Published in final edited form as:

Biol Psychiatry. 2010 January 1; 67(1): 20–27. doi:10.1016/j.biopsych.2009.08.019.

The Influence of *GABRA2*, Childhood Trauma and their Interaction on Alcohol, Heroin and Cocaine Dependence

Mary-Anne Enoch¹, Colin A Hodgkinson¹, Qiaoping Yuan¹, Pei-Hong Shen¹, David Goldman¹, and Alec Roy²

¹ Laboratory of Neurogenetics, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, MD, USA

² Psychiatry Service, Department of Veterans Affairs, New Jersey VA Health Care System, East Orange, NJ, USA

Abstract

Background—The *GABRA2* gene has been implicated in addiction. Early life stress has been shown to alter *GABRA2* expression in adult rodents. We hypothesized that childhood trauma, *GABRA2* variation and their interaction would influence addiction vulnerability.

Methods—African American men were recruited for this study: 577 patients with lifetime DSM-IV single and comorbid diagnoses of alcohol, cocaine and heroin dependence, and 255 controls. The Childhood Trauma Questionnaire (CTQ) was administered. Ten *GABRA2* haplotype-tagging SNPs were genotyped.

Results—We found that exposure to childhood trauma predicted substance dependence ($p < 0.0001$). Polysubstance dependence was associated with the highest CTQ scores ($p < 0.0001$). The African Americans had four common haplotypes (frequency: 0.11 – 0.30) within the distal haplotype block: two that correspond to the Caucasian and Asian yin-yang haplotypes and two not found in other ethnic groups. One of the unique haplotypes predicted heroin addiction whereas the other haplotype was more common in controls and appeared to confer resilience to addiction after exposure to severe childhood trauma. The yin-yang haplotypes had no effects. Moreover, the intron 2 SNP rs11503014, not located in any haplotype block and potentially implicated in exon splicing, was independently associated with addiction, specifically heroin addiction ($p < 0.005$). Childhood trauma interacted with rs11503014 variation to influence addiction vulnerability, particularly to cocaine ($p < 0.005$).

Conclusions—Our results suggest that at least in African American men, childhood trauma, *GABRA2* variation and their interaction play a role in risk-resilience for substance dependence.

Keywords

CTQ; childhood adversity; haplotype analyses; polysubstance dependence; gene-environment interaction; addiction

INTRODUCTION

Alcoholism and drug dependence are common, chronic disorders with considerable personal and societal costs (1). The 12 month prevalence for substance use disorders (abuse plus

dependence) in the United States is: alcohol 8.5%, cannabis 1.5%, opioids 0.4% and cocaine 0.3% (2,3). Alcoholism comorbidity in drug dependent individuals is high: 90% for cocaine, 74% for opioids and 68% for cannabis whereas only 13% of current alcoholics have a current drug use disorder (3). It has been shown that one common genetic factor has a strong influence on the risk for dependence on opiates, cocaine, cannabis and other illicit drugs and that most of the genetic and shared environmental risk factors are non-specific (4,5). Therefore there may be substantial shared vulnerability to addiction among substance dependent individuals although there is also evidence for substance-specific transmission factors (6).

A meta-analysis of studies in thousands of twin pairs has shown that the heritability of alcoholism is around 50%, and the heritability of cocaine and opiate addiction is around 60 – 70% (7). Therefore genetic and environmental influences on the development of addictive disorders are equally important. Preclinical studies indicate that *GABRA2*, the gene that encodes the GABA_A α_2 receptor subunit, may play a role in drug dependence (8–11). In human studies, the *GABRA2* distal haplotype block has been robustly associated with alcoholism, at least in Caucasians (12–21). The first findings came from the Collaborative Study on the Genetics of Alcoholism (COGA) (12). A subsequent re-analysis of the dataset showed that the association signal derived from alcoholics with comorbid illicit drug dependence (13). However, another study found that the association was strongest in alcoholics without drug dependence (19). The two published studies in African Americans were negative (22,23). HapMap data shows that, unlike Caucasian and Asian populations who have only two common *GABRA2* yin yang haplotypes, African populations have two unique haplotypes in addition to the yin yang haplotypes (24).

Early life stress has been shown to influence the expression of *GABRA2* in rats by permanently altering GABA_A α_2 subunit distribution in the hippocampus (25). Moreover, early life stress has been shown to affect ethanol consumption in adult rhesus monkeys and alcohol, cocaine and morphine consumption in rodents (26–28). Numerous studies in humans have provided support for a relationship between childhood trauma and the development of alcohol and drug dependence (29–36). For example, Widom et al (37) showed in a prospective cohort community study that middle aged men and women who had been abused in childhood were at greater risk not only for illicit drug use but also polysubstance abuse. Taken together, it seems reasonable to hypothesize that early life stress might interact with *GABRA2* variation to predict alcohol and drug dependence in humans.

Our study was therefore designed to test our hypothesis that *GABRA2*, childhood trauma and their interaction would contribute to vulnerability to substance dependence. To this end, the study sample included African American men recruited from a Veterans' Affairs substance abuse treatment program, the majority of whom had comorbid alcohol, heroin and cocaine dependency, and African American male controls. The sample had been exposed to considerable childhood trauma. In investigating *GABRA2* variation we focused both on the distal *GABRA2* haplotype block that has previously been associated with substance dependence (12–21) and on a novel intron 2 SNP, rs11503014, that is not located in any haplotype block and is potentially implicated in exon splicing.

METHODS AND MATERIALS

Participants

Originally, 635 African-American substance dependent men were recruited: 590 from the Substance Abuse Treatment Program (SATP) at the Department of Veteran Affairs New Jersey Healthcare System (VANJHCS), East Orange Campus and 45 men originally screened as controls (see below) who were found to have lifetime substance dependence. Most of the participants recruited from the SATP were inpatients on a 21 day residential treatment ward,

however some were recruited from the outpatient clinic or from the methadone clinic. Criteria for inclusion in the study were that participants were ≥ 18 years of age, met DSM-IV criteria for substance dependence, self-identified as African American and had been abstinent for at least two weeks. Exclusion criteria included mental retardation, dementia and acute psychosis. Patients were interviewed by a psychiatrist (A.R.) with the substance abuse section of the Structured Clinical Interview for DSM-IV (SCID) (38) to determine lifetime substance dependence diagnoses. The mean (SD) age of the patients was 45.6 (7.8) years.

