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Gene-environment resilience factors

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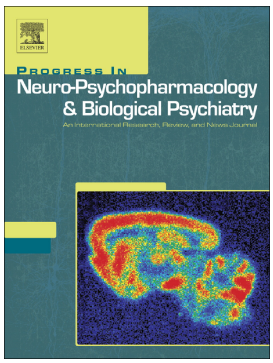

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Alcohol use disorders among adult children of alcoholics (ACOAs): gene-environment resilience factors

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Abstract

Both genetic and early environmental factors contribute to the pathogenesis of Alcohol Use Disorder (AUD). Gender and psychopathology symptoms might further moderate this association, resulting in an impairment of both the dopaminergic and serotoninergic pathways that sustain the binge, withdrawal and craving cycle.

In a sample of of adult children of alcoholic parents (ACOAs) (n=107) we compared those with and without an AUD, on socio-demographic variables, adverse childhood experiences, psychopathology symptoms and two polymorphisms associated with an impaired serotoninergic and dopaminergic neurotransmission (5HTTLPR and Taq1A/DRD2). A logistic regression revealed that an early caring environment might lower the risk of developing an AUD. When controlling for the actual psychopathology symptoms, being male and having the genotype associated with an impaired dopaminergic neurotransmission were still associated with AUD. Results were confirmed by an unsupervised approach that showed how the clusters characterised by being male and having the high risk genotypes were still associated with AUD compared to being female without the unfavourable dopamine genotype.Our results point to the need for implementing prevention strategies aimed at creating a caring environment especially in those families with an alcoholic parent. We further suggest that psycho-education as a symptom recognition and avoiding self-medication could improve the outcome in those subjects at higher risk, especially males.

Highlights

- Adverse childhood experiences at CECA-Q are associated with Alcohol Use Disorder
- The S-allele (5HTTLPR) and the T-allele (Taq1A/DRD2) were associated with AUD
- Male ACOAs are more likely to self-medicate with alcohol
- Female ACOAs could be more resilient to AUD

Introduction

Alcohol Use Disorders (AUDs) are among the most prevalent mental disorders worldwide, with a twelve-month and lifetime prevalence of 13.9% and 29.1%, respectively (American Psychiatric Association, 2013). AUDs have been found to be associated with significant rates of functional disability, physical morbidity and mortality (Grant et al., 2015).The aetiology of AUDs is multifactorial, including biological, psychological and social variables (Grant et al., 2015). Genetic and environmental factors contributed to the pathogenesis of AUD, with approximately the same weight (Goodwin et al., 1973).

Genetic factors can impact on risks for AUDs symptoms on a wide range of levels including alcohol consumption, alcohol metabolism and an alcohol related neural response to the reward system and negative affectivity (van Beek et al., 2012). Neurobiological models of addiction (Uhl et al., 2019) suggest that an impairment of both the dopaminergic and serotoninergic pathways could sustain the binge, withdrawal and craving cycle. When studying these neurotransmitters, two main variants were identified: the polymorphism 5HTTLPR (5-HTT gene-linked polymorphic region) related to the serotonin transporter gene (SERT or 5-HTT) (McHugh et al., 2010) and the single nucleotide polymorphism (SNP) Taq1A, rs1800497, related to the ANKK1/DRD2 gene (Wang et al., 2013). Regarding the first polymorphism, this variant determines a short (S) allele and a long (L) allele characterized by differentially transcriptional efficiency: the S allele was shown associated with lower gene expression and subsequent lower serotonin uptake activity compared to the L allele (Lesch et al., 1996). Individuals possessing genotype LL exhibited a significant lower risk of addition than those possessing SS/SL genotypes (Johnson et al., 2008). With regards to the second polymorphism, its minor allele (A1/T; Lys713) was found associated with a reduced number of D2 receptor availability in the brain of healthy subjects, highlighting a potential influence on promoter or regulatory gene elements that in turn affects dopamine D2 receptor expression (Pohjalainen et al., 1998). Carriers of CC genotype had a significant lower risk of addiction than those with CT or TT genotypes (Smith et al., 2008).
It is important to note that despite some of these genotypes are associated with a higher risk for AUD (Feinn et al., 2005; Munafo et al., 2007), many studies observed that engaged and supportive parenting moderates this genetic vulnerability (Brody et al., 2009; Gerra et al., 2010, 2007; van der Zwaluw et al., 2010). Gene Environment interactions (GxE) seems crucial in the pathogenesis of AUDs (Dick and Kendler, 2012). According to this diathesis-stress model (Moffitt et al., 2006) vulnerable, genetic, temperamental predispositions could be activated in the presence of independent environmental risk, which will lead to the clinical phenotypes of AUDs (Assary et al., 2020; Zucker et al., 2006).
Persons who grow up with alcoholic parents are exposed not only to the genetic load, but also to an inherited environment that increases their risk of AUDs(Leonard and Eiden, 2007).The term Adult Children of Alcoholics (ACoAs) defined those adults raised in a home with either one or two alcoholic parents (Woititz, 1984).Even though ACOAs have an up to 4-fold increase in the risk of developing AUD (Yoon et al., 2013), not all the ACOAs have an AUD.

Beyond genes and the early environment, psychopathology symptoms may play a crucial role in the development of an AUD (Castillo-Carniglia et al., 2019). Gender may moderate this association, even among ACOAs (Park and Schepp, 2015). Specifically, previous research suggested that male

ACOAs have a greater risk of problematic alcohol consumption and engage in externalising behaviours (Goodwin et al., 1973; Windle et al., 1995), while female ACOAs tend to display more internalising symptoms (Hibbard, 1993; Hinz, 1990) and insecure attachments (Kelley et al., 2008), but with more mature and effective coping strategies than men (Drapkin et al., 2015).

The only study to date that evaluated the genetic and psychological factors distinguishing between resilient and vulnerable ACOAs (Brown-rice et al., 2018) explored hazardous alcohol use in a college population, that could represent an already resilient and high-functioning subpopulation of ACOAs. This study aims tobetter understand which factors contribute to the family vulnerability for clinical AUD in a community-based population.
We hypothesise that even in those families with at least one alcoholic parent, an early care environment might moderate the genetic risk of AUD and that this relationship is further influenced by gender and psychopathology symptoms. We decided to explore whether a specific association of genetic factors, adverse childhood experiences and psychopathology symptoms might be associated with AUD in a sample of ACOAs. To do so we tested the association of the genotypes related to 5HTTLPR and Taq1A polymorphisms, experiences of neglect and abuse during childhood and general psychopathology symptoms in two groups of ACOAs with and without AUD.

Methods

The study was approved by the local Ethics Committee (Comitato Etico per Parma, AVEN, protocol #41912 of the 30/11/2016 and Comitato Etico Interaziendale dell'A.O. di Alessandria, protocol #ASL20.SerT.17.01 of the 18/05/2017) and was conducted according to the Helsinki Declaration

AUD group. The subjects referring to the out-patients service for alcohol detoxification were screened and those who satisfied a diagnosis of Alcohol Use Disorder according DSM-5 (APA, 2013) were included. The screening questionnaire included a question regarding the familiarity for Alcohol Use Disorder. Those that gave an affirmative answer were further questioned about this point. If at least one of the parents reported signs of an Alcohol Use Disorder they were defined as ACOAs.

Control group. Controls were those ACOAs that did not developed an AUD (DSM-5).Among those included in the AUD group, we asked whether they had children or sibling available to undergo a screening evaluation. Note that most of these relativeswere already in contact with the outpatients service as part of family psychological and support therapy.Those relatives with an AUD were included in the AUD group, whereas those without an AUD were included in the Control group. To avoid the confounding effect of "sick quitters" (Shaper et al., 1988), we also excluded those with a history of AUD.

Subjects in both groups were excluded if they were diagnosed with any major psychiatric disorders or showed a cognitive impairment or any other disorders that might affect comprehension of the informed consent or questionnaires.All subjects signed an informed consent after the study was fully explained. A separate consent form was approved by the ethical committee for offspring and

siblings that were contacted directly from the subjects screened positive for AUD. The screening procedure is depicted in Figure S1.
The final sample for the present study included one-hundred-and-seven ACOAs (n = 59 with AUD and n = 48 without AUD).

