HHS Public Access

Author manuscript

Clin Neurophysiol. Author manuscript; available in PMC 2021 June 01.

Published in final edited form as:

Clin Neurophysiol. 2020 June ; 131(6): 1180–1186. doi:10.1016/j.clinph.2020.02.031.

GRM8 Genotype is Associated with Externalizing Disorders and Greater Inter-Trial Variability in Brain Activation During a Response Inhibition Task

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Abstract

Objective: The present investigation tested the association of a novel measure of brain activation recorded during a simple motor inhibition task with a *GRM8* genetic locus implicated in risk for substance dependence.

Methods: 122 European-American adults were genotyped at rs1361995 and evaluated against DSM-IV criteria for Alcohol Dependence, Cocaine Dependence, Conduct Disorder, and Antisocial Personality Disorder. Also, their brain activity was recorded in response to rare, so-called "No-Go" stimuli presented during a continuous performance test. Brain activity was quantified with two indices:(1) the amplitude of the No-Go P300 electroencephalographic response averaged across trials; and (2) the inter-trial variability of the response.

Results: The absence of the minor allele at the candidate locus was associated with all of the evaluated diagnoses. In comparison to minor allele carriers, major allele homozygotes also demonstrated increased inter-trial variability in No-Go P300 response amplitude but no difference in average amplitude.

Conclusions: *GRM8* genotype is associated with Alcohol and Cocaine Dependence as well as personality risk factors for dependence. The association may be mediated through an inherited instability in brain function that affects cognitive control.

Keywords

GRM8; Response Inhibition; Alcohol Dependence; Cocaine Dependence; Conduct Disorder

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Author Contributions

The corresponding author supervised the collection of data, performed the analysis, and wrote the initial draft of this article. The co-author contributed to the authorship of the supporting research grant and revised the article before submission.

Conflict of Interest

No conflict declared.

1. Introduction

Many factors have been described that increase risk for onset or recurrence of Alcohol Dependence (Ciraulo et al., 2003, Hill and O'Brien, 2015). Prominent in the list are a family history of substance dependence (Lieb et al., 2002, Milne et al., 2009, Windle and Windle, 2018) and a childhood history of conduct problems (Hasin et al., 2011, Heron et al., 2013). Of great interest and unclear significance are specific genes that may underly the contributions of these factors. Unfortunately, progress in identifying powerful genetic predictors has been marred by failures to replicate some candidate gene and genome wide association findings (Derringer et al., 2011, Hart and Kranzler, 2015).

An example of a candidate gene association that has survived most replication attempts involves the glutamate receptor gene, *GRM8*, on chromosome 7. Two publications from the Collaborative Study on the Genetics of Alcoholism (COGA) have shown an association with Alcohol Dependence (AD). The analysis by Chen and colleagues (Chen et al., 2009) found linkage with multiple single nucleotide polymorphisms in the *GRM8* gene: an excess prevalence of major alleles at rs1361995, rs10487457, and rs10487459 was detected among 472 alcohol-dependent participants in comparison to 577 unaffected family members. Long and colleagues (Long et al., 2015) similarly found linkage in analyses of a younger group of participants--18-26 year old adults.

One major goal of the present study was to test the replicability of these associations in a different but smaller data set. To most readers, this replication attempt with only 122 cases may appear to be a weakness because fewer cases obviously reduces statistical power. Yet, in genetic association studies, sample size is not the only consideration. If the goal is to detect non-trivial and robust effects, then the same association should remain statistically significant in analyses employing fewer cases.

A related goal was to test replicability using a different ascertainment procedure. In COGA, affected cases were identified within densely-affected families and recruited from two disparate sources. Some cases (probands) were adults receiving AD treatment in either inpatient or outpatient settings. Other cases were members of the proband's family who were not in treatment and may never have been in treatment. They were only identified through research interviews. One could therefore assert that the ascertainment methods used in COGA yielded substantial variability in the severity of AD. In the present study, we recruited affected cases only from residential substance abuse treatment programs. By this method of ascertainment, we reduced the heterogeneity in the AD phenotype, focused on the most severe cases, and accordingly improved power.

The second major goal of this study was to determine if *GRM8* demonstrates the same breadth of association with multiple addiction-related phenotypes as seen for other candidategenes. The *CHRM2* gene, for example, has been linked to psychiatric disorders of both the internalizing and externalizing variety (Luo et al., 2005, Wang et al., 2004). The *GABRA2* gene on chromosome 4 has been implicated in risk for alcohol (Edenberg et al., 2004) and drug (Agrawal et al., 2006) dependence, conduct problems (Dick et al., 2006), and obesity (Bauer et al., 2012). The Taq1a polymorphism and nearby single nucleotide

polymorphisms (SNPs) on the *ANKK1* gene, which is adjacent to *DRD2* on chromosome 11, are likewise correlated with multiple disorders (Athanasoulia et al., 2014, Ponce et al., 2008, Wang et al., 2013, Wu et al., 2008, Yang et al., 2008). We hypothesized that *GRM8* would show a similar association with multiple disorders that overlap in their component features, such as high levels of risk-taking or impulsivity. Accordingly, we focused our analyses on disorders of the externalizing variety (Hicks et al., 2004, Kendler et al., 2003), including AD, Cocaine Dependence, Conduct Disorder, and Antisocial Personality Disorder.