Three hundred and twenty African American male controls were recruited from churches and a blood bank in Newark, NJ, (46%) and from among insulin-dependent diabetic outpatients seen at an ophthalmology clinic (54%) at the University of Medicine and Dentistry: New Jersey Medical School (UMDNJ, Newark, NJ). All controls had a semi-structured psychiatric interview and were without a lifetime history of any substance abuse or dependence or major Axis I psychiatric disorder. Their mean (SD) age was 34.0 (10.1) years.

After a full description of the study was provided, all participants gave written informed consent to the study that was approved by the Institutional Review Boards of the VANJHCS and UMDNJ.

Childhood Trauma Questionnaire (CTQ)

The CTQ (28 item version) (39,40) was completed by 495 patients with substance dependence and 145 controls. The CTQ yields scores for five traumas experienced in childhood: physical abuse, physical neglect, emotional abuse, emotional neglect and sexual abuse, as well as a total score. Reliability and validity of the CTQ has been demonstrated, including in drug abusers and African American populations (40–42). The total CTQ score ranges from 25 to 125. The CTQ was used as a continuous measure in all logistic regression analyses.

A dichotomous total CTQ score was derived for use in secondary analyses. A total CTQ score greater than or equal to one standard deviation above the mean CTQ score of controls (36.5 (10.3), i.e. ≥ 47) was designated 'high adversity' (N = 243); lower CTQ scores were designated 'low' adversity (N = 402).

Genotyping

A genomic region containing sequence 5 kb upstream and 1 kb downstream of *GABRA2* was retrieved from NCBI Human Build 35.1. Haplotype tagging SNPs were identified using a previously described design pipeline (43). Ten *GABRA2* SNPs were genotyped using the Illumina GoldenGate platform (43). Rs numbers for the 10 SNPs, the bases for alleles 1 – 2, together with the allele 2 frequencies, are shown in Figure 1. For each SNP, alleles 1 and 2 are located on opposite DNA strands.

Final Dataset Summary

The dataset is summarized in Figure 2. Missing DNA and CTQ data was random and showed no selection bias.

Assessment of Population Stratification Using Ancestry Informative Markers

The samples were genotyped for 186 ancestry markers (AIMS) (43). The same AIMS were genotyped in 1051 individuals from the 51 worldwide populations represented in the HGDP-CEPH Human Genome Diversity Cell Line Panel (<http://www.cephb.fr/HGDP-CEPH-Panel>). *Structure 2.2* (<http://pritch.bsd.uchicago.edu/software.html>) was run simultaneously using the AIMS genotypes from our sample and the 51 CEPH populations to identify population substructure and compute individual ethnic factor scores. This ancestry assessment identifies

seven ethnic factors (43). In our study sample the predominant mean (median) ethnic factor scores were: African: 0.77 (0.81); European: 0.09 (0.04); Mid East and Asian: 0.06 (0.04).

Statistical Analyses

Logistic regression analyses were undertaken using JMP 7 software. Backward stepwise regression was performed with variables being eliminated from the model in an iterative process. CTQ scores and ethnic factor scores were therefore included as covariates in the final model if they had significant effects (the European factor had a significant effect on heroin addiction in most analyses). The interaction term was included in the final model when significant. Logistic regression models with nominal variables yielded likelihood ratio χ^2 results. The Fit Least Squares method was performed to determine differences in CTQ scores between patients with substance dependence and controls.

There were significant differences between the mean (SD) age of the patients (45.6 (7.8)) and the controls (34.0 (10.1)), $F = 211$, $p < 0.0001$. Nevertheless, there was no correlation between age and CTQ score in the patients ($r = 0.07$, $p = 0.211$) or the controls ($r = 0.09$, $p = 0.276$). Inclusion of age as a covariate had no effect on outcomes.

Haplotype frequencies were estimated using a Bayesian approach implemented with PHASE (44). Haploview version 2.04 Software (Whitehead Institute for Biomedical Research, USA) was used to produce LD matrices.

In the logistic regression analyses for the effects of CTQ scores and (a) haplotypes and (b) rs11503014 on substance dependence, the only tests that were independent were the tests where the outcomes were single diagnoses of alcohol, cocaine and heroin dependence, resulting in a Bonferroni corrected significant p value of $p < 0.008$ for the whole model tests. A more stringent correction for all 14, non-independent logistic regression analyses would result in a Bonferroni corrected significant p value of $p < 0.004$ for each of the logistic regression whole model tests.

RESULTS

Effects of Childhood Trauma on Addiction

The total group of patients with substance dependence had a significantly higher mean total CTQ score than the controls: 48.7 (16.8) vs 36.5 (10.3); $F(1,643) = 72$, $p < 0.0001$. Further analysis showed that CTQ scores (mean (SD)) were higher in men with at least two addictions: no addiction (i.e. controls): 36.5 (10.3); one addiction: 46.1 (16.5); two addictions: 50.9 (16.8); three addictions: 49.2 (16.8); $F(3,641) = 27$, $p < 0.0001$. For further details see Supplementary Table 1.

GABRA2 Haplotype Block Structure

The *GABRA2* haplotype block structure is shown in Figure 1. Although the African American haplotype block structure is not as well defined as in Caucasians, African Americans nevertheless have the same two haplotype blocks with a region of recombination in intron 3. Logistic regression analyses were performed with the distal block (block 2) haplotypes, since this is the *GABRA2* region that has previously been associated with alcohol and drug dependence (12–21).

