25:47–58.

20. Nakamura M, Ueno S, Sano A, et al. The human serotonin transporter gene linked polymorphism (5-HTTLPR) shows ten novel allelic variants. *Mol Psychiatry* 2000;5:32–8.

21. Parsley RV, Hastings RS, Oquendo MA, et al. Effect of a triallelic functional polymorphism of the serotonin-tranporter-linked region on expression of serotonin transporter in the human brain. *Am J Psychiatry* 2006;163:48–51.

22. Zalszum G, Huang YY, Oquendo MA, et al. Association of a triallelic serotonin transporter gene promoter region (5-HTTLPR) polymorphism with stressful life events and severity of depression. *Am J Psychiatry* 2006;163:1588–93.

23. Enoch MA, Gorodetsky E, Hodgkinson C, et al. Functional genetic variants that increase synaptic serotonin and 5-HT3 receptor sensitivity predict alcohol and drug dependence. *Mol Psychiatry* 2011;16:139–49.

24. Pascale E, Ferraguti G, Codazzo C, et al. Alcohol dependence and serotonin transporter functional polymorphisms 5-HTTLPR and rs25531 in an Italian population. *Alcohol Clin Exp Res* 2015;50:255–69.

25. Covault J, Tennen H, Arminelli S, et al. Interactive effects of the serotonin transporter 5-HTTLPR polymorphism and stressful life events on college student drinking and drug use. *Biol Psychiatry* 2007;61:609–16.

26. Kim J, Park A, Giatt SJ, et al. Interaction effects between the 5-hydroxytryptamine transporter-linked polymorphic region (5-HTTLPR) genotype and family conflict on adolescent alcohol use and misuse. *Addiction* 2015;110:289–99.

27. Windle M, Kogan SM, Lee S, et al. Neighborhood × Serotonin Transporter Linked Polymorphic Region (5-HTTLPR) interactions for substance use among ages 10 to 24 years using a harmonized data set of African American children. *Dev Psychopathol* 2018;26:415–31.

28. Enoch MA. The influence of gene-environment interactions on the development of alcoholism and drug dependence. *Curr Psychiatry Rep* 2012;14:150–8.

29. Gerra G, Zaldivar C, Castaldini L, et al. Relevance of perceived childhood neglect, 5-HTT gene variants and hypothalamus-adrenal axis dysregulation to substance abuse susceptibility. *Am J Med Genet B Neuropsychiatr Genet* 2010;153B:75–22.

30. Vaske J, Newsome J, Wright JP. Interaction of serotonin transporter linked polymorphic region and childhood neglect on criminal behavior and substance use for males and females. *Dev Psychopathol* 2012;24:181–93.

31. Kaufman J, Yang BZ, Douglas-Palumberi H, et al. Genetic and environmental predictors of early alcohol use. *Biol Psychiatry* 2007;61:1228–34.

32. Nilsson KW, Sjöberg RL, Damber M, et al. Role of the serotonin transporter gene and family function in adolescent alcohol consumption. *Addict Biol* 2005;29:864–70.

33. Little J, Higgins JP, Ioannidis JP, et al. Strengthening the Reporting of Genetic Association studies (STREGA): an extension of the STROBE Statement. *Ann Intern Med* 2009;150:206–15.

34. Kessler RC, Aguilar-Gaxiola S, Alonso J, et al. The WHO World Mental Health (WMH) Surveys. Psychiatr Stuttg 2009;5:5–9.

35. Navarro-Mateu F, Tormo MJ, Salmerón D, et al. Prevalence of mental disorders in the South-East of Spain and the European regions most affected by the economic crisis: the cross-sectional PEGASUS-Murcia project. *PLoS One* 2015;10:e0137293.

36. Navarro-Mateu F, Moran-Sanchez I, Alonso J, et al. Cultural adaptation of the Latin American version of the World Health Organization Composite International Diagnostic Interview (WHO-CIDI) (v 3.0) for use in Spain. *Gac Sanit* 2012;27:325–31.

37. Kessler RC, Üstün TB. The World Mental Health (WMH) Survey Initiative version of the World Health Organization (WHO) Composite International Diagnostic Interview (CIDI). *Int J Methods Psychiatr Res* 2004;13:93–121.

38. Bruffaerts R, Demyttenaere K, Borges G, et al. Childhood adversities as risk factors for onset and persistence of suicidal behaviour. *Br J Psychiatry* 2010;197:20–7.

39. Green JG, McLaughlin KA, Berglund PA, et al. Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Arch Gen Psychiatry* 2010;67:113–23.

40. Mielman TA, Alim T, Brown DD, et al. Serotonin polymorphisms and posttraumatic stress disorder in a trauma exposed African American population. *Depress Anxiety* 2009;26:993–7.

41. Knol MJ, van der Tweel I, Grobbee DE, et al. Estimating interaction on an additive scale between continuous determinants in a logistic regression model. *Int J Epidemiol* 2007;36:1111–8.

42. Navarro-Mateu F, García-Moles H, et al. Meta-analyses of the 5-HTTLPR polymorphisms and post-traumatic stress disorder. *PLoS One* 2013;8:e66227.

43. Bender R, Lange S. Adjusting for multiple testing-when and how? *J Clin Epidemiol* 2001;54:343–9.

44. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990;1:43–6.

45. Ramos-Olazagasti MA, Bird HR, Canino GJ, et al. Childhood adversity and early initiation of alcohol use in two representative samples of puerto rican youth. *J Youth Adolesc* 2016;46:28–44.

46. Konkoly-Thege B, Horwood LJ, et al. Relationship between interpersonal trauma exposure and addictive behaviours: a systematic review. *BMC Psychiatry* 2017;17:164.