The third major goal was to record an objective measure of brain function and assess its relationship with the *GRM8* gene. Chen and colleagues (Chen et al., 2009) adopted this approach in their 2008 analysis of COGA data. In comparison to minor allele homozygotes, they found that major allele homozygotes at representative SNPs showed smaller amplitude 4–5 Hz oscillations in electroencephalographic (EEG) responses to novel stimuli presented during a selective attention task.

We have a similar interest in demonstrating EEG differences as a factor that may mechanistically connect the higher risk genotype to the disorder. However, our interest is in a novel approach to measurement. More specifically, we are not similarly interested in EEG responses averaged across trials of a cognitive task because averages are insensitive to momentary lapses and periods of overcompensation. Our focus is on inter-trial variability.

There is a compelling rationale justifying this novel focus. Specific research areas, including Attention-Deficit Hyperactivity Disorder and healthy aging (Garrett et al., 2013, Tamm et al., 2012), have already demonstrated the value of measuring inter-trial variability as an early and sensitive indicator of abnormalities in brain function--particularly when the brain disorder or condition is not severe. We have previously shown that it differentiates HIV-1 seropositive and seronegative groups (Bauer, 2018a) and reveals previously undetected but hypothesized interactions between HIV-1 serostatus and drug abuse (Bauer, 2018b). Other investigators have shown that greater inter-trial variability predicts balance and gait difficulties among older adults (Graveson et al., 2016) as well as increased risk of all-cause mortality over a 17-year monitoring period (Batterham et al., 2014).

A critical reader may ask if greater variability in either task performance or brain activity is simply a reflection of random noise or a temporary state. The evidence to date suggests that it is a stable characteristic. It correlates across different tasks (Hultsch et al., 2008). Also, it correlates from session-to-session and day-to-day (Rabbitt et al., 2001). We (Bauer, manuscript in preparation) recently evaluated the test-retest reliability of P300 amplitude inter-trial variability over a 1-year interval and detected an intra-class correlation coefficient (ICC) equivalent to the ICC of P300 average amplitude.

For these reasons, we suspect that inter-trial variability is as stable a trait as many other putative indicators of brain function. It may be useful in developing new phenotypes for candidate gene or genome-wide association studies. In this investigation, we hypothesized that greater variability during a challenge to cognitive control would be associated with the *GRM8* genotype implicated in risk for Alcohol Dependence.

2. Methods

2.1 Recruitment

A total of 171 participants were recruited from either residential substance use treatment programs or the community. Patients in residential treatment were recruited for a study focused on an examination of genetic and neurophysiological predictors of risk for relapse to substance use. Community residents were recruited using advertisements that described a study of genetic and neurophysiological correlates of cognitive function. Treatment program and community residents were compensated for their time and effort with gift cards redeemable at fast food or big box stores.

2.2 Evaluation Procedures

The initial phase of the evaluation of recruits was an interview conducted by telephone. The interview was structured to identify exclusion criteria that would likely complicate the interpretation of the participants' cognitive abilities and their electroencephalographic data. Recruits were not enrolled if they reported a history of seizures, neurosurgery, head injury with loss of consciousness greater than 30 minutes, schizophrenia, bipolar disorder, mental retardation, dementia, or significant medical disorders, including HIV-1 infection, or cardiovascular, hepatic, immunologic, or renal disease. Uncorrected deficits in vision or hearing were also reasons for exclusion from further participation.

The next phase was performed in-person on a subsequent day at the University of Connecticut Health Center (UCHC). It began with the review of informed consent and other documents approved by the UCHC Institutional Review Board. Urine and breath samples were then collected and assayed to exclude volunteers complicated by recent exposure to alcohol, cocaine, amphetamine, marijuana, or heroin.

During the meeting, detailed information was collected about personal and family histories of psychological problems, including substance use. The Computerized Diagnostic Interview Schedule for DSM-IV [CDIS-4; (Robins, 2002)] was used to detect psychiatric disorders. Additional information about psychological and drug use characteristics was garnered from medical records, interviews, and questionnaires, including the Michigan Alcoholism Screening Test [MAST; (Selzer, 1971)], Drug Abuse Screening Test [DAST; (Skinner, 1982)], Fagerstrom Test for Nicotine Dependence [FTND; (Heatherton et al., 1991)], Beck Depression and Anxiety Inventories (Beck, 1996, Leyfer et al., 2006)], Family History Assessment Module [FHAM; (Rice et al., 1995)], and the Wender Utah Rating Scale [WURS; (Ward et al., 1993)]. The Kaufman Brief Intelligence Test [KBIT; (Kaufman, 1990)] was employed to provide an estimate of general cognitive function.