Block 2 Haplotype Analyses

There were 12 haplotypes with ≥ 0.01 frequency that accounted for 0.95 of the haplotype diversity. Only four haplotypes had a frequency ≥ 0.05 and these accounted for 0.79 of the haplotype diversity in the total sample: (a) 2222111 (0.30); (b) 1111222 (0.23); (c) 2112122 (0.15); and (d) 2111111 (0.11). The yin-yang haplotypes 2222111 and 1111222 correspond to

the two haplotypes found in Caucasians and Asians (24). The 2112122 and 2111111 haplotypes are unique to individuals of African descent. Haplotype frequencies for each substance dependence group are given in Table 1. There were no differences in CTQ scores between the haplotypes ($p = 0.34$).

Logistic regression analyses were performed to determine the effects of all four *GABRA2* haplotypes and childhood trauma (total CTQ score) on alcohol, cocaine and heroin dependence. The results are presented in Table 2. Childhood trauma had a significant effect on patients with alcohol dependence and cocaine dependence but there was no gene effect. There was a significant effect of childhood trauma and the 2111111 haplotype in the total group of patients with substance dependence. Childhood trauma had no effect in the total group of individuals with heroin dependence however haplotypes 2111111 and 2112122 both had significant effects. The direction of the haplotypic effects can be discerned from Table 1: haplotype 2112122 was more abundant in individuals with heroin dependence whereas haplotype 2111111 was more common in controls compared with individuals with alcohol, heroin or cocaine dependence. The full model contributed to 10% of the variance in any addiction and up to 13% of the variance for heroin addiction alone: in both of these models, genetic and environmental effects had a significant impact.

The two yin yang haplotypes were not associated with substance dependence. There were no significant interaction effects in the logistic regression analyses. The seven logistic regression whole model tests were significant when corrected for multiple testing ($p < 0.004$).

Secondary Analyses in the Total Group of Patients—Within the logistic regression analysis for the total group of patients and controls, the comparison of haplotype 2111111 with the other three haplotypes showed a trend interactive effect with CTQ scores ($p = 0.087$). Therefore we asked whether the protective effect of haplotype 2111111 might differ with exposure to childhood adversity. In secondary analyses we used the dichotomous CTQ high/low variable (high childhood adversity designated as CTQ total score ≥ 1 S.D. above mean score of controls; low adversity being < 1 S.D. above mean CTQ score of controls). Figure 3 shows that carriers of the other three haplotypes who had been exposed to high childhood adversity were predictably more likely to have developed substance dependence than those exposed to low childhood adversity ($\chi^2 = 6.7 - 11$, $p = 0.009 - 0.0009$, 1df). In carriers of the 2111111 haplotype, high childhood adversity was associated with a numerically higher but non-significant ($p = 0.44$) frequency of addiction indicating that this haplotype may confer resilience to childhood adversity.

Incidental Analyses—For the sake of completeness, the following analyses were undertaken.

Block 2 SNP Analyses: None of the seven block 2 SNPs were associated with alcohol, cocaine or heroin dependence.

GABRA2 Block 1 Haplotype Analyses: There were three common haplotypes: 22 (0.39); 12 (0.34); and 11 (0.27). As expected from published studies (12–21) there were no haplotype or SNP associations with any form of addiction.

GABRA2 SNP rs11503014 Association with Addiction

Main Effects: Gene and Stressor—The intron 2 SNP, rs11503014, is not located within a haplotype block (Figure 1). The homozygote 11 frequency (0.33) and the heterozygote 12 frequency (0.36) in heroin addicted men were very similar and together differed significantly from the homozygote 22 frequency (0.23) ($p = 0.003$). The mean total CTQ score for the

homozygote 11 and heterozygote 12 individuals were very similar (46.1, SD = 15.2 and 46.9, SD = 18.5 respectively) and together differed from that of the homozygous 22 individuals (43.6, SD = 15.5), $p = 0.039$. Thus these results provided the justification for combining the 11 and 12 genotypes in order to increase statistical power in the smaller data subsets.

Table 3 shows that there was a significant effect of rs11503014 in the total group of patients and there was a specific effect on heroin addiction. The effects of childhood trauma were greater on alcohol and cocaine dependence than on heroin dependence. The seven logistic regression whole model tests were significant when corrected for multiple testing ($p < 0.004$).

Gene × Environment Interaction—There was a significant gene × total CTQ score interaction effect on cocaine addiction for the group of patients with cocaine dependence only ($p = 0.003$) and for all patients with cocaine dependence ($p = 0.039$). There was also a trend effect in the total group of patients ($p = 0.076$) (Table 3).

Secondary Analyses: In order to illustrate the direction of the significant interaction for cocaine dependence we used the previously described CTQ high/low childhood adversity variable. Figure 4 Panel B shows that high childhood adversity was associated with increased cocaine dependence in both genotype groups, however, individuals with the rs11503014 11/12 genotype tended to have the greater risk compared with individuals with the 22 genotype ($\chi^2 = 3.7$, 1df, $p = 0.054$). The impact of high childhood adversity on cocaine addiction alone was only apparent in individuals with the 11/12 genotype ($\chi^2 = 5.0$, 1df, $p = 0.026$) (Figure 4, Panel A). The trend effect in the total group of patients was in the same direction as for the patients with cocaine dependence.

Gene-Environment Correlation—CTQ scores did not differ significantly ($p = 0.072$) between rs11503014 11/12 and 22 genotypes when ‘any addiction’ was included as a between factor variable. Thus there was no evidence of a gene-environment correlation.

DISCUSSION

In the present study we confirmed our hypothesis that *GABRA2*, childhood trauma and their interaction influence vulnerability to substance dependence, at least in African American men. Firstly, we found that the patients with heroin, alcohol and cocaine dependence had experienced significantly more childhood trauma than the controls. Furthermore, our results showed that the greater the severity of childhood trauma the greater the likelihood of polysubstance dependence. The latter finding is supported by previous studies (37). Secondly, our results showed that *GABRA2* variation predicted addiction vulnerability, particularly for heroin dependence. Thirdly, an interaction between childhood trauma and *GABRA2* variation was found to influence addiction risk, particularly for cocaine dependence.