47. Kendall S, Jackson HJ, Hulbert CA, et al. Childhood trauma and psychotic disorders: a systematic, critical review of the evidence. *Schizophr Bull* 2009;36:688–709.

48. Matheson SL, Shepherd AM, Pinchbeck RM, et al. Childhood adversity in schizophrenia: a systematic meta-analysis. *Psychol Med* 2013;43:225–38.

49. McGrath JJ, McLaughlin KA, Saha S, et al. The association between childhood adversities and subsequent first onset of psychotic experiences: a cross-national analysis of 23 998 respondents from 17 countries. *Psychol Med* 2017;47:1230–45.

50. Nanni V, Uher R, Danese A. Childhood maltreatment predicts unfavorable course of illness and treatment outcome in depression: a meta-analysis. *Am J Psychiatry* 2012;169:141–51.

51. Nelson J, Klumparendt A, Doebler P, et al. Childhood maltreatment and characteristics of adult depression: meta-analysis. *Br J Psychiatry* 2017;210:96–104.

52. Norman RE, Byambaa M, De R, et al. The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. *PLoS Med* 2012;9:e1001349.

53. Abarca NE, Garro AC, Pearlman DN. Relationship between breastfeeding and asthma prevalence in young children exposed to adverse childhood experiences. *J Asthma* 2019;56:1–10.

54. Basu A, McLaughlin KA, Misra S, et al. Childhood maltreatment and health impact: the examples of cardiovascular disease and type 2 diabetes mellitus in adults. *Clin Nutr* 2017;36:125–39.

55. Huang H, Yan P, Shan Z, et al. Adverse childhood experiences and risk of type 2 diabetes: a systematic review and meta-analysis. *Metabolism* 2015;64:1408–18.

56. Shekelle MA. Childhood adversities and chronic conditions: examination of mediators, recall bias and age at diagnosis. *Int J Public Health* 2018;63:181–92.

57. Tamayo T, Christian H, Rathmann W. Impact of early psychosocial factors (childhood socioeconomic factors and adversities) on future risk of type 2 diabetes, metabolic disturbances and obesity: a systematic review. *BMC Public Health* 2010;10:525.

58. Cuipiers P, Smit F, Unger F, et al. The disease burden of childhood adversities in adults: a population-based study. *Child Abuse Negl* 2011;35:937–45.

59. Gerra G, Garofano L, Santoro G, et al. Association between low-activity serotonin transporter genotype, sex, and serotonin function and heroin dependence: behavioral and personality correlates. *Am J Med Genet B Neuropsychiatr Genet* 2004;128B:37–42.
62. Hammoumi S, Payen A, Favr E JD, et al. Does the short variant of the serotonin transporter linked polymorphic region constitute a marker of alcohol dependence? Alcohol 1999;17:107–12.

63. Limosin F, Loze JY, Boni C, et al. Male-specific association between the 5-HTTLPR S allele and suicide attempts in alcohol-dependent subjects. J Psychiatr Res 2005;39:179–82.

64. Mingione CJ, Hefner JL, Blom TJ, et al. Childhood adversity, serotonin transporter (5-HTTLPR) genotype, and risk for cigarette smoking and nicotine dependence in alcohol dependent adults. Drug Alcohol Depend 2012;123:201–6.

65. Kendler KS, Gardner C, Dick DM. Predicting alcohol consumption in adolescence from alcohol-specific and general externalizing genetic risk factors, key environmental exposures and their interaction. Psychol Med 2011;41:1507–16.

66. Skowronek MH, Laucht M, Hohm E, et al. Interaction between the dopamine D4 receptor and the serotonin transporter promoter polymorphisms in alcohol and tobacco use among 15-year-olds. Neurogenetics 2006;7:239–46.

67. Palmisano M, Pandey SC. Epigenetic mechanisms of alcoholism and stress-related disorders. Alcohol 2017;60:7–18.

68. Park BY, Lee BC, Jung KH, et al. Epigenetic changes of serotonin transporter in the patients with alcohol dependence: methylation of an serotonin transporter promoter CpG island. Psychiatry Investig 2011;8:130–3.

69. Hartt J, Rutter M. Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. J Child Psychol Psychiatry 2004;45:260–73.

70. Taylor A, Kim-Cohen J. Meta-analysis of gene-environment interactions in developmental psychopathology. Dev Psychopathol 2007;19:1029–37.

71. Haro JM, Arbabzadeh-Bouchez S, Brugha TS, et al. Concordance of the Composite International Diagnostic Interview Version 3.0 (CIDI 3.0) with standardized clinical assessments in the WHO World Mental Health Surveys. Int J Methods Psychiatr Res 2006;15:167–80.

72. Kessler RC, Abelson J, Demler O, et al. Clinical calibration of DSM-IV diagnoses in the World Mental Health (WMH) version of the World Health Organization (WHO) Composite International Diagnostic Interview (WMH-CIDI). Int J Methods Psychiatr Res 2004;13:122–39.

73. Gatt JM, Burton KL, Williams LM, et al. Specific and common genes implicated across major mental disorders: a review of meta-analysis studies. J Psychiatr Res 2015;60:1–13.

74. Jiang HY, Qiao F, Xu XF, et al. Meta-analysis confirms a functional polymorphism (5-HTTLPR) in the serotonin transporter gene conferring risk of bipolar disorder in European populations. Neurosci Lett 2013;549:191–6.

75. Marotta PL. Childhood adversities and substance misuse among the incarcerated: implications for treatment and practice in correctional settings. Subst Use Misuse 2017;52:717–33.