Psychosocial Evaluation. The included subjects underwent an extensive interview performed by twomedical doctors (one per centre) trained by the clinical psychiatrists (PO and MLG) in the administration of the SCID-5 Substance Use module and with experience in treating subjects with substance abuse (MZ and VZ).Socio-demographic and family history information were collected at the enrolment. We also recorded the employment status, having economic difficulty (coded as 0=no and 1=yes), having divorced parents and whether this happened during childhood or adolescence. We further asked whether the onset of the AUD was before the 25 years of age, whether there were other substance use disorders and whether they were taking any psychopharmacotherapy.
Subsequently, the participantscompleted two questionnaires: The Symptoms Check List 90 (SCL-90) (Derogatis, 1994) to evaluate the actual psychopathology symptoms and the Child Experience of Care and Abuse Questionnaire (CECA-Q) (Bifulco et al., 2005)which assesses adverse childhood experiences.
The SCL-90 is a 90-item 5-point likert scale that evaluates general psychopathology symptoms. The questions load on a total score and on nine main subscales: somatisation, obsessive-compulsivity, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism. SCL-90 showed a good internal consistency in our sample (Cronbach's alpha =.967).Most of the literature agrees on a single factor structure that measures a general psychopathological factor (Preti et al., 2012). For this reason, we included only the total score at SCL-90.
The self-report Child Experience of Care and Abuse Questionnaire (CECA-Q) was used to measure, retrospectively, possible experiences of neglect and abuse during infancy and adolescence in both patients and controls. The questionnaire assesses the lack of parental care (neglect and antipathy), parental physical abuse and sexual abuse from any adult before age 17. CECA-Q subscales include mother neglect, father neglect, mother antipathy, father antipathy, mother physical abuse, father physical abuse and sexual abuse.
We computed a single total score adding on a continuous scale, neglect, antipathy, punishment and sexual abuse scores for both mother and father.Combining in a continuous measure experiences of neglect or abuse from different perpetrators allow to examine dose–response effects and to explore the relation between adverse childhood experiences and other continuous variables, such as symptom scores. Moreover, for each subscale a cut-off of two has been used (Gerra et al., 2019)in accordance with cut-off guidelines, to identify those that experienced that specific maltreatment.The use of cut-off scores has been demonstrated to achieve higher specificity and the higher cut-off scores have been associated with depression outcomes (Bifulco et al., 2005). The use of both a continuous and categorical approach offers greater potential for a reliable measurement of risk.

Blood sample collection. Blood samples were collected using FTA classic cards (Whatman). The procedure was performed by the two out-patients service for alcohol detoxification as part of the

routine diagnosis process and following the FTA Classic Cards Purification Protocol. In brief, blood spot samples were obtained from a finger puncture, two 1.2-mm diameter disks were punched from the blood spot of each filter paper and transferred to 0.2 ml PCR tubes, washed three times with the FTA Purification Reagent and twice with TE-1 buffer (10 mM Tris-Cl, 0.10 mM EDTA; pH 8.0) and then air dried in the same tube. Two FTA matrix disks for each sample with genomic DNA (gDNA) were subsequently subjected to PCR amplification.

Genotyping. Genotyping was carried out in the same laboratory at the University of Parmacomparing the two groups of recruited participants: ACOAs with AUD and ACOAs without AUD. All the samples collected were processed following the same pipeline and parameters and applying randomization to avoid biases. The polymorphic regions were amplified through a standard PCR protocol, using human oligonucleotide primers previously selected (**Table S1**) (Grandy et al., 1993; Stoltenberg et al., 2002). The master mix was assembled and directly added to the FTA disks in the PCR tubes. For the Taq1A polymorphism a standard PCR protocol was applied: the PCR reaction was performed in a 25 µl volume containing two 1.2 mm FTA cards disks, 200 µM of each dATP, dTTP, dCTP and dGTP, 0.25 µM of each primer, 1x PCR Buffer, 1.5 mM $MgCl_2$ and 1 U of Platinum Taq (DNA Pol Invitrogen 5U/µl). The gDNA denaturation (thermal cycler at 94°C for 2min) was followed by 35 cycles of PCR amplification (denaturing 94°C for 30 s, annealing 60°C for 30 s, extension 72°C for 30 s); the samples were then incubated for an additional 7 min at 72°C and maintained the reaction at 4°C. The PCR products were subjected to restriction digestion, using the enzyme TaqI, before electrophoresis analysis. For the 5HTTLPR polymorphism, being in a high GC content region and high secondary structure, further optimization of the standard protocol was required. To avoid nonspecific amplicons, touchdown PCR was performedincluding 50 mM Tris/HCl (pH8.8) and 12.5 mM $(NH_4)_2SO_4$ in the PCR mix, in combination with the denaturing agent dimethyl sulfoxide (10% DMSO) in a 25 µl final volume. After an initial denaturation step at 94°C for 2 min, 20 cycles were performed consisting of three steps: denaturation (94°C, 30 sec), annealing (60°C, 30 sec, decreasing 0.5°C/cycle to 50°C) and extension (72°C, 30 sec) followed by 30 additional cycles in which the denaturation step was 94°C for 30 sec, the annealing temperature was 50°C and the final elongation step was performed at 72°C for 7 min. DNA amplicons in case of 5HTTLPR were directly loaded on agarose gel electrophoresis.

Analysis

Following the main study aim, all the ACOAs were separated based on the current Alcohol Use Disorder (AUD) diagnosis (American Psychiatric Association, 2013). The control group were defined as such not only if it did not satisfy the criteria for Alcohol Use Disorder, but also if they had to drink 14 units or less over the course of three days or more. Because most of the variables were non-normally distributed, when comparing those with and without AUD we adopted the Mann-Whitney test for continuous variables and the Fisher exact test for categorical variables.

In order to perform the descriptive and inferential analyses, the genetic variables where subjected to dichotomization. The short allele being associated with an increased risk for a variety of disorders, including alcoholism, the homozygous S/S and heterozygous S/L genotypes related to the 5HTTLPR were identified as risk genotypes and coded as 1. The homozygous L/L genotype coded as 0. The DRD2 Taq1A polymorphism, in which the T allele was associated to AUD and

psychiatric conditions, the homozygous T/T and heterozygous C/T genotypes were coded as 1. Whereas the homozygous C/C genotype was coded as 0.
Correlation between the independent variables included in the model has been explored through Spearman correlations.

For a better description of the data, here we adopted two different approaches: a supervised one (logistic regression) and an unsupervised one (cluster analysis). In logistic regressions the independent variables are selected by the researcher and separately entered in the model to answer questions based on the relevant literature. In our study, for example we looked at whether the effect of adverse childhood experience remained significantly associated with the AUD after controlling for the genotypes. The main limitation of this approach is that when variables are correlated (e.g., adverse childhood experiences and psychopathology symptoms) the stability of the model is threatened by collinearity issues. Cluster analysis on the other hand, groups the subjects based on the similarity of data points in an unsupervised manner. The obtained clusters, should then be more independent and allow a more naturalistic description of the sample and its association with the outcome.

Logistic Regression models. To evaluate which genetic and environmental variables were more strongly associated with AUD, we built a series of logistic regression models with AUD as a dependent variable. Specifically, being interested in the effect of a caring early environment we entered the CECA-Q in the first model controlling for which parent was alcoholic. Those subjects where the alcoholic parent was unknown (n=6) were excluded from this analysis. In the second model we entered the genotypes of interest, namely SS/SL genotypes (5HTTLPR) and TT/CT genotypes (Taq1A/DRD2). In the third model we included the subject sex. In the fourth and last model the psychopathology symptoms severity. Given the non-normal distribution of the SCL-90 and CECA-Q scores, we log transformed (base n=2) them before entering in the logistic regression model as an independent variable. Because some subjects had a score of zero, we added a constant before the logarithmic transformation.

The final model was then as follows:
$p(AUD) = 1/ 1+e^{-X}$
where X= b0 + b1*ACE + b2*alcoholic parent + b3*5-HTT genotype + b4*Taq1A DRD2 genotype + b5*gender + b6*symptoms at SCL-90 + error

AUD is coded as 0=control group and 1=current AUD; ACE=adverse childhood experiences as Log transformation of CECA-Q total score; alcoholic parent coded as 1=mother (set as referral category), 2=father and 3=both; 5-HTT genotype coded as 0=LL and 1=SL or SS; Taq1A DRD2 coded as 0=CC and 1=CT or TT; symptoms at SCL-90= Log transformation of the SCL-90 total score; gender coded as 0=female; 1=male.

Interaction terms. To test the hypothesis of a moderation effect of adverse childhood experiences over the genetic load in determining AUD, we further explored the predictive ability of two interaction terms added to the above model. These were ACE*5-HTTLPR genotype and ACE*Taq1A/DRD2 genotype. We then explored the interaction between genes and gender

because this could help in specifying whether gender or genes are more important, or gender and genes combined.
Lastly, because it is also possible that the effect of adverse childhood experience on the AUD is mediated by the actual comorbidity as measured by the SCL-90 total score we further explored this interaction (CECA-Q*SCL-90).
All the mediation and the moderation have been explored with the PROCESS macro v3.5 for SPSS (Hayes, 2017).

Cluster Analysis. First, as an exploratory analysis, we performed a cluster analysis entering all five variables of interest: adverse childhood experiences at CECA-Q, 5-HTT and Taq1A DRD2 genotypes dichotomised, gender and symptoms at SCL-90. The aim was to evaluate whether an unsupervised classification of the subjects based on their clinical, genetic and socio-demographic characteristic would predict the association with AUD.
Having both categorical and continuous measures, we adopted a two-step approach with an automatic selection of clusters number. We kept the Log-likelihood as a distance measure when the similarity between clusters is computed according to the Bayesian Information Criterion to account for categorical variables. Continuous variables were standardized before the clustering.Even though the continuous variables were not normally distributed, we did not transform data as doing so before any estimation-modelling, might result in data losing its properties and modelling may not reflect the underlying phenomena.

Cluster Validity. To test the validity of the clusters extracted, we adopted two approaches. Firstly, we performed both a k-mean and a hierarchical clustering with a Ward method with Euclidean distance to account for binary data. We entered as a fixed number of clusters those obtained through the primary cluster analysis. The similarity between the clusters obtained with both these two confirmatory approaches and those resulting from the two-step analysis has been explored through Chi-squared analysis. Secondly, we split the sample into two random halves and repeated the two-step cluster analysis in each sub-sample. Again, the similarity between these clusters and the original ones has been tested through Chi-squared analysis.