Earlier studies, together with HapMap, have identified the same two *GABRA2* haplotype blocks within Caucasians, Asians, Native Americans and African Americans. The previously reported significant association signals with alcoholism have been within the haplotype block that extends downstream from intron 3 (called block 2 in our study). In Caucasians, Asians and Native Americans there are two major yin-yang haplotypes within this block that account for nearly all of the haplotype diversity (24). Individuals of African origin also have these two yin-yang haplotypes however in addition they have two common haplotypes that are not present in other populations. In the present study these two unique haplotypes were associated with addiction vulnerability: one haplotype was associated with heroin dependence and the other haplotype was more common in controls and may be protective against addiction. In contrast, we found no association between the yin-yang haplotypes and alcohol dependence or drug dependence unlike the many earlier studies in Caucasians (12–21). The two previously reported

studies in African Americans showed no association between the yin-yang haplotypes and alcohol dependence (22) or polysubstance abuse (23). Moreover, a recent, dense genomewide linkage scan for alcohol dependence in African Americans did not find a linkage peak at the GABA_A receptor gene complex on chromosome 4 (45), unlike earlier studies in Caucasians (46) and Native Americans (47).

In this study we also found that an intron 2 SNP, rs11503014 that is not in LD with any SNPs in haplotype blocks 1 or 2, was associated with heroin dependence. Rs11503014 is not located within a haplotype block in the three HapMap populations or within Finnish Caucasian and Plains Indian samples for which we have genotyped the same SNPs as in the present study (data not shown). Rs11503014 is located nearby to an alternatively spliced exon 2 and within an exon (5' UTR) of the alternative *GABRA2* transcript NM_001114175. The DNA sequence within which rs11503014 is located is similar to the exonic splicing enhancers: srp55, srp40, sf2 and sc35. Thus it is theoretically possible that rs11503014 may be implicated in exon splicing. Our results therefore suggest that *GABRA2* may have at least two independent loci that are implicated in the vulnerability to heroin dependence.

It has been shown that there is a common genetic factor for addiction to illicit drugs, including cocaine and opiates (4,5). However alcohol and drug dependence also have substantial disorder-specific genetic loading (6). In the present study we detected both a general and specific effect for *GABRA2*: one of the uniquely African haplotypes appeared to be protective against any addiction, however it was specifically protective for heroin dependence. In contrast, the other uniquely African haplotype predicted heroin dependence only. Likewise, rs11503014 variation was predictive for any addiction and specifically for heroin addiction.

Opiates and cocaine have different mechanisms of action in the CNS and may interact differently with *GABRA2* variation and early life stress to influence the vulnerability to heroin or cocaine dependence. Our finding of a *GABRA2*-heroin dependence association is backed up by preclinical studies that indicate that GABA_A receptors may influence the actions of opiates; for example, hyperpolarization of GABAergic neurons in the ventral tegmental area (where GABA_A α_2 receptors are highly expressed) by opiates results in increased firing of dopamine neurons within the dopamine reward pathway (8–10,48–52).

In our study, childhood trauma had the least impact on heroin dependence. In contrast, we found a strong effect of childhood trauma on cocaine dependence. Although there was no main effect of *GABRA2*, severe childhood trauma was associated with cocaine dependence only in individuals with the rs11503014 11/12 genotypes. Preclinical studies support our findings: rats subjected to early life stress are more sensitive to cocaine, demonstrate increased cocaine self-administration (27,53–55) and, perhaps in line with this, have an altered pattern of distribution of GABA_A receptor α_2 subunits (25) that have been implicated in cocaine sensitization (56). Therefore, based on these preclinical findings, one speculative explanation for the gene $\times$ environment (G $\times$ E) interaction in our study might be that if rs11503014 (or a tightly linked SNP) is indeed implicated in exon splicing and thus might influence *GABRA2* expression, carriers of the variant allele might be more sensitive to early life stress and subsequent vulnerability to cocaine addiction. However, one caveat should be discussed. Due to the limited sample size and loss of power stemming from categorization of variables we did not expect strong effects of G $\times$ E interactions and indeed the RSquare values (reflecting the proportion of the total uncertainty that is attributed to the model fit) of the whole model tests for rs11503014 (0.13 for cocaine dependence only, 0.06 for all cocaine dependence) are modest indicating that, if there were no *a priori* biological hypothesis, the likelihood of expected cross validation in other samples on a statistical basis alone might be low.

Strengths of the present study include the large sample size of African-American subjects, a group that has been under-represented in genetic studies of addiction. Moreover, since the dataset included individuals with polysubstance dependence as well as individuals addicted to a single substance we were able to parse out both specific and general influences of *GABRA2* variation on addiction. Furthermore, it should be noted that we corrected for the effects of population stratification by using ethnic factor scores derived from 186 AIMS as covariates in our analyses. The European factor had a significant effect on heroin addiction in most analyses but no effect on cocaine addiction or alcoholism.

There are some limitations to the present study. Data on other Axis 1 diagnoses were not available for the patients. Since it is known that there is high comorbidity between substance dependence and other psychiatric disorders, particularly major depression, and the controls were free of all Axis 1 diagnoses, it is possible that the signals for association found in our study derived from hidden comorbidity. Nevertheless it should be noted that the extensive literature on association studies with *GABRA2* (12–21) has largely focused on alcohol and drug dependence and no published study has yet shown a *GABRA2* association with depression. Moreover, Covault et al's 2004 (19) study showed that the association with alcoholism became stronger when alcoholics with major depression were removed.