The final phase of evaluation focused on the measurement of each participant's brain activity during tasks challenging different aspects of cognitive function. The task that is the focus of the present analysis is a version of the classic Continuous Performance Test (Beck et al., 1956). It involved an instruction to press a button in response to regularly and frequently-occurring "Go" stimuli and withhold the button press when rare, "No-Go" stimuli appeared. In total, 200 Go stimuli and 50 No-Go stimuli were presented in an interleaved series. The stimuli were the numerals "1" (Go) and "0" (No-Go) presented for 200 ms each

at a rate of one stimulus every 1.3 sec. They subtended a visual angle of 2.86 degrees and were presented in a white font on a computer screen in a darkened room.

Throughout the task, the electroencephalogram was recorded from 31 electrodes positioned over the scalp. Eyeblinks and eye movements were also recorded with a pair of electrodes placed diagonally above and below the left eye. The EEG and eye movement channels were appropriately amplified (EEG gain = 10K, EOG gain = 2K) using a Compumedics Neuroscan Inc. (Charlotte, NC) NuAmp® amplifier and SCAN® version 4.1 data collection software. The data collection system routed the EEG and EOG channels to an A/D converter and sampled each channel at a rate of 250 Hz for 50 ms preceding and 750 ms following the onset of each No-Go stimulus.

The off-line processing of the EEG data was aggressive in identifying and removing artifacts that may have compromised valid estimation of No-Go P300 amplitude on individual trials. To this end, EEG epochs were initially screened for the absence of A/D converter overflow and motion artifacts by imposing a voltage acceptance window of −75 to +75μv. They were also passed through a bandpass filter favoring frequencies in the delta and theta frequency bands (highpass cutoff=0.5 Hz, 12 db/octave roll-off; low pass cutoff=8 Hz, 48 db/octave roll-off) known to contribute disproportionately to P300 activity. Subsequently, EEG epochs were mathematically corrected for correlated activity in the eye movement channel using the Semlitsch algorithm (Semlitsch et al., 1986). The final processing step involved the removal of voltage offsets by subtracting the average voltage during the 50 ms pre-stimulus period from the voltages at each post-stimulus sampling point.

Two methods were used to summarize the amplitude of the No-Go P300 response at 2 electrode sites (Fz, Cz) where the No-Go and Go P300 responses reliably differ (Salisbury et al., 2004). The first method was conventional. At each time point throughout the 750 ms epoch, the voltage of the EEG was averaged across all acceptable trials (n=25–35). No-Go P300 response amplitude was calculated as the average voltage over a time window of 250 to 500 ms. The second measurement method involved the calculation of the standard deviations of the EEG voltages across all acceptable No-Go epochs (trials) at each sampling point. The standard deviations were averaged over the same 250–500 ms time window used for calculating the central tendency of P300 amplitude. The standard deviation data were transformed using the formula, (100+SD of amplitude)/(100+avg of amplitude), because across-trial variability was found to be linearly correlated with the across-trial average voltage. The constant was added to the numerator and denominator to eliminate division-by-zero errors.

2.3 Genotype Analysis Procedures

A subset of the 171 participants were included in the analyses described below. Because the analyses were focused on examining genetic associations, and many SNP allele frequencies are influenced by race and ancestral origin, it was necessary to exclude data from participants who were Black or Hispanic by self-report. Accordingly, DNA from peripheral blood was processed only for the 122 participants who reported European-American ancestry.

Three intronic SNPs within the *GRM8* region were genotyped in a batch procedure. These SNPs were chosen because the prior report by Chen and colleagues (Chen et al., 2009) found that they were more robustly associated with an EEG frequency contributing to the P300 response than other *GRM8* SNPs. Chen and colleagues also found an association between these SNPs and a diagnosis of Alcohol Dependence defined by ICD-10 criteria.

Genotyping revealed that the three SNPs showed 100% concordance—a finding consistent with other published data {Genomes Project, 2012}. It was therefore appropriate to choose one SNP, rs1361995, as a representative marker and discard the other SNPs, rs10487457 and rs10487459. At rs1361995, the distribution of genotype frequencies was consistent with Hardy Weinberg equilibrium expectations: χ^2=0.97, p=0.32. There were 50 major allele homozygotes (CC), 60 heterozygotes (CT), and 12 minor allele homozygotes (TT). To address the statistical analysis problem arising from the presence of only 12 minor allele homozygotes, we combined this small cell with the heterozygotes. The composite group, defined as minor allele carriers was compared to the remaining cell--the at-risk major allele homozygotes--in all of the analyses reported below.

2.4 Data Analysis

The initial analyses used simple χ^2 and t-tests to contrast the demographic and psychiatric characteristics of the major allele homozygotes and minor allele carriers (Table 1). Associations of genotypes with diagnoses and severity scores were examined with logistic regressions employing age and sex as covariates.

A different approach was used for testing associations of genotypes with the inter-trial mean and variability of No-Go P300 amplitude because these hypotheses had not previously been explored. As a protection against spurious findings, effects of genotype were initially tested with a multivariate analysis of variance. Univariate tests were performed if and only if the multivariate test was significant.