The association of each cluster with AUD has been tested through a single logistic regression with AUD (AUD vs control) as dependent variable and included the extracted clusters as independent variables.

Results

Descriptive. We recruited 107 ACOAs (n=59, 55.1% with AUD). Descriptive of the sample divided on the AUDdiagnosis is depicted in **Table 1**. We listed in the table the risk genotypes SS/SL and TT/CT referred to 5HTTLPR and Taq1A/DRD2 polymorphisms, respectively. The genotype frequencies in the full samples did not differ significantly from those predicted by Hardy–Weinberg equilibrium. Those with an AUD, compared to healthy ACOAs were older, more likely to be men and they also reported higher subjective ratings of adverse childhood experiences at CECA-Q, specifically regarding the neglect and antipathy of the father. Spearman's correlations

between age, symptoms at SCL-90 and adverse childhood experience at CECA-Q are reported in **Table S5**.

Table 1. Socio demographic variables in the ACOAs divided on the AUD

	AUD (n=59)	**Control (n=48)**	**U/Chi-sq**	**p-value**
Age, years (s.d.)	55.53 (9.72)	38.25 (12.76)	420.5	<.001
Male (%)	43 (72.9)	16 (33.3)	16.73	<.001
Unemployed (%)	12 (20.3)	10 (20.8)	.004	.999
Economic problems (%)	19 (52.8)	17 (47.2)	.122	.837
Recruitment Centre (Parma) (%)	34 (57.6%)	23 (47.9)	1.00	.317
Onset AUD <25 y.o. (%)	36 (61)	-		
Other SUD (%)	13 (22)	-		
Treatment				
None (%)	22 (37.3)	-		
Specific for SUD (%)	31 (52.5)	-		
Other (%)	6 (10.2)	4 (8.3 %)		
Alcoholic parent			2.092	.580
Father (%)	36 (61)	2? (5?.3)		
Mother (%)	10 (16.9)	13 (27.1)		
Both (%)	9 (15.3)	5 (10.4)		
Divorced parents (%)	13 (22)	10 (20.8)	.023	.880
in childhood (%)	13 (100)	6 (66.7)	5.02	.055
CECA-Q total score(s.d.)	2.01 (2.18)	1.31 (2.05)	1091	.035
Mother				
Antipathy (%)	8 (13.6)	5 (10.4)	.245	.769
Neglect (%)	15 (25.4)	9 (19.1)	.588	.491
Punishment (%)	9 (15.3)	5 (10.4)	.545	.570
Father				
Antipathy (%)	21 (35.6)	7 (14.6)	6.04	.016
Neglect (%)	28 (47.5)	12 (25.5)	5.353	.027
Punishment (%)	9 (18.8)	14 (23.7)	.389	.638
Sexual abuse (%)	6 (10.2)	4 (8.3)	.105	.999
SCL-90 total score(s.d.)	16.71 (5.54)	15.10 (4.04)	1187	.151
SS/SL genotypes (%)	46 (78)	34 (70.8)	.714	.503
TT/CT genotypes (%)	22 (37.3)	11 (22.9)	2.56	.142

Note. SUD= Substance Use Disorder; CECA-Q: Childhood Experience of Care and Abuse Questionnaire; cat= categorical score at CECA-Q; Economic problems as explicitly stated by the participants; SCL: Symptoms checklist 90 items. SS/SL referring to the 5-HTT genotype; TT/CT referring to the Taq1A DRD2 genotype. For six subjects (AUD: n=4, 6.8%; non-AUD: n=2, 4.2%) data regarding the alcoholic parent were not available.

Logistic Model. The association between psychopathology symptoms and childhood experiences was significant but not high enough (all Spearman's rho<.5) to prevent the building of a model with both questionnaires. When controlling for which was the alcoholic parent (Model 1) and

when including the two genotypes (Model 2), both adverse childhood experience (OR=1.56 95%CI=1.01-2.40, p=.045) and having the T-alleles (OR=2.66, 95%CI=1.021-6.96; p=.045) were associated with AUD. Results were overlapping when considering having both the parents as a referral category in the variable alcoholic parent to test for the addictive effect (Model 1. ACE: OR=3.651, 95%CI= .904- 14.748, p=.069; alcoholic parent: p=.494, alcoholic mother: OR=.757, 95%CI=.222-2.573, p=.655; alcoholic father: OR=.464, 95%CI=.115-1.875, p=.281). This means that doubling the adverse childhood experiences was associated with a 60% greater probability (p=OR/1+OR) of developing an AUD and having the T-allele was associated with a three-fold increase in developing AUD. No interaction emerged between the risk genotypes and the adverse childhood experience in predicting AUD (see **Table S6**). The association between ACE and AUD was still significant (OR=1.684; 95%CI=1.041-2.714, p=.034) when including gender (Model 3) but disappeared when considering the psychopathology symptoms (Model 4). Symptoms at SCL-90 did not mediated the relationship between adverse childhood experiences at CECA-Q and the development of AUD (**Table S7**). Similarly there was no interaction between gender and genes (**Table S8**). Both being male and greater psychopathology symptoms were associated with AUD. On average our results suggest that one out of seven female ACOAs developed AUD (OR=0.15, 95%CI=.061-0.392, p<.001) whereas doubling the severity of symptoms was associated with a seven-fold increase in the risk of developing an AUD (OR=7.028; 95%CI=1.29-38.43; p=0.024). Again, having the T-allele was associated at a trend level with a three-fold risk of having an AUD (OR= 2.874, 95%CI=.959-8.608; p=.059) (**Table S2**).

When entering the subjects' age in the model (**Table S3**), this was significantly associated to AUD (OR=1.15, 95%CI=1.08-1.229, p<.001) but also gender (OR=0.123; 95%CI=.034-0.447; p=001) and symptom severity at a trend level (OR=7.653; 95%CI=.91-64.6, p=.061).

Cluster Analysis. The Cluster analysis revealed a four-cluster solution with a good fit. The ratio between the smallest and the biggest clusters was 1.94. In order of importance in the clustering the variables were the two genotypes, gender, the symptoms at SCL-90 and the adverse childhood experience at CECA-Q.

We defined the four groups: (a) Males (n=32, 29.9%); (b) SS/SL and TT/CT high risk genotypes, being more likely to have both the S- and T- alleles (n=33, 30.8%); (c) LL and CC low risk genotypes, that is being less likely to have the S-allele for the 5HTTLPR polymorphism or the T-allele for the Taq1A/DRD2 polymorphism (n=17, 15.9%); and (d) Females (n=25, 23.4%).

These four clusters differed significantly from each other in the AUD (Chi-sq=14.18; p=.003) (**Table 2**). Among the cluster extracted, belonging to the cluster named Female was associated with an OR of 0.224 (95% CI=0.084-0.599; p=.003). This means that compared to all the other clusters only one female ACOA in five will develop an AUD.

The four clusters interestingly did not differ in the adverse childhood experience: among participants exposed to the same early environmental risk factors, the cluster analysis showed that genetic factors determine the development of AUD. Specifically, being female seems to represent the greater protective factor and having favourable genotypes (low risk genotypes) did not significantly differ from being female, for the risk of developing an AUD (**Figure 1**). Moreover, the clusters did not differ in the psychopathology symptoms, suggesting that the protective factors did not necessarily prevent ACOAs to experience psychological distress.

Table 2. Socio demographic variables in the ACOAs divided in the four clusters

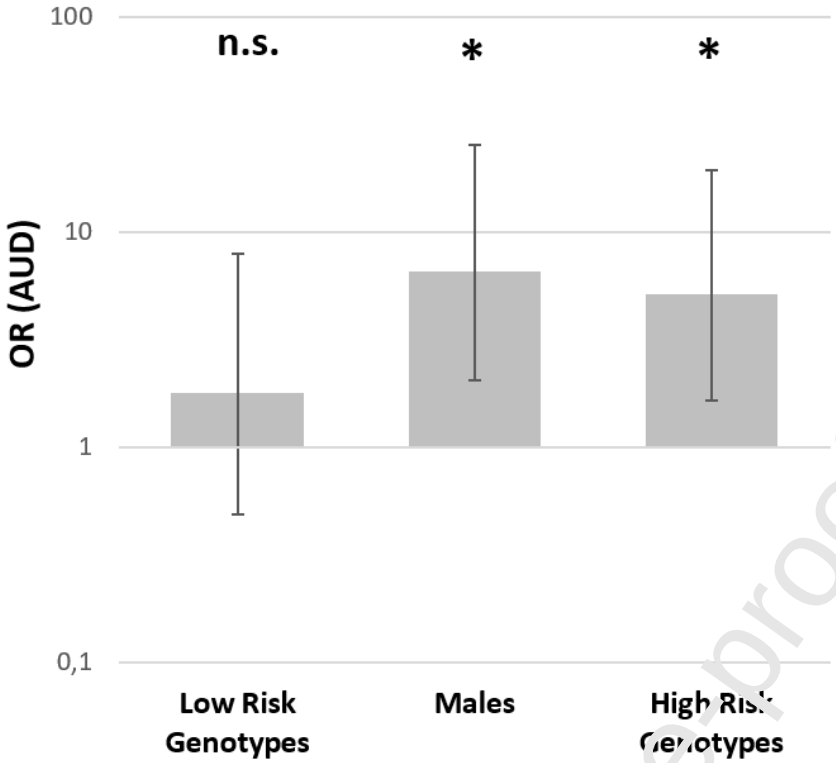
	Male (n=32)	**High risk genotypes (n=33)**	**Low risk genotypes (n=17)**	**Female (n=25)**
AUD	23 (71.9)	22 (66.7)	7 (41.2)	7 (28)
Age, years(s.d.)	49.4 (12.98)	51.97 (12.44)	46 (14.01)	41.36 (15.79)
Male (%)	32 (100)	20 (60.6)	7 (41.2)	0(0)
CECA-Q total score (s.d.)	1.37 (1.38)	1.51 (1.62)	2.94 (3.88)	1.52 (1.73)
SCL-90total score (s.d.)	15.11 (3.93)	15.66 (5.1)	16.96 (6.91)	16.88 (4.48)
SS/SL genotypes (%)	32(100)	22(66.7)	10(58.9)	25(100)
TT/CT genotypes (%)	0(0)	33(100)	0(0)	0(0)