Measures of childhood trauma were retrospectively derived from the CTQ. Longitudinal measures are preferable; for example a recent study (57) suggests that the *GABRA2* × childhood trauma interaction might be moderated across development by other environmental factors. Nevertheless, the CTQ is widely used and has been shown to have high reliability and validity in both sexes, in different ethnic groups and in psychiatric patients (42,58–61). Although the overall dataset was large some of the analytical subsets were smaller hence the G×E interactions that we have detected may be an underestimate. Moreover, because of these power issues we had to combine the rs11503014 11/12 genotypes in all analyses. The controls were derived from two sources and although their mean age was appreciably lower than that of patients they had largely passed through the peak age of risk for onset of addictive disorders.

In conclusion, the present study has shown that childhood trauma is a strong predictor for alcohol, cocaine and heroin dependence in African American men. Together with childhood trauma, *GABRA2* variation influences risk and resilience for all addiction but most strongly for heroin dependence. Our results suggest that *GABRA2* may have at least two independent loci that are implicated in the vulnerability to heroin addiction. The two *GABRA2* risk – resilience haplotypes are unique to African Americans. There were no findings with the yin-yang haplotypes that confer addiction risk in Caucasians. Moreover, since there is evidence for sexual dimorphism in the influence of *GABRA2* on addiction vulnerability with previous results being significant only in men (16), the results of our study may not extend to African American women. Thus the findings of the present study may be unique to African-American men and await replication in other similar datasets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by the Intramural Research Program of the National Institute on Alcohol Abuse and Alcoholism, NIH and in part by grant RO1 DA 10336-02 to AR from the National Institute of Drug Abuse, NIH.

FINANCIAL DISCLOSURES

The authors declare that, except from income received from our primary employers, no financial support or compensation has been received from any individual or corporate entity over the past 2 years for research or

professional services and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

References

1. McLellan AT, Lewis DC, O'Brien CP, Kleber HD. Drug dependence, a chronic medical illness: implications for treatment, insurance, and outcomes evaluation. *JAMA* 2000;284:1689–1695. [PubMed: 11015800]
2. Grant BF, Hasin DS, Chou SP, Stinson FS, Dawson DA. Nicotine dependence and psychiatric disorders in the United States: results from the national epidemiologic survey on alcohol and related conditions. *Arch Gen Psychiatry* 2004;61:1107–1115. [PubMed: 15520358]
3. Stinson FS, Grant BF, Dawson DA, Ruan WJ, Huang B, Saha T. Comorbidity between DSM-IV alcohol and specific drug use disorders in the United States: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Drug Alcohol Depend* 2005;80:105–116. [PubMed: 16157233]
4. Tsuang MT, Bar JL, Harley RM, Lyons MJ. The Harvard twin study of substance abuse: what we have learned. *Harvard Rev Psychiatry* 2001;9:267–279.
5. Kendler KS, Jacobson KC, Prescott CA, Neale MC. Specificity of genetic and environmental risk factors for use and abuse/dependence of cannabis, cocaine, hallucinogens, sedatives, stimulants, and opiates in male twins. *Am J Psychiatry* 2003;160:687–695. [PubMed: 12668357]
6. Hicks BM, Krueger RF, Iacono WG, McGue M, Patrick CJ. Family transmission and heritability of externalizing disorders: a twin-family study. *Arch Gen Psychiatry* 2004;61:922–928. [PubMed: 15351771]
7. Goldman D, Oroszi G, Ducci F. The genetics of addictions: uncovering the genes. *Nat Rev Genet* 2005;6:521–532. [PubMed: 15995696]
8. Johnson SW, North RA. Opioids excite dopamine neurons by hyperpolarization of local interneurons. *J Neurosci* 1992;12:483–488. [PubMed: 1346804]
9. Steffensen SC, Stobbs SH, Colago EE, Lee RS, Koob GF, Gallegos RA, et al. Contingent and non-contingent effects of heroin on mu-opioid receptor-containing ventral tegmental area GABA neurons. *Exp Neurol* 2006;202:139–151. [PubMed: 16814775]
10. Zarrindast MR, Heidari-Darvishani A, Rezayof A, Fathi-Azarbaijani F, Jafari-Sabet M, Hajizadeh-Moghaddam A. Morphine-induced sensitization in mice: changes in locomotor activity by prior scheduled exposure to GABAA receptor agents. *Behav Pharmacol* 2007;18:303–310. [PubMed: 17551323]
11. Morris HV, Dawson GR, Reynolds DS, Atack JR, Rosahl TW, Stephens DN. Alpha2-containing GABA(A) receptors are involved in mediating stimulant effects of cocaine. *Pharmacol Biochem Behav* 2008;90:9–18. [PubMed: 18358520]
12. Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, et al. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor, are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet* 2004;74:705–714. [PubMed: 15024690]
13. Agrawal A, Edenberg HJ, Foroud T, Bierut LJ, Dunne G, Hinrichs AL, et al. Association of GABRA2 with drug dependence in the collaborative study of the genetics of alcoholism sample. *Behav Genet* 2006;36:640–650. [PubMed: 16622805]
14. Dick DM, Bierut L, Hinrichs A, Fox L, Bucholz KK, Kramer J, et al. The role of GABRA2 in risk for conduct disorder and alcohol and drug dependence across developmental stages. *Behav Genet* 2006;36:577–590. [PubMed: 16557364]
15. Soyka M, Preuss UW, Hesselbrock V, Zill P, Koller G, Bondy B. GABA-A2 receptor subunit gene (GABRA2) polymorphisms and risk for alcohol dependence. *J Psychiatr Res* 2008;42:184–191. [PubMed: 17207817]
16. Enoch M-A, Schwartz L, Albaugh B, Virkkunen M, Goldman D. Dimensional Anxiety Mediates Linkage of GABRA2 Haplotypes with Alcoholism. *Am J Med Genet Part B: Neuropsychiatr Genet* 2006;141B:599–607.
17. Bauer LO, Covault J, Harel O, Das S, Gelernter J, Anton R, et al. Variation in GABRA2 predicts drinking behavior in project MATCH subjects. *Alcohol Clin Exp Res* 2007;11:1780–1787. [PubMed: 17949392]