The final phase of the analysis was designed to evaluate the relevance of the No-Go P300 response to behavior. It involved the calculation of correlation coefficients between task performance metrics, including reaction times and error rates, and No-Go P300 features that were statistically significant in the univariate analyses.

3. Results

Table 1 presents the results of simple comparisons of the two genotype groups on background characteristics. Comparisons revealed no significant group differences in age (t=0.4, p=0.7) or gender (χ^2=2.4, p=0.1) composition. The groups were also similar in years of education (t=−1.1, p=0.2), estimated intelligence from the KBIT (t=0.5, p=0.5), Attention Deficit Hyperactivity Disorder ratings from the Wender Scale (t=1.2, p=0.2), nicotine dependence severity (t=1.4, p=0.1), and scores on the Beck Depression (t=0.8, p=0.3) and Anxiety (t=0.1, p=0.9) Inventories.

Two symptom severity scales did differentiate the groups. MAST scores were greater (t=2.3, p=0.02) among participants who were major allele homozygotes [mean(sd)=8.9(6.9)] versus

minor allele carriers [mean(sd)=6.0(6.6)]. The groups similarly differed (t=2.2, p=0.02) in scores on the DAST [12.9(7.4) versus 9.7(7.9)].

Table 2 shows the associations of genotype with DSM-4 diagnoses of Alcohol Dependence, Cocaine Dependence, childhood Conduct Disorder, and Antisocial Personality Disorder. Simple χ^2 tests with no adjustment for covariates revealed statistically significant findings for all of these disorders. The findings from more appropriate, logistic regression analyses adjusted for age and sex were also statistically significant: Alcohol Dependence (OR=3.3, 95% CI=1.3–8.1, p=0.007), Cocaine Dependence (OR=2.9, 95% CI=1.3–6.6, p=0.007), Conduct Disorder (OR=2.6, 95% CI=1.2–5.6, p=0.018), Antisocial Personality Disorder (OR=2.2, 95% CI=1.1–5.2, p=0.049). Participants with 2 copies of the major allele were more likely than minor allele carriers to meet criteria for these diagnoses.

Table 3 and Figure 1 show that *GRM8* genotype was likewise associated with differences in the No-Go P300 response. The test for joint significance via MANOVA (Wilks' lambda=0.90, F=3.04, p=0.02) indicated that univariate tests could be performed. They showed that the inter-trial variability in No-Go P300 amplitude at Fz (F=12.17, p<0.001) and Cz (F=4.37, p<0.04) sites was significantly greater in the at-risk major allele homozygote group versus the minor allele carrier group. The differences between the groups in analyses of amplitude averaged across trials were not statistically significant. There were also no significant differences between the groups in task performance, although there was a trend for major allele homozygotes to demonstrate faster reaction times on Go trials.

The final set of analyses examined the correlations of inter-trial variability in No-Go P300 amplitude with reaction times and error rates. The correlations with omission and commission error rates did not approach or attain statistical significance. However, No-Go P300 amplitude variablity was significantly correlated with reaction time variability (Fz: r=0.29, p<0.05; Cz: r=0.17, p<0.05) and average reaction time (Fz: r=0.49, p<0.05; Cz: r=0.33, p<0.05).

4. Discussion

It was reassuring to discover from the present analyses that the association between *GRM8* genotype and Alcohol Dependence could be replicated. The need for replication in psychiatry and neuroscience has become more evident in recent years. For example, an interesting review (Tajika et al., 2015) of the literature following the publication of 83 highly-cited intervention studies in psychiatry found that only 16 of the original study findings had been confirmed using similar methods. Eleven studies were replicated with substantially smaller effects. More significantly, sixteen studies were followed by other studies detecting an opposite change. In addition, 40 studies had never been the subject of a replication attempt.

In the field of psychiatric genetics, the reproducibility problem has been met with a call (Duncan et al., 2019) for larger sample sizes and genome-wide scans. The call for larger samples is reasonable. Yet, a larger sample does not adequately address other problems likely to affect reproducibility and generalization. One problem irrelevant to sample size is

ascertainment bias, in which the associations between phenotypes and genotypes are found to vary with the manner in which participants are recruited, screened, enrolled, or evaluated. Regardless of sample size, there is a need for replication studies that employ different ascertainment methods than were employed in the original studies, such as COGA.

Another problem affecting reproducibility is the excessive heterogeneity in the phenotype attending a large sample size. In practical terms, the push for more participants is likely to loosen enrollment criteria and invite multiple subcategories of patients who may vary markedly in genetic risk patterns, epigenetic factors, and disease severity. The unintended effect of a large sample size may be a cascade in which an increasingly large sample is needed to provide adequate power to separate hidden subcategories. It is noteworthy that the present sample was modest in size and that patients were a homogenous group. Because they were recruited from residential substance abuse treatment programs, their levels of symptom severity and personality dysfunction were likely greater and more uniform than the affected cases recruited into COGA.