Note. SUD= Substance Use Disorder; CECA-Q: Childhood Experience of Care and Abuse Questionnaire; Economic problems as explicitly stated by the participants; SCL: Symptoms checklist 90 items. SS/SL referring to the 5-HTT genotype: TT/CT referring to the Taq1A DRD2 genotype.
Groups are compared through interactions at Kruskal Wallis test and Fisher exact test.The interactions of group with CECA-Q and SCL-90 were not significant (p>.05). Post-hoc not reported.

The clusters were valid and consistent even when adopting other clustering approaches (i.e., hierarchical and k-mean clustering) or using the same two-step approach in the sample randomly divided into two halves (see **Supplementary** for details).

We then evaluated whether the four clusters extracted with the two-step analysis were associated with AUD. We set the last cluster (i.e. Female), that was the one associated with a resilience through AUD as the referral category. Being male and having both the high risk genotypes, that is, being more likely to have both the S-allele for the 5HTTLPR polymorphism and the T-allele for the Taq1A DRD2 polymorphism, were associated with a higher risk of AUD (**Figure 1**).
The cluster characterised mostly by males was still associated with AUD, even when controlling for the subjects' age, which was not included in the cluster analyses (**Table S4**).

Figure 1. Odd Ratio of developing AUD for each Cluster



Note. On the Y-axis, the Odd Ratio (OR) on a logarithmic scale associated with the AUD when the cluster Female is set as referral category. On the X-axis the three remaining clusters. n.s. non-significant association with AUD.* p-value <.05. Bars reflect the 95% CI.

Discussion

Our findings show that overall being a male ACOA and having both the high risk genotypes were associated with an increased risk of developing and looking for treatment for an AUD. Conversely, resilience to AUD, among ACOAs seems to be mediated by a favourable genotype for the dopaminergic transmission and by being female.

When exploring which variable within each cluster was more strongly associated with AUD we further demonstrate that within a family with an alcoholic parent, a more affectionate environment in early childhood and, specifically, a more caring father were associated with a lower risk of developing AUD, regardless of which parent was alcoholic. When considering the genotypes, being a carrier of the T-allele for the Taq1A/DRD2 polymorphism was associated with a higher risk beyond the protective caring factors in childhood. However, the effect of the early environment disappeared when considering gender and the actual psychopathology.

It is possible that male and female ACOAs could have different clinical manifestation of the same genetic vulnerability. Winokur (Winokur, 1974) postulated a subtype of depressive illness, called Depressive Spectrum Disease, that would lead to alcoholism in males and depression in females. The results from the Collaborative Study of Depression supported the hypothesis, showing higher rates of AUD in the families of depressed women (Winokur and Coryell, 1991). In line with this evidence, our results showed that also ACOAs females experience psychological distress but, in

this group, the same level of psychopathology symptoms did not increase the risk of AUD. The result that male ACOAs are more likely to develop an AUD has been previously observed (Goodwin et al., 1973; Windle et al., 1995). By contrast other authors, found that internalising symptoms are causal for AUD in women (Epstein et al., 2019; Jeong et al., 2019). It is possible that this discrepancy relies on the samples studied. Resiliency in ACOAs is especially important to overcome the major challenges associated with being raised by parents with AUD (Carle and Chassin, 2004; Palmer, 1997) that can be thought of as a chronic stressor (Hussong et al., 2008).
Whereas female ACOAs report more mature and effective coping styles than men (Drapkin et al., 2015) male ACOAs adopt less efficient coping strategies such as self-medicating with alcohol. This might be because men are less likely to acknowledge internalised symptoms (Martin et al., 2013) and drinking conforms more to masculine norms (Patró-Hernández et al., 2020). As a cascade effect, self-medication with alcohol might then prevent the adoption of more mature and efficient coping strategies,exacerbating the problem (Marmorstein et al., 2010).

The association between AUD and the risk genotypes of 5HTTLPR and Taq1A polymorphisms confirms previous studies in ACOAs (Brown-rice et al., 2018; Ratsma et al., 2001) and in longitudinal cohorts (Enoch et al., 2016).
Interestingly, the cluster with both the low risk genotypes LL and CC, that does not have the short-allele for the serotonin transporter nor the t-substitution in the dopamine receptor, was not associated with resilience through AUD. Similarly the cluster associated with the lower risk of developing AUD had the high risk genotype for the serotonin transporter. On the contrary, having both the high risk genotypes was a risk factor. This could suggest a synergic effect between the two impaired neurotransmission pathways.
The S allele could determine specific impairments in emotion processing (Pezawas et al., 2005) that results in increased levels of anxiety (Lesch et al., 1996) and depression (Otten et al., 2018). This pathway would account for the similar level of psychopathology in all the clusters independently from the risk of developing AUD. Psychopathology symptoms by itself might not be enough to induce the AUD but the association with higher levels of reward-dependence (Heinrich et al., 2016) related to the presence of T alleles in Taq1A/DRD2 polymorphism could deteriorate the already maladaptive decision making (O'Brien et al., 2014) and result in an AUD (Oo et al., 2016).

Our findings that adverse childhood experiences are associated with an increased risk of AUD confirm the well-known key role of family in the development and progression of alcohol use disorder. Parental monitoring, parent-child relationship quality, parental support and parental involvement are all longitudinal protective factors of alcohol misuse (Yap et al., 2017). Moreover, a secure parent-child attachment has been consistently found as a main resilience factor for the development of any mental health problem in children of a parent or parents with alcohol or drug use disorders (Wlodarczyk et al., 2017).
A variety of evidence demonstrated that negative early childhood experiences of abuse and neglect are involved in the susceptibility to substance use disorders from cocaine (Gerra et al., 2009) to heroin (Gerra et al., 2016a, 2014, 2017) and nicotine (Gerra et al., 2016b) or THC (Gerra et al., 2019).
The specific way in which a childhood history of neglect might be associated to an increased risk of AUD could be because of a complex neurobiological derangement including HPA axis and

dopamine system dysfunctions, that are related to a decreased resiliency when facing stressful events (Gerra et al., 2010, 2009, 2008; Somaini et al., 2011).
The effect of adverse childhood experiences was independent from which parent was alcoholic (Jääskeläinen et al., 2016) suggesting a broader disruption of parental support and family care.

Our results also underline how the effect of childhood maltreatment disappears when controlling for psychopathology symptoms. This, together with the high correlation between adverse childhood experiences and general psychopathology symptoms might suggest developmental psychopathological pathways. It has been shown how abuse and neglect in childhood are related to both emotion dysregulation (Gerra et al., 2014) and internalising and externalising symptoms (Gerra et al., 2009).

The main study limitation is the sample size that could have been too small to detect small effects. However, despite further research with greater sample is needed to confirm and generalize our findings, this is one of the few studies that specifically focused on ACOAs with AUD seeking treatment.
Secondly, the gender differences could represent a sampling bias. Nevertheless, the higher prevalence of men among the ACOAs with AUD and the over-representation of female in the control group could also be object of interpretations: female gender could represent a protective factor in ACOAs for developing AUD or, considering that we recruited ACOAs with AUD from an alcohol detoxification program, it could be possible that females are less likely to seek treatment. This could reflect the many recognized barriers that women face to access substance abuse treatment, such as gender insensitivity in program content, threat of legal sanction including loss of child custody and societal intolerance and stigmatization of alcohol or drug-dependent women, suggesting, beyond a sampling bias, the need for more female orientated services (Alvanzo et al., 2014), focused on overcoming those gender-specific barriers and develop female-tailored treatment options (Epstein et al., 2019). A possible critique on the association between poor parenting and AUD relies on the retrospective nature of the questionnaires. It has been shown that the subjective experience of maltreatment is more strongly linked to mental health problems than the actual objective court-documented evidence of maltreatment (Danese and Widom, 2020). It is to note that in the present research we considered a candidate gene approach. Despite genome wide studies are considered the best method to yield reliable results in novel datasets in psychiatry (Duncan et al., 2019) and contrasting findings were evidenced on the role of Taq1A and 5HTTLPR in alcoholism development (Noble et al., 1993; Tartter & Ray, 2011), the candidate genes approach, based on prior biological knowledge, is still essential to replicate and to correlate the fundamental discoveries of genetics of addiction to environmental factors.
Overall, we suggest that an increased caring environment in childhood reduces the risk of developing an AUD even when growing up in a family with at least one alcoholic parent. This, together with being female and able to recognise and face even mild levels of psychopathology symptoms might further offer protection from developing AUD. Interestingly from a genetic perspective, the serotonin or the dopamine polymorphisms association seem to increase the risk of AUD.
Our results point to the need for implementing prevention strategies aimed at creating a caring environment especially in those families with an alcoholic parent. This could not only decrease the

risk of AUD (Hurley et al., 2019), but also overall improve emotion regulation strategies and lessen psychopathology symptoms.