18. Pierucci-Lagha A, Covault J, Feinn R, Nellissery M, Hernandez-Avila C, Oncken C, et al. GABRA2 alleles moderate the subjective effects of alcohol, which are attenuated by finasteride. *Neuropsychopharmacology* 2005;30:1193–1203. [PubMed: 15702134]
19. Covault J, Gelernter J, Hesselbrock V, Nellissery M, Kranzler HR. Allelic and haplotypic association of GABRA2 with alcohol dependence. *Am J Med Genet B Neuropsychiatr Genet* 2004;129B:104–109. [PubMed: 15274050]
20. Lappalainen J, Krupitsky E, Remizov M, Pchelina S, Taraskina A, Zvartau E, et al. Association between alcoholism and gamma-amino butyric acid alpha2 receptor subtype in a Russian population. *Alcohol Clin Exp Res* 2005;29:493–498. [PubMed: 15834213]
21. Fehr C, Sander T, Tadic A, Lenzen KP, Anghelescu I, Klawe C, et al. Confirmation of association of the GABRA2 gene with alcohol dependence by subtype-specific analysis. *Psychiatr Genet* 2006;16:9–17. [PubMed: 16395124]
22. Covault J, Gelernter J, Jensen K, Anton R, Kranzler HR. Markers in the 5'-Region of GABRG1 Associate to Alcohol Dependence and are in Linkage Disequilibrium with Markers in the Adjacent GABRA2 Gene. *Neuropsychopharmacology* 2008;33:837–848. [PubMed: 17507911]
23. Drgon T, D'Addario C, Uhl GR. Linkage disequilibrium, haplotype and association studies of a chromosome 4 GABA receptor gene cluster: candidate gene variants for addictions. *Am J Med Genet B Neuropsychiatr Genet* 2006;141B:854–860. [PubMed: 16894595]
24. Enoch M-A. The role of GABA_A Receptors in the Development of Alcoholism. *Pharmacol Biochem Behav* 2008;90:95–104. [PubMed: 18440057]
25. Hsu FC, Zhang GJ, Raol YS, Valentino RJ, Coulter DA, Brooks-Kayal AR. Repeated neonatal handling with maternal separation permanently alters hippocampal GABAA receptors and behavioral stress responses. *Proc Natl Acad Sci USA* 2003;100:12213–12218. [PubMed: 14530409]
26. Higley JD, Hasert MF, Suomi SJ, Linnoila M. Nonhuman primate model of alcohol abuse: effects of early experience, personality, and stress on alcohol consumption. *Proc Natl Acad Sci U S A* 1991;88:7261–7265. [PubMed: 1871131]
27. Moffett MC, Vicentic A, Kozel M, Plotsky P, Francis DD, Kuhar MJ. Maternal separation alters drug intake patterns in adulthood in rats. *Biochem Pharmacol* 2007;73:321–330. [PubMed: 16962564]
28. Vazquez V, Penit-Soria J, Durand C, Besson MJ, Giros B, Daugé V. Brief early handling increases morphine dependence in adult rats. *Behav Brain Res* 2006;170:211–218. [PubMed: 16567006]
29. Arnow BA. Relationships between childhood maltreatment, adult health and psychiatric outcomes, and medical utilization. *J Clin Psychiatry* 2004;65(Suppl 12):10–5. [PubMed: 15315472]
30. Dodge KA, Bates JE, Pettit GS. Mechanisms in the cycle of violence. *Science* 1990;250:1678–1683. [PubMed: 2270481]
31. Heffernan K, Cloitre M, Tardiff K, Marzuk PM, Portera L, Leon AC. Childhood trauma as a correlate of lifetime opiate use in psychiatric patients. *Addict Behav* 2000;25:797–803. [PubMed: 11023022]
32. Hyman SM, Garcia M, Sinha R. Gender specific associations between types of childhood maltreatment and the onset, escalation and severity of substance use in cocaine dependent adults. *Am J Drug Alcohol Abuse* 2006;32:655–664. [PubMed: 17127554]
33. Nelson EC, Heath AC, Lynskey MT, Bucholz KK, Madden PA, Statham DJ, et al. Childhood sexual abuse and risks for licit and illicit drug-related outcomes: a twin study. *Psychol Med* 2006;36:1473–1483. [PubMed: 16854248]
34. Rothman EF, Edwards EM, Heeren T, Hingson RW. Adverse childhood experiences predict earlier age of drinking onset: results from a representative US sample of current or former drinkers. *Pediatrics* 2008;122:298–304.
35. Shin SH, Edwards EM, Heeren T. Child abuse and neglect: Relations to adolescent binge drinking in the national longitudinal study of Adolescent Health (AddHealth) Study. *Addict Behav* 2009;34:277–280. [PubMed: 19028418]
36. Verona E, Sachs-Ericsson N. The intergenerational transmission of externalizing behaviors in adult participants: the mediating role of childhood abuse. *J Consult Clin Psychol* 2005;73:1135–1145. [PubMed: 16392986]
37. Widom CS, Marmorstein NR, White HR. Childhood victimization and illicit drug use in middle adulthood. *Psychol Addict Behav* 2006;20:394–403. [PubMed: 17176174]