To a critical reader, it should also be reassuring that the present study found an association of *GRM8* SNP genotype with other addiction-related phenotypes. Indeed, because glutamate receptors are widely distributed throughout the brain (Niswender and Conn, 2010), it is logical that altered function/expression of glutamate receptor genes would be associated with numerous disorders and phenotypes. It is also noteworthy but of unknown significance that *GRM8* is located in a region, 7q31.3-q32, in proximity to the *CHRM2* gene, 7q33, which has been repeatedly associated with alcohol (Wang et al., 2004) and other drug (Dick et al., 2007a, Luo et al., 2005) dependence, as well as Conduct Disorder, Antisocial Personality Disorder, and a general externalizing factor (Dick et al., 2008). *CHRM2* has also been linked to Major Depressive Disorder (Wang et al., 2004), lower IQ (Dick et al., 2007b, Gosso et al., 2007), and an abnormal EEG response found in both externalizing and internalizing disorders (Bauer and Hesselbrock, 2001, 2003, Houston et al., 2003, 2004) — reduced P300 amplitude or, more specifically, reduced power in EEG frequencies that contribute to the P300 (Jones et al., 2006, Jones et al., 2004).

The demonstration of an association between *GRM8* and inter-trial variability in P300 amplitude during a response inhibition task is the most novel finding of the present study. It is remarkable that P300 amplitude variability was greater among participants with the higher risk genotype for Alcohol and Cocaine Dependence as well as Conduct and Antisocial Personality Disorder, which are known risk factors for dependence {Bahlmann, 2002}. Yet, no differences were found between the groups in the average amplitude of the No-Go P300 response. In a previous study (Bauer, 2018a) comparing HIV-1 seropositive and seronegative participants performing a time estimation task, we similarly found group differences in motor potentials that were evident in the inter-trial variability analysis but not when average amplitude was analyzed.

Another interesting finding came from the calculation of correlations between P300 amplitude variability and task performance. The significant correlations found between P300 inter-trial variability and both reaction time and reaction time variability suggest that the neurophysiological differences found presently are not behaviorally silent. In this data set,

the differences affected response timing only. They did not appear to affect the perception and discrimination of stimuli respectively reflected in the omission and commission error rates.

4.1 Limitations

Unfortunately, the design of the present study does not allow us to argue convincingly that greater variability in the No-Go P300 response is a mechanism connecting *GRM8* genotype to Alcohol or Cocaine Dependence. The complicating issue is the possibility that variability in the response is caused by the pharmacological effects of alcohol or cocaine or medications prescribed for treating these or other disorders. Exerting statistical control over severity of use by specifying MAST or DAST scores as covariates will not resolve the issue because *GRM8* genotype also affects these severity measures (see Table 1). A more convincing demonstration of neural response variability as an intervening phenotype requires the study of participants with genetic risk for dependence but without significant exposure to alcohol, other substances of abuse, and psychoactive medications.

Another limitation is our present inability to defend a specific mechanism connecting *GRM8* gene polymorphisms to cellular changes and, in turn, to functional changes contributing to both impaired response inhibition and externalizing disorders. Such a theory is beyond the scope of current knowledge. Admittedly, there is a published investigation demonstrating statistically significant acute effects of glutamine on response selection and sequence learning in college students (Jongkees et al., 2017). There are also reports documenting effects of the glutamate receptor antagonist, acamprosate, on alcohol withdrawal (Boeijinga et al., 2004) and craving (Hammarberg et al., 2009) among alcohol-dependent patients. In addition, there is a large preclinical literature (Hayton et al., 2010). Unfortunately, to date, there is remarkably little information implicating altered glutamate neurotransmission in externalizing disorders other than Alcohol Dependence or in the motor or cognitive control problems experienced by individuals at-risk.

Proposing a specific mechanism involving the *GRM8* gene is premature for another reason. The reason relates to the growing evidence that no single gene polymorphism can sufficiently explain a complex phenotype such as an externalizing disorder or a diminished or highly variable No-Go P300 response. As we have already noted, externalizing disorders are also associated with other genes, including GABRA2 and CHRM2 among others. The challenge and likely solution to this complexity is to identify a reliable candidate, as we have done presently, and examine its interplay with other genes and brain function findings.

4.2 Conclusions

The findings of the present investigation suggest that there is merit in conducting tests of the reproducibility of genetic associations with alcohol misuse, regardless of the size and scope of the original study. There is no substitute for independent confirmation. Also, the present investigation revealed two new findings. The first noteworthy finding was the demonstration that *GRM8* SNP genotypes previously associated with Alcohol Dependence were also associated with Cocaine Dependence, Conduct Disorder, and Antisocial Personality Disorder. The other notable finding was an association between *GRM8* and greater

variability in neural activity across trials of a response inhibition task. It remains to be determined if studies of variability will prove to be more valuable than studies of average response amplitude for revealing group or quantitative risk score differences in future single gene or genome-wide association studies.