Similarly, our data suggest that substance abuse treatment programs may fail to address ACOAs and specific treatment focusing on ACOAs risk and resilient factors might be needed (Kingree and Thompson, 2000).

On a psychobiological perspective a more finely tuned model for alcohol use disorder might be needed. This should confirm the role of these genetic liabilities in shaping underlying cognitive processes crucial to developing and maintaining AUDs (Mews and Calipari, 2017).

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References

Alvanzo, A.A.H., Storr, C.L., Mojtabai, R., Green, K.M., Pacek, L.R., La Flair, L.N., Cullen, B.A., Crum, R.M., 2014. Gender and race/ethnicity differences for initiation of alcohol-related service use among persons with alcohol dependence. Drug Alcohol Depend. 140, 48–55. https://doi.org/10.1016/j.drugalcdep.2014.03.010

American Psychiatric Association, 2013. Diagnostic and statistical manual of mental disorders (DSM-5), Washington, DC. American Psychiatric Pub.

Assary, E., Zavos, H.M.S., Krapohl, E., Keers, R., Pluess, M., 2020. Genetic architecture of Environmental Sensitivity reflects multiple heritable components: a twin study with adolescents. Mol. Psychiatry. https://doi.org/10.1038/s41380-020-0783-8

Bifulco, A., Bernazzani, O., Moran, P.M., Jacobs, C., 2005. The childhood experience of care and abuse questionnaire (CECA.Q): validation in a community series. Br. J. Clin. Psychol. 44, 563–581. https://doi.org/10.1348/0144665505X35344

Brody, G.H., Beach, S.R.H., Philibert, R.A., Chen, Y.F., Lei, M.K., Murry, V.M., Brown, A.C., 2009. Parenting moderates a genetic vulnerability factor in longitudinal increases in youths' substance use. J. Consult. Clin. Psychol. 77, 1–11. https://doi.org/10.1037/a0012996

Brown-rice, K.A., Scholl, I.L., Fercho, K.A., Pearson, K., Kallsen, N.A., Davies, G.E., Ehli, E.A., Olson, S., Schweinle, A., Baugh, L.A., Forster, G.L., 2018. Progress in Neuropsychopharmacology & Biological Psychiatry Neural and psychological characteristics of college students with alcoholic parents di ff er depending on current alcohol use. Prog. Neuropsychopharmacol. Biol. Psychiatry 81, 284–296. https://doi.org/10.1016/j.pnpbp.2017.09.010

Carle, A.C., Chassin, L., 2004. Resilience in a community sample of children of alcoholics: Its prevalence and relation to internalizing symptomatology and positive affect. J. Appl. Dev. Psychol. 25, 577–595. https://doi.org/https://doi.org/10.1016/j.appdev.2004.08.005

Castillo-Carniglia, A., Keyes, K.M., Hasin, D.S., Cerdà, M., 2019. Psychiatric comorbidities in alcohol use disorder. Lancet Psychiatry 6, 1068–1080. https://doi.org/10.1016/S2215-0366(19)30222-6

Danese, A., Widom, C.S., 2020. Objective and subjective experiences of child maltreatment and their relationships with psychopathology. Nat. Hum. Behav. https://doi.org/10.1038/s41562-020-0880-3

Derogatis, L.R., 1994. SCL-90-R Administration, Scoring and Procedures Manual . Minneapolis, MN: National Computer Systems. National Computer Systems. Inc, Minneapolis, MN.

Dick, D.M., Kendler, K.S., 2012. The impact of gene-environment interaction on alcohol use

disorders. Alcohol Res. 34, 318–324.
Drapkin, M.L., Eddie, D., Buffington, A.J., Mccrady, B.S., 2015. Alcohol-Speci fi c Coping Styles of Adult Children of Individuals with Alcohol Use Disorders and Associations with Psychosocial Functioning. Alcohol Alcohol. 50, 463–469. https://doi.org/10.1093/alcalc/agv023
Enoch, M.-A., Kitzman, H., Smith, J.A., Anson, E., Hodgkinson, C.A., Goldman, D., Olds, D.L., 2016. A Prospective Cohort Study of Influences on Externalizing Behaviors Across Childhood: Results From a Nurse Home Visiting Randomized Controlled Trial. J. Am. Acad. Child Adolesc. Psychiatry 55, 376–382. https://doi.org/10.1016/j.jaac.2016.02.007
Epstein, E.E., Mccrady, B.S., Hallgren, K.A., Cook, S., Jensen, N.K., Hildebrandt, T., 2019. Therapy for Alcohol Dependent Women. Psychol. Addict. Behav. 32, 1–15. https://doi.org/10.1037/adb0000330.A
Feinn, R., Nellissery, M., Kranzler, H.R., 2005. Meta-analysis of the association of a functional serotonin transporter promoter polymorphism with alcohol dependence. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 133B, 79–84. https://doi.org/10.1002/ajmg.b.30132
Gerra, G., Leonardi, C., Cortese, E., Zaimovic, A., Dell'Agnello, G., Manfredini, M., Somaini, L., Petracca, F., Caretti, V., Baroni, C., Donnini, C., 2008. Adrenocorticotropic hormone and cortisol plasma levels directly correlate with childhood neglect and depression measures in addicted patients. Addict. Biol. 13, 95–104. https://doi.org/10.1111/j.1369-1600.2007.00086.x
Gerra, G., Leonardi, C., Cortese, E., Zaimovic, A., Dell'agnello, G., Manfredini, M., Somaini, L., Petracca, F., Caretti, V., Raggi, M.A., Donnini, C., 2009. Childhood neglect and parental care perception in cocaine addicts: relation with psychiatric symptoms and biological correlates. Neurosci. Biobehav. Rev. 33, 601–610. https://doi.org/10.1016/j.neubiorev.2007.08.002
Gerra, G., Manfredini, M., Somaini, L., Marenzani, I., Leonardi, C., Donnini, C., 2016a. Sexual Dysfunction in Men Receiving Methadone Maintenance Treatment: Clinical History and Psychobiological Correlates. Eur. Addict. Res. 22, 163–175. https://doi.org/10.1159/000441470
Gerra, G., Manfredini, M., Somaini, L., Milano, G., Ciccocioppo, R., Donnini, C., 2016b. Perceived parental care during childhood, ACTH, cortisol and nicotine dependence in the adult. Psychiatry Res. 245, 458–465. https://doi.org/10.1016/j.psychres.2016.09.001
Gerra, G., Somaini, L., Manfredini, M., Raggi, M.A., Saracino, M.A., Amore, M., Leonardi, C., Cortese, E., Donnini, C., 2014. Dysregulated responses to emotions among abstinent heroin users: Correlation with childhood neglect and addiction severity. Prog. Neuropsychopharmacol. Biol. Psychiatry 48, 220–228. https://doi.org/10.1016/j.pnpbp.2013.10.011
Gerra, G., Zaimovic, A., Castaldini, L., Garofano, L., Manfredini, M., Somaini, L., Leonardi, C., Gerra, M.L., Donnini, C., 2010. Relevance of perceived childhood neglect, 5-HTT gene variants and hypothalamus-pituitary-adrenal axis dysregulation to substance abuse susceptibility. Am. J. Med. Genet. Part B, Neuropsychiatr. Genet. Off. Publ. Int. Soc. Psychiatr. Genet. 153B, 715–722. https://doi.org/10.1002/ajmg.b.31038
Gerra, G., Zaimovic, A., Garofano, L., Ciusa, F., Moi, G., Avanzini, P., Talarico, E., Gardini, F., Brambilla, F., Manfredini, M., Donnini, C., 2007. Perceived parenting behavior in the childhood of cocaine users: relationship with genotype and personality traits. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 144B, 52–57. https://doi.org/10.1002/ajmg.b.30388
Gerra, L.M., Gerra, G., Mercolini, L., Manfredini, M., Somaini, L., Pieri, C.M., Antonioni, M., Protti, M., Ossola, P., Marchesi, C., 2017. Increased oxytocin levels among abstinent heroin addicts: Association with aggressiveness, psychiatric symptoms and perceived childhood neglect. Prog. Neuropsychopharmacol. Biol. Psychiatry 75, 70–76.