38. Spitzer, RL.; Williams, JBW.; Gibbon, M. Structured Clinical Interview for DSM-IV (SCID). New York: New York State Psychiatric Institute, Biometrics Research; 1995.
39. Bernstein, DP.; Fink, L. Childhood Trauma Questionnaire: A retrospective self-report manual. San Antonio, TX: The Psychological Corporation; 1998.
40. Bernstein DP, Stein JA, Newcomb MD, Walker E, Pogge D, Ahluvalia T, et al. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. *Child Abuse Negl* 2003;27:169–190. [PubMed: 12615092]
41. Scher CD, Stein MB, Asmundson GJ, McCreary DR, Forde DR. The childhood trauma questionnaire in a community sample: psychometric properties and normative data. *J Trauma Stress* 2001;14:843–857. [PubMed: 11776429]
42. Thombs BD, Lewis C, Bernstein DP, Medrano MA, Hatch JP. An evaluation of the measurement equivalence of the Childhood Trauma Questionnaire--Short Form across gender and race in a sample of drug-abusing adults. *J Psychosom Res* 2007;63:391–398. [PubMed: 17905047]
43. Hodgkinson CA, Yuan Q, Xu K, Shen PH, Heinz E, Lobos EA, et al. Addictions Biology: Haplotype-Based Analysis for 130 Candidate Genes on a Single Array. *Alcohol Alcohol* 2008;43:505–515. [PubMed: 18477577]
44. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–1169. [PubMed: 14574645]
45. Gelernter J, Kranzler HR, Panhuysen C, Weiss RD, Brady K, Poling J, Farrer L. Dense genomewide linkage scan for alcohol dependence in African Americans: significant linkage on chromosome 10. *Biol Psychiatry* 2009;65:111–115. [PubMed: 18930185]
46. Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, et al. Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Gene* 1998;81:207–215.
47. Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, et al. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet* 1998;81:216–221. [PubMed: 9603607]
48. Laviolette SR, Nader K, van der Kooy D. Motivational state determines the functional role of the mesolimbic dopamine system in the mediation of opiate reward processes. *Behav Brain Res* 2002;129:17–29. [PubMed: 11809491]
49. Nader K, van der Kooy D. Deprivation state switches the neurobiological substrates mediating opiate reward in the ventral tegmental area. *J Neurosci* 1997;17:383–390. [PubMed: 8987763]
50. Okada H, Matsushita N, Kobayashi K, Kobayashi K. Identification of GABAA receptor subunit variants in midbrain dopaminergic neurons. *J Neurochem* 2004;89:7–14. [PubMed: 15030384]
51. Steiger JL, Russek SJ. GABAA receptors: building the bridge between subunit mRNAs, their promoters, and cognate transcription factors. *Pharmacol Ther* 2004;101:259–281. [PubMed: 15031002]
52. Wisden W, Laurie DJ, Monyer H, Seeburg PH. The distribution of 13 GABAA receptor subunit mRNAs in the rat brain. I. Telencephalon, diencephalon, mesencephalon. *J Neurosci* 1992;12:1040–1062. [PubMed: 1312131]
53. Brake WG, Zhang TY, Diorio J, Meaney MJ, Gratton A. Influence of early postnatal rearing conditions on mesocorticolimbic dopamine and behavioural responses to psychostimulants and stressors in adult rats. *Eur J Neurosci* 2004;19:1863–1874. [PubMed: 15078560]
54. Meaney MJ, Brake W, Gratton A. Environmental regulation of the development of mesolimbic dopamine systems: a neurobiological mechanism for vulnerability to drug abuse? *Psychoneuroendocrinology* 2002;27:127–138. [PubMed: 11750774]
55. Marquardt AR, Ortiz-Lemos L, Lucion AB, Barros HM. Influence of handling or aversive stimulation during rats' neonatal or adolescence periods on oral cocaine self-administration and cocaine withdrawal. *Behav Pharmacol* 2004;15:403–412. [PubMed: 15343067]
56. Chen Q, Lee TH, Wetsel WC, Sun QA, Liu Y, Davidson C, et al. Reversal of cocaine sensitization-induced behavioral sensitization normalizes GAD67 and GABAA receptor alpha2 subunit expression, and PKC zeta activity. *Biochem Biophys Res Commun* 2007;356:733–738. [PubMed: 17382295]

57. Dick DM, Latendresse SJ, Lansford JE, Budde JP, Goate A, Dodge KA, et al. Role of GABRA2 in trajectories of externalizing behavior across development and evidence of moderation by parental monitoring. *Arch Gen Psychiatry* 2009;66:649–657. [PubMed: 19487630]
58. Bernstein DP, Fink L, Handelsman L, Foote J, Lovejoy M, Wenzel K, et al. Initial reliability and validity of a new retrospective measure of child abuse and neglect. *Am J Psychiatry* 1994;151:1132–1136. [PubMed: 8037246]
59. Bernstein DP, Ahluvalia T, Pogge D, Handelsman L. Validity of the Childhood Trauma Questionnaire in an adolescent psychiatric population. *J Am Acad Child Adolesc Psychiatry* 1997;36:340–348. [PubMed: 9055514]
60. Goodman LA, Thompson KM, Weinfurt K, Corl S, Acker P, Mueser KT, et al. Reliability of reports of violent victimization and posttraumatic stress disorder among men and women with serious mental illness. *J Trauma Stress* 1999;12:587–599. [PubMed: 10646178]
61. Fergusson DM, Horwood LJ, Woodward LJ. The stability of child abuse reports: a longitudinal study of the reporting behaviour of young adults. *Psychol Med* 2000;30:529–544. [PubMed: 10883709]

FIGURE 1. GABRA2 haplotype block structure

The rs numbers of the 10 SNPs and the bases for alleles 1 – 2 are given, together with the allele 2 frequencies. For each SNP, alleles 1 and 2 are located on opposite DNA strands. The numbers in the squares refer to linkage disequilibrium (LD) measured as D' between each pair of SNPs. Haplotype blocks were defined using a setting of average pairwise D' within-block of ≥ 0.80 .

FIGURE 2.

Description of dataset: patients with substance dependence (alcohol, cocaine, heroin) and controls (no addiction). CTQ: childhood trauma questionnaire. Genotype data: 542 men; 360 patients and 182 controls. CTQ data: 640 men; 495 patients, 145 controls. Genotype + CTQ data: 350 men; 278 patients, 72 controls.

FIGURE 3. GABRA2 block 2 haplotypes: the effects of childhood adversity on risk for addiction to heroin, alcohol or cocaine

High childhood adversity defined as total CTQ score ≥ 1 S.D. above mean CTQ score of controls; lower CTQ scores were designated 'low' adversity.