Funding Sources

This research was supported in part by PHS grants P50AA027055 and R01DA17666. The funding source had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the report; or the decision to submit this article for publication.

References

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(5):640–50. [PubMed: 16622805]

Athanasoulia AP, Sievers C, Uhr M, Ising M, Stalla GK, Schneider HJ. The effect of the ANKK1/DRD2 Taq1A polymorphism on weight changes of dopaminergic treatment in prolactinomas. Pituitary 2014;17(3):240–5. [PubMed: 23740147]

Batterham PJ, Bunce D, Mackinnon AJ, Christensen H. Intra-individual reaction time variability and all-cause mortality over 17 years: a community-based cohort study. Age Ageing 2014;43(1):84–90. [PubMed: 23934546]

Bauer LO. HIV/AIDS and an overweight body mass are associated with excessive intra-individual variability in response preparation. J Neurovirol 2018a;24(5):577–86. [PubMed: 29777461]

Bauer LO. Inter-trial variability in brain activity as an indicator of synergistic effects of HIV-1 and drug abuse. Drug Alcohol Depend 2018b;191:300–8. [PubMed: 30170301]

Bauer LO, Hesselbrock VM. CSD/BEM localization of P300 sources in adolescents “at-risk”: evidence of frontal cortex dysfunction in conduct disorder. Biol Psychiatry 2001;50(8):600–8. [PubMed: 11690595]

Bauer LO, Hesselbrock VM. Brain maturation and subtypes of conduct disorder: interactive effects on p300 amplitude and topography in male adolescents. J Am Acad Child Adolesc Psychiatry 2003;42(1):106–15. [PubMed: 12500083]

Bauer LO, Yang BZ, Houston RJ, Kranzler HR, Gelernter J. GABRA2 genotype, impulsivity, and body mass. Am J Addict 2012;21(5):404–10. [PubMed: 22882390]

Beck AT, Steer RA, Brown GK Beck Depression Inventory, Version II Manual. San Antonio, TX: Psychological Corporation/Harcourt Brace, 1996.

Beck LH, Bransome ED Jr., Mirsky AF, Rosvold HE, Sarason I. A continuous performance test of brain damage. J Consult Psychol 1956;20(5):343–50. [PubMed: 13367264]

Boeijinga PH, Parot P, Soufflet L, Landron F, Danel T, Gendre I, et al. Pharmacodynamic effects of acamprosate on markers of cerebral function in alcohol-dependent subjects administered as pretreatment and during alcohol abstinence. Neuropsychobiology 2004;50(1):71–7. [PubMed: 15179024]

Chen AC, Tang Y, Rangaswamy M, Wang JC, Almasy L, Foroud T, et al. Association of single nucleotide polymorphisms in a glutamate receptor gene (GRM8) with theta power of event-related oscillations and alcohol dependence. Am J Med Genet B Neuropsychiatr Genet 2009;150B(3):359–68. [PubMed: 18618593]

Ciraulo DA, Piechniczek-Buczek J, Iscan EN. Outcome predictors in substance use disorders. Psychiatr Clin North Am 2003;26(2):381–409. [PubMed: 12778840]

Derringer J, Krueger RF, Manz N, Porjesz B, Almasy L, Bookman E, et al. Nonreplication of an association of SGIP1 SNPs with alcohol dependence and resting theta EEG power. Psychiatr Genet 2011;21(5):265–6. [PubMed: 21317682]

Dick DM, Agrawal A, Wang JC, Hinrichs A, Bertelsen S, Bucholz KK, et al. Alcohol dependence with comorbid drug dependence: genetic and phenotypic associations suggest a more severe form of the

disorder with stronger genetic contribution to risk. Addiction 2007a;102(7):1131–9. [PubMed: 17567401]

Dick DM, Aliev F, Kramer J, Wang JC, Hinrichs A, Bertelsen S, et al. Association of CHRM2 with IQ: converging evidence for a gene influencing intelligence. Behav Genet 2007b;37(2):265–72. [PubMed: 17160701]

Dick DM, Aliev F, Wang JC, Grucza RA, Schuckit M, Kuperman S, et al. Using dimensional models of externalizing psychopathology to aid in gene identification. Arch Gen Psychiatry 2008;65(3):310–8. [PubMed: 18316677]

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(4):577–90. [PubMed: 16557364]

Duncan LE, Ostacher M, Ballon J. How genome-wide association studies (GWAS) made traditional candidate gene studies obsolete. Neuropsychopharmacology 2019;44(9):1518–23. [PubMed: 30982060]

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(4):705–14. [PubMed: 15024690]

Garrett DD, Samanez-Larkin GR, MacDonald SW, Lindenberger U, McIntosh AR, Grady CL. Moment to-moment brain signal variability: a next frontier in human brain mapping? Neurosci Biobehav Rev 2013;37(4):610–24. [PubMed: 23458776]