https://doi.org/10.1016/j.pnpbp.2017.01.005
Gerra, M.C., Manfredini, M., Cortese, E., Antonioni, C.M., Leonardi, C., Magnelli, F., Somaini, L., Jayanthi, S., Cadet, J.L., Donnini, C., 2019. Genetic and Environmental Risk Factors for Cannabis Use: Preliminary Results for the Role of Parental Care Perception. Subst. Use Misuse 54, 1–11. https://doi.org/10.1080/10826084.2018.1531430
Goodwin, D.W., Schulsinger, F., Hermansen, L., Guze, S.B., Winokur, G., 1973. Alcohol Problems in Adoptees Raised Apart From Alcoholic Biological Parents. Arch. Gen. Psychiatry 28, 238–243. https://doi.org/10.1001/archpsyc.1973.01750320068011
Grandy, D.K., Zhang, Y., Civelli, O., 1993. PCR detection of the TaqA RFLP at the DRD2 locus. Hum. Mol. Genet. 2, 2197. https://doi.org/10.1093/hmg/2.12.2197-a
Grant, B.F., Goldstein, R.B., Saha, T.D., Chou, S.P., Jung, J., Zhang, H., Pickering, R.P., Ruan, W.J., Smith, S.M., Huang, B., Hasin, D.S., 2015. Epidemiology of DSM-5 Alcohol Use Disorder: Results From the National Epidemiologic Survey on Alcohol and Related Conditions III. JAMA Psychiatry 72, 757–766. https://doi.org/10.1001/jamapsychiatry.2015.0584
Heinrich, A., Muller, K.U., Banaschewski, T., Barker, G.J., Bokde, A.L.W., Bromberg, U., Buchel, C., Conrod, P., Fauth-Buhler, M., Papadopoulos, D., Gallinat, J., Garavan, H., Gowland, P., Heinz, A., Ittermann, B., Mann, K., Martinot, J.-L., Paus, T., Pausova, Z., Smolka, M., Strohle, A., Rietschel, M., Flor, H., Schumann, G., Nees, F., 2016. Prediction of alcohol drinking in adolescents: Personality-traits, behavior, brain responses, and genetic variations in the context of reward sensitivity. Biol. Psychol. 118, 79–87. https://doi.org/10.1016/j.biopsycho.2016.05.002
Hayes, A.F., 2017. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach. Guilford publications.
Hibbard, S., 1993. Adult children of alcoholics: narcissism, shame, and the differential effects of paternal and maternal alcoholism. Psychiatry 56, 153–162. https://doi.org/10.1080/00332747.1993.11024629
Hinz, L.D., 1990. College student adult children of alcoholics: psychological resilience or emotional distance? J. Subst. Abuse 2, 449–457. https://doi.org/10.1016/s0899-3289(12)80005-8
Hurley, E., Dietrich, T., Rundle-Thiele, S., 2019. A systematic review of parent based programs to prevent or reduce alcohol consumption in adolescents. BMC Public Health 19, 1451. https://doi.org/10.1186/s12889-019-7733-x
Hussong, A.M., Bauer, D.J., Huang, W., Chassin, L., Sher, K.J., Zucker, R.A., 2008. Characterizing the life stressors of children of alcoholic parents. J. Fam. Psychol. 22, 819–832. https://doi.org/10.1037/a0013704
Jääskeläinen, M., Holmila, M., Notkola, I.-L., Raitasalo, K., 2016. Mental disorders and harmful substance use in children of substance abusing parents: A longitudinal register-based study on a complete birth cohort born in 1991. Drug Alcohol Rev. 35, 728–740. https://doi.org/10.1111/dar.12417
Jeong, J., Joo, S., Hahn, C., Kim, D., Kim, T., 2019. Gender-Specific Association between Alcohol Consumption and Stress Perception, Depressed Mood, and Suicidal Ideation: The 2010 – 2015 KNHANES. Psychiatry Investig. 16, 386–396. https://doi.org/10.30773/pi.2019.02.28
Johnson, B.A., Javors, M.A., Roache, J.D., Seneviratne, C., Bergeson, S.E., Ait-Daoud, N., Dawes, M.A., Ma, J.Z., 2008. Can serotonin transporter genotype predict serotonergic function, chronicity, and severity of drinking? Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 209–216. https://doi.org/10.1016/j.pnpbp.2007.07.030
Kelley, M.L., French, A., Schroeder, V., Bountress, K., Fals-stewart, W., Steer, K., Cooke, C.G., 2008. Mother-Daughter and Father-Daughter Attachment of College Student ACOAs. Subst. Use Misuse 43, 1562–1573. https://doi.org/10.1080/10826080802240906

Kingree, J.B., Thompson, M., 2000. Mutual help groups, perceived status benefits, and well-being: a test with adult children of alcoholics with personal substance abuse problems. Am. J. Community Psychol. 28, 325–342. https://doi.org/10.1023/A:1005153304710

Leonard, K.E., Eiden, R.D., 2007. Marital and family processes in the context of alcohol use and alcohol disorders. Annu. Rev. Clin. Psychol. 3, 285–310. https://doi.org/10.1146/annurev.clinpsy.3.022806.091424

Lesch, K.P., Bengel, D., Heils, A., Sabol, S.Z., Greenberg, B.D., Petri, S., Benjamin, J., Muller, C.R., Hamer, D.H., Murphy, D.L., 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274, 1527–1531. https://doi.org/10.1126/science.274.5292.1527

Marmorstein, N.R., Iacono, W.G., Malone, S.M., 2010. Longitudinal associations between depression and substance dependence from adolescence through early adulthood. Drug Alcohol Depend. 107, 154–160. https://doi.org/10.1016/j.drugalcdep.2009.10.002

Martin, L.A., Neighbors, H.W., Griffith, D.M., 2013. The experience of symptoms of depression in men vs women: analysis of the National Comorbidity Survey Replication. JAMA Psychiatry 70, 1100–1106. https://doi.org/10.1001/jamapsychiatry.2013.1985

McHugh, R.K., Hofmann, S.G., Asnaani, A., Sawyer, A.T., Otto, M.W., 2010. The serotonin transporter gene and risk for alcohol dependence: a meta-analytic review. Drug Alcohol Depend. 108, 1–6. https://doi.org/10.1016/j.drugalcdep.2009.11.017

Mews, P., Calipari, E.S., 2017. Cross-talk between the epigenome and neural circuits in drug addiction. Prog. Brain Res. 235, 19–63. https://doi.org/10.1016/bs.pbr.2017.08.012

Moffitt, T.E., Caspi, A., Rutter, M., 2006. Measured Gene-Environment Interactions in Psychopathology: Concepts, Research Strategies, and Implications for Research, Intervention, and Public Understanding of Genetics. Perspect. Psychol. Sci. 1, 5–27. https://doi.org/10.1111/j.1745-6916.2006.00002.x

Munafo, M.R., Matheson, I.J., Flint, J., 2007. Association of the DRD2 gene Taq1A polymorphism and alcoholism: a meta-analysis of case-control studies and evidence of publication bias. Mol. Psychiatry 12, 454–461. https://doi.org/10.1038/sj.mp.4001938

O'Brien, J.W., Lichenstein, S.D., Hill, S.Y., 2014. Maladaptive decision making and substance use outcomes in high-risk individuals: preliminary evidence for the role of 5-HTTLPR variation. J. Stud. Alcohol Drugs 75, 643–652. https://doi.org/10.15288/jsad.2014.75.643

Oo, K.Z., Aung, Y.K., Jenkins, M.A., Win, A.K., 2016. Associations of 5HTTLPR polymorphism with major depressive disorder and alcohol dependence: A systematic review and meta-analysis. Aust. N. Z. J. Psychiatry 50, 842–857. https://doi.org/10.1177/0004867416637920

Otten, R., van der Zwaluw, C.S., Engels, R.C., 2018. Testing bidirectional relationships between alcohol use and depressive symptoms: What is the role of the serotonin transporter gene? Alcohol 66, 69–75. https://doi.org/10.1016/j.alcohol.2017.08.003

Palmer, N., 1997. Resilience in adult children of alcoholics: a nonpathological approach to social work practice. Health Soc. Work 22, 201–209. https://doi.org/10.1093/hsw/22.3.201

Park, S., Schepp, K.G., 2015. Understanding Korean Families With Alcoholic Fathers in a View of Confucian Culture. J. Addict. Nurs. 26, 111–119. https://doi.org/10.1097/JAN.0000000000000085

Patró-Hernández, R.M., Nieto Robles, Y., Limiñana-Gras, R.M., 2020. The relationship between Gender Norms and Alcohol Consumption: A Systematic Review. Adicciones 32, 145–158. https://doi.org/10.20882/adicciones.1195