* $p < 0.01$

FIGURE 4. Interaction between *GABRA2* rs11503014 and childhood adversity; influence on cocaine dependence

High childhood adversity defined as total CTQ score ≥ 1 S.D. above mean CTQ score of controls; lower CTQ scores were designated 'low' adversity.

** $P < 0.05$; * $p = 0.05$.

GABRA2 block 2 haplotype frequencies in heroin, cocaine and alcohol dependent patients and controls

TABLE 1

Subjects	N	GABRA2 Block 2 Haplotype Frequencies			
		2222111	1111222	2112122	2111111
Controls	308	0.34	0.28	0.19	0.19
Total group of patients	563	0.38	0.32	0.20	0.11
All patients with heroin dependence	250	0.36	0.31	0.23	0.10
Patients with only heroin dependence	66	0.41	0.26	0.26	0.07
All patients with alcohol dependence	357	0.38	0.32	0.19	0.11
Patients with only alcohol dependence	99	0.41	0.33	0.16	0.10
All patients with cocaine dependence	341	0.38	0.31	0.19	0.11
Patients with only cocaine dependence	88	0.40	0.33	0.18	0.09

Since these are haplotype analyses the N's are the number of chromosomes (2 per individual). The four haplotypes for which frequencies are given here account for 0.79 of block 2 haplotypes in the total sample.

TABLE 2
The influence of *GABRA2* block 2 haplotypes and childhood trauma on heroin, alcohol and cocaine dependence

Patient groups	N's for group comparisons	European Effect		Haplotype 21111111		Haplotype 21121222		Childhood Trauma		Whole		Model
		χ^2	P value	χ^2	P value	χ^2	P value	χ^2	P value	P value	Df	Var
All patients with heroin dependence	250/612 ^b	13.0	0.0003	4.2	0.042	6.5	0.011			0.0004	4	0.02
Patients with only heroin dependence	43/120 ^a	5.8	0.016	3.7	0.054	4.8	0.028	7.9	0.005	0.0001	5	0.13
All patients with alcohol dependence	580/700 ^c							47.3 [*]	<0.0001	<0.0001	1	0.04
Patients with only alcohol dependence	148/294 ^a							52.1 [*]	<0.0001	<0.0001	1	0.10
All patients with cocaine dependence	660/620 ^d							115 [*]	<0.0001	<0.0001	1	0.08
Patients with only cocaine dependence	77/120 ^a			3.3	0.072			17.8	<0.0001	0.0002	4	0.09
Total group of patients	433/120 ^a			4.3	0.037			53.1	<0.0001	<0.0001	4	0.10

The Table summarizes the results of 7 logistic regression models.

Results are for effect likelihood ratio (L-R) tests and are given for $p < 0.1$.

^{*} F values; results are from the Fit Least Squares method.

The Ns for some analyses are higher because genotypes or CTQ scores were not included in some models as detailed below.

Since these are haplotype analyses the N's are the number of chromosomes (2 per individual) in patients/comparison group as follows:

^a Comparison with controls. For alcoholism only, genotype was not included in the final model since there was no significant gene or G×E effect.

^b Comparison with: patients without heroin dependence + controls; CTQ score was not included in the final model since there was no significant effect.

^c Comparison with: patients without alcohol dependence + controls; genotype was not included in the final model since there was no significant gene or G×E effect.

^d Comparison with: patients without cocaine dependence + controls.

The European factor effect was included when significant to correct for population stratification.

Childhood trauma effect is the effect of the continuous measure: total CTQ score.

The 4 haplotypes included in the model represent 79% of the total haplotype diversity.

There were no significant effects for the other two haplotypes. There were no significant gene-environment interactions.

TABLE 3
The influence of *GABRA2* rs11503014 and childhood trauma on heroin, alcohol and cocaine dependence

Patient Groups	N's Patients/Comparison Group	European Effect		Gene Effect		Childhood Trauma Effect		G × E Interaction		Whole P value	Model	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P		Df	Var
All patients with heroin dependence	157/385 ^b	6.5	0.011	8.7	0.003	-	-	-	-	0.0005	2	0.02
Patients with only heroin dependence	27/72 ^a	4.8	0.029	3.2	0.073	5.8	0.016	-	-	0.0012	3	0.14
All patients with alcohol dependence	290/350 ^c	-	-	-	-	23.6 [*]	< 0.0001	-	-	< 0.0001	1	0.04
Patients with only alcohol dependence	74/145 ^a	-	-	-	-	26.0 [*]	< 0.0001	-	-	< 0.0001	1	0.11
All patients with cocaine dependence	184/166 ^d	-	-	-	-	23.7	< 0.0001	4.3	0.039	< 0.0001	3	0.06
Patients with only cocaine dependence	47/72 ^a	-	-	-	-	16.0	< 0.0001	8.8	0.003	0.0002	3	0.13
Total group of patients	278/72 ^a	-	-	5.4	0.020	32.7	< 0.0001	3.1	0.076	< 0.0001	3	0.10

The table summarizes the results of seven logistic regression models.

χ^2 results are for effect likelihood ratio (L-R) tests and are given for p values < 0.1.

* F values: results are from the Fit Least Squares method.

The Ns for some analyses are higher because genotypes or CTQ scores were not included in some models as detailed below.

N's are the numbers of patients/comparison group as follows:

^a Comparison with controls. For patients with only alcohol dependence, genotype was not included in the final model since there was no significant gene or G×E effect.

^b Comparison with: patients without heroin dependence + controls; CTQ score was not included in the final model since there was no significant effect.

^c Comparison with: patients without alcohol dependence + controls; genotype was not included in the final model since there was no significant gene or G×E effect.

^d Comparison with: patients without cocaine dependence + controls.

The European factor effect was included when significant to correct for population stratification.

Childhood trauma effect is the effect of the continuous measure: total CTQ score.