Gosso FM, de Geus EJ, Polderman TJ, Boomsma DI, Posthuma D, Heutink P. Exploring the functional role of the CHRM2 gene in human cognition: results from a dense genotyping and brain expression study. BMC Med Genet 2007;8:66. [PubMed: 17996044]

Graveson J, Bauermeister S, McKeown D, Bunce D. Intraindividual Reaction Time Variability, Falls, and Gait in Old Age: A Systematic Review. J Gerontol B Psychol Sci Soc Sci 2016;71(5):857–64. [PubMed: 25969471]

Hammarberg A, Jayaram-Lindstrom N, Beck O, Franck J, Reid MS. The effects of acamprosate on alcohol-cue reactivity and alcohol priming in dependent patients: a randomized controlled trial. Psychopharmacology (Berl) 2009;205(1):53–62. [PubMed: 19319508]

Hart AB, Kranzler HR. Alcohol Dependence Genetics: Lessons Learned From Genome-Wide Association Studies (GWAS) and Post-GWAS Analyses. Alcohol Clin Exp Res 2015;39(8):1312–27. [PubMed: 26110981]

Hasin D, Fenton MC, Skodol A, Krueger R, Keyes K, Geier T, et al. Personality disorders and the 3-year course of alcohol, drug, and nicotine use disorders. Arch Gen Psychiatry 2011;68(11):1158–67. [PubMed: 22065531]

Hayton SJ, Lovett-Barron M, Dumont EC, Olmstead MC. Target-specific encoding of response inhibition: increased contribution of AMPA to NMDA receptors at excitatory synapses in the prefrontal cortex. J Neurosci 2010;30(34):11493–500. [PubMed: 20739571]

Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict 1991;86(9):1119–27. [PubMed: 1932883]

Heron J, Maughan B, Dick DM, Kendler KS, Lewis G, Macleod J, et al. Conduct problem trajectories and alcohol use and misuse in mid to late adolescence. Drug Alcohol Depend 2013;133(1):100–7. [PubMed: 23787037]

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(9):922–8. [PubMed: 15351771]

Hill SY, O'Brien J. Psychological and Neurobiological Precursors of Alcohol Use Disorders in High Risk Youth. Curr Addict Rep 2015;2(2):104–13. [PubMed: 26301172]

Houston RJ, Bauer LO, Hesselbrock VM. Depression and familial risk for substance dependence: a P300 study of young women. Psychiatry Res 2003;124(1):49–62. [PubMed: 14511795]

Houston RJ, Bauer LO, Hesselbrock VM. P300 evidence of cognitive inflexibility in female adolescents at risk for recurrent depression. Prog Neuropsychopharmacol Biol Psychiatry 2004;28(3):529–36. [PubMed: 15093961]

Hultsch DF, Strauss E, Hunter MA, MacDonald SW. Intraindividual variability, cognition, and aging In: Craik IM, Salthouse TA, editors. The handbook of aging and cognition. 3rd ed. New York, NY: Psychology Press; 2008 p. 491–556.

Jones KA, Porjesz B, Almasy L, Bierut L, Dick D, Goate A, et al. A cholinergic receptor gene (CHRM2) affects event-related oscillations. Behav Genet 2006;36(5):627–39. [PubMed: 16823639]

Jones KA, Porjesz B, Almasy L, Bierut L, Goate A, Wang JC, et al. Linkage and linkage disequilibrium of evoked EEG oscillations with CHRM2 receptor gene polymorphisms: implications for human brain dynamics and cognition. Int J Psychophysiol 2004;53(2):75–90. [PubMed: 15210286]

Jongkees BJ, Immink MA, Colzato LS. Influences of glutamine administration on response selection and sequence learning: a randomized-controlled trial. Sci Rep 2017;7(1):2693. [PubMed: 28578427]

Kaufman AS, Kaufman NL Kaufman Brief Intelligence Test. Circle Pines, MN: American Guidance Services, 1990.

Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. Arch Gen Psychiatry 2003;60(9):929–37. [PubMed: 12963675]

Leyfer OT, Ruberg JL, Woodruff-Borden J. Examination of the utility of the Beck Anxiety Inventory and its factors as a screener for anxiety disorders. J Anxiety Disord 2006;20(4):444–58. [PubMed: 16005177]

Lieb R, Merikangas KR, Hofler M, Pfister H, Isensee B, Wittchen HU. Parental alcohol use disorders and alcohol use and disorders in offspring: a community study. Psychol Med 2002;32(1):63–78. [PubMed: 11883731]

Long EC, Aliev F, Wang JC, Edenberg HJ, Nurnberger J Jr., Hesselbrock V, et al. Further Analyses of Genetic Association Between GRM8 and Alcohol Dependence Symptoms Among Young Adults. J Stud Alcohol Drugs 2015;76(3):414–8. [PubMed: 25978827]

Luo X, Kranzler HR, Zuo L, Wang S, Blumberg HP, Gelernter J. CHRM2 gene predisposes to alcohol dependence, drug dependence and affective disorders: results from an extended case-control structured association study. Hum Mol Genet 2005;14(16):2421–34. [PubMed: 16000316]