Pezawas, L., Meyer-Lindenberg, A., Drabant, E.M., Verchinski, B.A., Munoz, K.E., Kolachana, B.S., Egan, M.F., Mattay, V.S., Hariri, A.R., Weinberger, D.R., 2005. 5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for

depression. Nat. Neurosci. 8, 828–834. https://doi.org/10.1038/nn1463
Preti, A., Giovanni, M., Rita, D., 2019. Factor structure models of the SCL-90-R : Replicability across community samples of adolescents. Psychiatry Res. 272, 491–498. https://doi.org/10.1016/j.psychres.2018.12.146
Ratsma, J.E., van der Stelt, O., Schoffelmeer, A.N., Westerveld And, A., Boudewijn Gunning, W., 2001. P3 event-related potential, dopamine D2 receptor A1 allele, and sensation-seeking in adult children of alcoholics. Alcohol. Clin. Exp. Res. 25, 960–967.
Shaper, A.G., Wannamethee, G., Walker, M., 1988. Alcohol and mortality in British men: explaining the U-shaped curve. Lancet 2, 1267–1273. https://doi.org/10.1016/s0140-6736(88)92890-5
Smith, L., Watson, M., Gates, S., Ball, D., Foxcroft, D., 2008. Meta-analysis of the association of the Taq1A polymorphism with the risk of alcohol dependency: a HuGE gene-disease association review. Am. J. Epidemiol. 167, 125–138. https://doi.org/10.1093/aje/kwm281
Somaini, L., Donnini, C., Manfredini, M., Raggi, M.A., Saracino, M.A., Gerra, M.L., Amore, M., Leonardi, C., Serpelloni, G., Gerra, G., 2011. Adverse childhood experiences (ACEs), genetic polymorphisms and neurochemical correlates in experimentation with psychotropic drugs among adolescents. Neurosci. Biobehav. Rev. 35, 1771–1778. https://doi.org/10.1016/j.neubiorev.2010.11.008
Stoltenberg, S.F., Twitchell, G.R., Hanna, G.L., Cook, E.H., Fitzgerald, H.E., Zucker, R.A., Little, K.Y., 2002. Serotonin transporter promoter polymorphism, peripheral indexes of serotonin function, and personality measures in families with alcoholism. Am. J. Med. Genet. 114, 230–234. https://doi.org/10.1002/ajmg.10187
Uhl, G.R., Koob, G.F., Cable, J., 2019. The neurobiology of addiction. Ann. N. Y. Acad. Sci. 1451, 5–28. https://doi.org/10.1111/nyas.13989
van Beek, J.H.D.A., Kendler, K.S., de Moor, M.H.M., Geels, L.M., Bartels, M., Vink, J.M., van den Berg, S.M., Willemsen, G., Boomsma, D.I., 2012. Stable genetic effects on symptoms of alcohol abuse and dependence from adolescence into early adulthood. Behav. Genet. 42, 40–56. https://doi.org/10.1007/s10519-011-9488-8
van der Zwaluw, C.S., Engels, R.C.M.E., Vermulst, A.A., Franke, B., Buitelaar, J., Verkes, R.J., Scholte, R.H.J., 2010. Interaction between dopamine D2 receptor genotype and parental rule-setting in adolescent alcohol use: evidence for a gene-parenting interaction. Mol. Psychiatry 15, 727–735. https://doi.org/10.1038/mp.2009.4
Wang, F., Simen, A., Arias, A., Lu, Q.-W., Zhang, H., 2013. A large-scale meta-analysis of the association between the ANKK1/DRD2 Taq1A polymorphism and alcohol dependence. Hum. Genet. 132, 347–358. https://doi.org/10.1007/s00439-012-1251-6
Windle, M., Windle, R.C., Scheidt, D.M., Miller, G.B., 1995. Physical and sexual abuse and associated mental disorders among alcoholic inpatients. Am. J. Psychiatry 152, 1322–1328. https://doi.org/10.1176/ajp.152.9.1322
Winokur, G., 1974. The division of depressive illness into depression spectrum disease and pure depressive disease. Int. Pharmacopsychiatry 9, 5–13. https://doi.org/10.1159/000468111
Winokur, G., Coryell, W., 1991. Familial alcoholism in primary unipolar major depressive disorder. Am. J. Psychiatry 148, 184–188. https://doi.org/10.1176/ajp.148.2.184
Wlodarczyk, O., Schwarze, M., Rumpf, H.-J., Metzner, F., Pawils, S., 2017. Protective mental health factors in children of parents with alcohol and drug use disorders: A systematic review. PLoS One 12, e0179140. https://doi.org/10.1371/journal.pone.0179140
Woititz, J.G., 1984. Adult children of alcoholics. Alcohol. Treat. Q. 1, 71–99.
Yap, M.B.H., Cheong, T.W.K., Zaravinos-Tsakos, F., Lubman, D.I., Jorm, A.F., 2017. Modifiable parenting factors associated with adolescent alcohol misuse: a systematic review and meta-analysis of longitudinal studies. Addiction 112, 1142–1162.

https://doi.org/10.1111/add.13785
Yoon, G., Westermeyer, J., Kuskowski, M.A., Nesheim, L., 2013. Impact of the number of parents with alcohol use disorder on alcohol use disorder in offspring: a population-based study. J. Clin. Psychiatry 74, 795–801. https://doi.org/10.4088/JCP.13m08350
Zucker, R.A., Wong, M.M., Clark, D.B., Leonard, K.E., Schulenberg, J.E., Cornelius, J.R., Fitzgerald, H.E., Homish, G.G., Merline, A., Nigg, J.T., O'Malley, P.M., Puttler, L.I., 2006. Predicting risky drinking outcomes longitudinally: what kind of advance notice can we get? Alcohol. Clin. Exp. Res. https://doi.org/10.1111/j.1530-0277.2006.00033.x

Supplementary

Data Availability. Data are available on Mendeley Data at http://dx.doi.org/10.17632/xm7vnt3w5v.1

Cluster Validity. To test the validity of the clusters extracted with the two-step approach we performed the same analysis with two other approaches fixing the number of clusters at four: the hierarchical and the k-mean.
The hierarchical cluster analysis extracted almost the same four clusters (cluster 1, n=28, 26.2%; cluster 2, n=23, 21.5%; cluster 3, n=25, 23.4% and cluster 4, n=31, 29%, chi-squared=218.9; p<.001).
The k-mean extracted four clusters, slightly more unbalanced in size (cluster 1, n=11, 10.3%; cluster 2, n=56, 52.3%; cluster 3, n=2, 1.9% and cluster 4, n=38, 35.5%) but that did not differ significantly from those extracted with the two-step approach (chi-squared=19.18; p=.018).
We then randomly split the sample in half (sub-sample #1, n=49 and sub-sample #2 n=58). These two sample were not significantly different in sex (chi-squared=.158; p=.691), 5-HTT genotype (chi-squared=3.124; p=.113), Taq1A DRD2 genotype (chi-squared=1.568; p=.223), CECA-Q total score (U=1412.5; p=.956) and SCL-90 total score (U=1402; p=.905). The two-step cluster analysis extracted three clusters in both samples. These were not significantly different from the four cluster extracted (sample 1: chi-squared=98; p<.001; sample 2: chis-squared=98.04; p<.001) and the analysis simply merged two clusters (i.e. low risk genotypes and female).

Table S1. The polymorphic variants analysed, DNA sequence variations and functional consequences. Forward (FW) and reverse (RV) primers used in the PCR reactions, restriction enzymes and references are reported.

Gene	Polymorphism	DNA Consequence	Functional Consequence	Primers FW and RV (5'–3')
DRD2/ANKK1	Taq1A - rs1800497	C>T	Missense: Glu=> Lys	FW-CCGTCGACGGCTGGCCAAGTTGTCTA RV-CCGTCGACCCTTCCTGAGTGTCATCA
SLC6A4	5HTTLPR	deletion/insertion of a 44-base pairs sequence	Promoter	FW-TGAATGCCAGCACCTAACCC RV- TTCTGGTGCCACCTA

		GACGC

Table S2. Predictors of AUD

		OR (95%CI)	Wald	p-value
Model 1	ACE	1.48 (.970-2.24)	3.31	.069
	Alcoholic parent		1.409	.494
	father	1.631 (.614-4.336)	.963	.327
	both	2.156 (.533-8.716)	1.162	.281
Model 2	ACE	1.558 (1.010-2.401)	4.014	.045
	Alcoholic parent		.685	.710
	father	1.450(.531-3.96)	.526	.468
	both	1.706 (.394-7.382)	.511	.475
	5-HTT genotype	1.65 (.625-4.355)	1.022	.312
	Taq1A DRD2 genotype	2.666 (1.021-6.963)	4.011	.045
Model 3	ACE	1.681(1.041-2.714)	4.511	.034
	Alcoholic parent		.232	.890
	father	1.234 (.418-3.64)	.145	.704
	both	1.421(.306-6.594)	.201	.654
	5-HTT genotype	1.378(.474-4.007)	.348	.555
	Taq1A DRD2 genotype	2.661 (.920-7.648)	3.304	.069
	Gender (Female)	.155(.061-.392)	15.530	<.001
Model 4	ACE	1.213(.702-2.103)	.486	.486
	Alcoholic parent		.575	.750
	father	1.558(.495-4.905)	.575	.448
	both	1.362(.280-6.635)	.146	.702
	5-HTT genotype	1.248(.409-3.807)	.151	.697
	Taq1A DRD2 genotype	2.874 (.959-8.608)	3.556	.059
	Gender (Female)	.107 (.038-.302)	17.810	<.001
	Symptoms at SCL-90	7.028 (1.285-38.439)	5.059	.024

Note.ACE=adverse childhood experiences as the base 2 Log transformation of the CECA-Q total score. Alcoholic parent coded as: 1= mother (set as a referral category), 2= father and 3= both. We excluded from the regression those subjects (n=6) who did not declare who the alcoholic parent was. 5-HTT genotype coded as 0=LL and 1=SL or SS. Taq1A DRD2 genotype coded as 0=CC and 1=CT or TT. Gender coded as 0=female (set as a referral category). 1=male Symptoms at SCL-90= Base 2 Logtransformation of the SCL-90 total score.

Table S3. Predictors of AUD controlling for subjects' age

	OR (95%CI)	Wald	p-value
ACE	.886(.431-1.823)	.108	.742
Alcoholic parent		.741	.691
father	1.370(.321-5.847)	.181	.670
both	2.838(.262-30.69)	.737	.390
5-HTT genotype	1.522(.330-7.021)	3290	.590
Taq1A DRD2 genotype	2.243(.607-8.284)	1.468	.226
Gender (Female)	.123(.034-.447)	10.14	.001
Symptoms at SCL-90	7.656(.907-64.619)	3.498	.061
Age	1.154(1.083-1.229)	19.56	<.001

Note. ACE=adverse childhood experiences as the base 2 Log transformation of the CECA-Q total score. Alcoholic parent coded as: 1= mother (set as a referral category), 2= father and 3= both. We excluded from the regression those subjects (n=6) who did not declare who the alcoholic parent was. 5-HTT genotype coded as 0=LL and 1=SL or SS. Taq1A DRD2 genotype coded as 0=CC and 1=CT or TT. Gender coded as 0=female (set as a referral category). 1=male Symptoms at SCL-90= Base 2 Log transformation of the SCL-90 total score. Model Chi-squared=68.77; p<.001; R2 Nagelkerke =.660; R2 Cox & Snell=.494;

Table S4. Association between clusters and AUD controlling for subjects' age

	OR (95%CI)	**Wald**	**p-value**
Clusters		7.317	.062
Low risk Genotypes	1.609 (.301-8.606)	.309	.578
High risk Genotypes	3.288 (.771-14.025)	2.586	.108
Males	7.208 (1.534-33.862)	6.261	.012
Age	1.143 (1.082-1.207)	23.09	<.001

Note. Clusters coded as: 0= female; 1=low risk genotypes; 2=males; 2= high risk genotypes. Constant B=-7.18, SE=1.52, p<.001; Chi-sq=56.06; p<.001; R2 Nagelkerke =.546; R2 Cox & Snell=.408;

Table S5. Spearman correlation coefficients (rho) between the continuous variables entered in the model

	Age	SCL-90 (total score)
SCL-90 (total score)	.071 (.465)	-
CECA-Q (total score)	.210 (.030)	.442 (<.001)

Note. In parenthesis the p-value

Table S6. Moderation model with the interaction between adverse childhood experiences at CECA-Q and the two genotypes in predicting AUD

	b(95%CI)	**s.e.**	**Z**	**p**
Constant	-.601 (-1.81, .607)	.616	-.975	.329

5-HTT genotype	.488 (-.886, 1.86)	.701	.696	.486
ACE	1.44 (-1.119, 4.01)	1.31	1.10	.169
ACE*5-HTT genotype	-.078 (-3.11, 2.95)	1.54	-.051	.959

Note. ACE=adverse childhood experiences at CECA-Q, log-transformed;

	b(95%CI)	**s.e.**	**Z**	**p**
Constant	-.375 (-1.06, .313)	.351	-1.06	.285
Taq1A DRD2 genotype	.440 (-.823, 1.71)	.345	.683	.494
ACE	1.13 (-.420, 2.69)	.794	1.43	.152
ACE*Taq1A DRD2 genotype	1.04 (-2.23, 4.33)	1.67	.626	.531

Note. ACE=adverse childhood experiences at CECA-Q, log-transformed;

Table S7. Mediation modelwith symptoms at SCL-90 mediating the relationship between adverse childhood experiences at CECA-Q and AUD

	b(95%CI)	**s.e.**	**T**	**p**
X predicts M	.202 (.135, .269)	.034	5.99	<.001
M|X predict Y	1.02 (-.524, 2.56)	.786	1.29	.196
X|M predict Y	1.67 (-2.27, 5.60)	2.00	.832	.405

Note. Y= AUD coded as 0=no and 1=yes; X=adverse childhood experiences at CECA-Q, log-transformed; M=symptoms at SCL-90, log-transformed. Indirect effect of X on Y: Effect size=.3374 (95%CI=-.416, 1.32).

Table S8. Moderation model with the interaction between gender and the two genotypes in predicting AUD

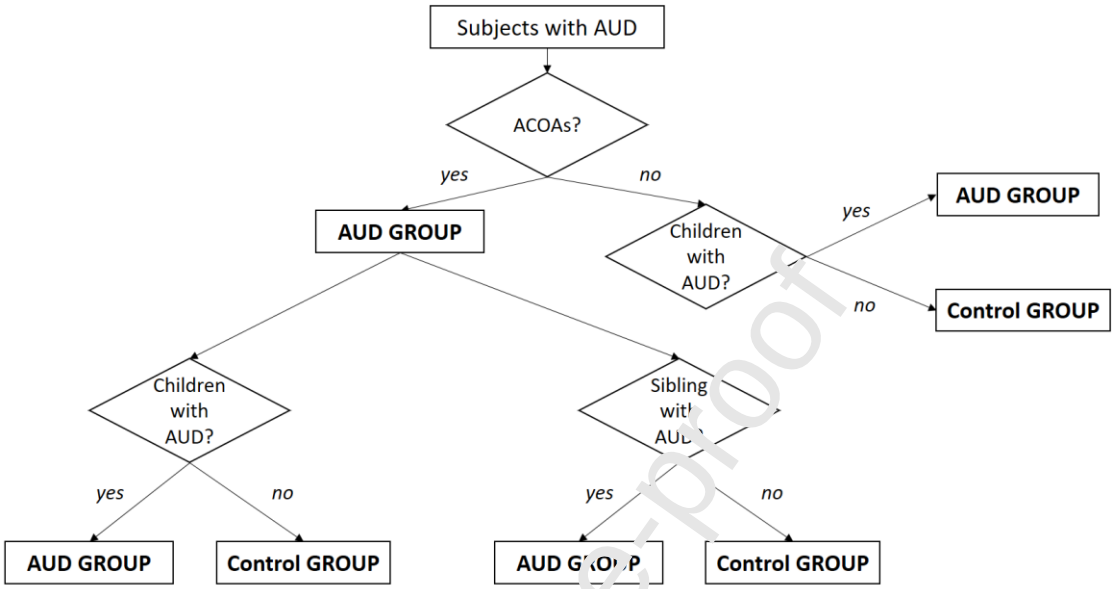
	b(95%CI)	**s.e.**	**Z**	**p**
Constant	2.53 (-.08, 5.16)	1.33	1.89	.058
5-HTT genotype	.150 (-2.86, 3.17)	1.54	.097	.922
gender	-1.72 (-3.38, -.074)	.843	-2.04	.040
gender*5-HTT genotype	.079 (-1.83, 1.99)	.976	.081	.935

Note. Gender coded as 1=male and 2=female

	b(95%CI)	**s.e.**	**Z**	**p**
Constant	2.53 (.993,4.08)	.788	3.21	.001
Taq1A DRD2 genotype	.388 (-2.5, 3.28)	1.47	.263	.792
gender	-1.72 (-2.72, -.727)	.51	-3.38	<.001
gender*Taq1A DRD2 genotype	.186 (-1.65, 2.02)	.939	.198	.842

Note. Gender coded as 1=male and 2=female

Figure S1. Screening Procedures



Paolo Ossola: Conceptualization, Methodology, Formal analysis, Data Curation, Writing - Original Draft, Visualization, Project administration; **Maria Carla Gerra**: Conceptualization, Methodology, Formal analysis, Investigation, Writing - Original Draft, Visualization, Funding acquisition; **Maria Lidia Gerra:** Conceptualization, Methodology, Investigation, Writing - Original Draft; **Giulia Milano** Conceptualization, Investigation, Writing - Review & Editing; **Marta Zatti:** Conceptualization, Investigation, Writing - Review & Editing; **Valeria Zavan:** Conceptualization, Investigation, Writing - Review & Editing; **Riccardo Volpi:** Conceptualization, Resources, Writing - Review & Editing, Supervision; **Carlo Marchesi:** Conceptualization, Resources, Writing - Review & Editing, Supervision; **Claudia Donnini:** Conceptualization, Methodology, Resources, Writing - Review & Editing, Supervision, Funding acquisition; **Gilberto Gerra:** Conceptualization, Methodology, Writing - Original Draft, Supervision, Funding acquisition; **Cristiana Di Gennaro:** Conceptualization, Investigation, Writing - Review & Editing, Supervision, Project administration.

Declarations of interest: none

The study was approved by the local Ethics Committee (Comitato Etico per Parma, AVEN, protocol #41912 of the 30/11/2016 and Comitato Etico Interaziendale dell'A.O. di Alessandria, protocol #ASL20.SerT.17.01 of the 18/05/2017)
All subjects signed an informed consent after the study was fully explained.
The study was conducted according to the Helsinki Declaration of 1972, as revised in 2013.

- Adverse childhood experiences at CECA-Q are associated with Alcohol Use Disorder
- The S-allele (5HTTLPR) and the T-allele (Taq1A/DRD2) were associated with AUD
- Male ACOAs are more likely to self-medicate with alcohol
- Female ACOAs could be more resilient to AUD