Milne BJ, Caspi A, Harrington H, Poulton R, Rutter M, Moffitt TE. Predictive value of family history on severity of illness: the case for depression, anxiety, alcohol dependence, and drug dependence. Arch Gen Psychiatry 2009;66(7):738–47. [PubMed: 19581565]

Niswender CM, Conn PJ. Metabotropic glutamate receptors: physiology, pharmacology, and disease. Annu Rev Pharmacol Toxicol 2010;50:295–322. [PubMed: 20055706]

Ponce G, Hoenicka J, Jimenez-Arriero MA, Rodriguez-Jimenez R, Aragues M, Martin-Sune N, et al. DRD2 and ANKK1 genotype in alcohol-dependent patients with psychopathic traits: association and interaction study. Br J Psychiatry 2008;193(2):121–5. [PubMed: 18669994]

Rabbitt P, Osman P, Moore B, Stollery B. There are stable individual differences in performance variability, both from moment to moment and from day to day. Quart J Exper Psychol A, Hum Exp Psychol 2001;54(4):981–1003.

Rice JP, Reich T, Bucholz KK, Neuman RJ, Fishman R, Rochberg N, et al. Comparison of direct interview and family history diagnoses of alcohol dependence. Alcohol Clin Exp Res 1995;19(4):1018–23. [PubMed: 7485811]

Robins LN, Cottler LB, Bucholz KK, Compton WM, North CS, Rourke KM Diagnostic Interview Schedule for the DSM-IV (DIS-IV). St. Louis, MO: Washington University, 2002.

Salisbury DF, Griggs CB, Shenton ME, McCarley RW. The NoGo P300 ‘anteriorization’ effect and response inhibition. Clin Neurophysiol 2004;115(7):1550–8. [PubMed: 15203056]

Selzer ML. The Michigan alcoholism screening test: the quest for a new diagnostic instrument. Am J Psychiatry 1971;127(12):1653–8. [PubMed: 5565851]

Semlitsch HV, Anderer P, Schuster P, Presslich O. A solution for reliable and valid reduction of ocular artifacts, applied to the P300 ERP. Psychophysiology 1986;23(6):695–703. [PubMed: 3823345]

Skinner HA. The drug abuse screening test. Addict Behav 1982;7(4):363–71. [PubMed: 7183189]

Tajika A, Ogawa Y, Takeshima N, Hayasaka Y, Furukawa TA. Replication and contradiction of highly cited research papers in psychiatry: 10-year follow-up. Br J Psychiatry 2015;207(4):357–62. [PubMed: 26159600]

Tamm L, Narad ME, Antonini TN, O'Brien KM, Hawk LW Jr., Epstein JN. Reaction time variability in ADHD: a review. Neurotherapeutics 2012;9(3):500–8. [PubMed: 22930417]

Wang F, Simen A, Arias A, Lu QW, Zhang H. A large-scale meta-analysis of the association between the ANKK1/DRD2 Taq1A polymorphism and alcohol dependence. Hum Genet 2013;132(3):347–58. [PubMed: 23203481]

Wang JC, Hinrichs AL, Stock H, Budde J, Allen R, Bertelsen S, et al. Evidence of common and specific genetic effects: association of the muscarinic acetylcholine receptor M2 (CHRM2) gene with alcohol dependence and major depressive syndrome. Hum Mol Genet 2004;13(17):1903–11. [PubMed: 15229186]

Ward MF, Wender PH, Reimherr FW. The Wender Utah Rating Scale: an aid in the retrospective diagnosis of childhood attention deficit hyperactivity disorder. Am J Psychiatry 1993;150(6):885–90. [PubMed: 8494063]

Windle M, Windle RC. Parental Divorce and Family History of Alcohol Disorder: Associations with Young Adults' Alcohol Problems, Marijuana Use, and Interpersonal Relations. Alcohol Clin Exp Res 2018;42(6):1084–95. [PubMed: 29693716]

Wu CY, Wu YS, Lee JF, Huang SY, Yu L, Ko HC, et al. The association between DRD2/ANKK1, 5-HTTLPR gene, and specific personality trait on antisocial alcoholism among Han Chinese in Taiwan. Am J Med Genet B Neuropsychiatr Genet 2008;147B(4):447–53. [PubMed: 17948892]

Yang BZ, Kranzler HR, Zhao H, Gruen JR, Luo X, Gelernter J. Haplotypic variants in DRD2, ANKK1, TTC12, and NCAM1 are associated with comorbid alcohol and drug dependence. Alcohol Clin Exper Res 2008;32(12):2117–27. [PubMed: 18828801]

Significance:

The present study focuses on a metric and brain mechanism not typically considered or theorized in studies of patients with substance use disorders.

Highlights

- Previous studies have demonstrated an association between *GRM8* genotype and Alcohol Dependence.
- The present study revealed associations with several other externalizing disorders.
- The same *GRM8* genotype was associated with greater inter-trial variability in the No-Go P300 response.

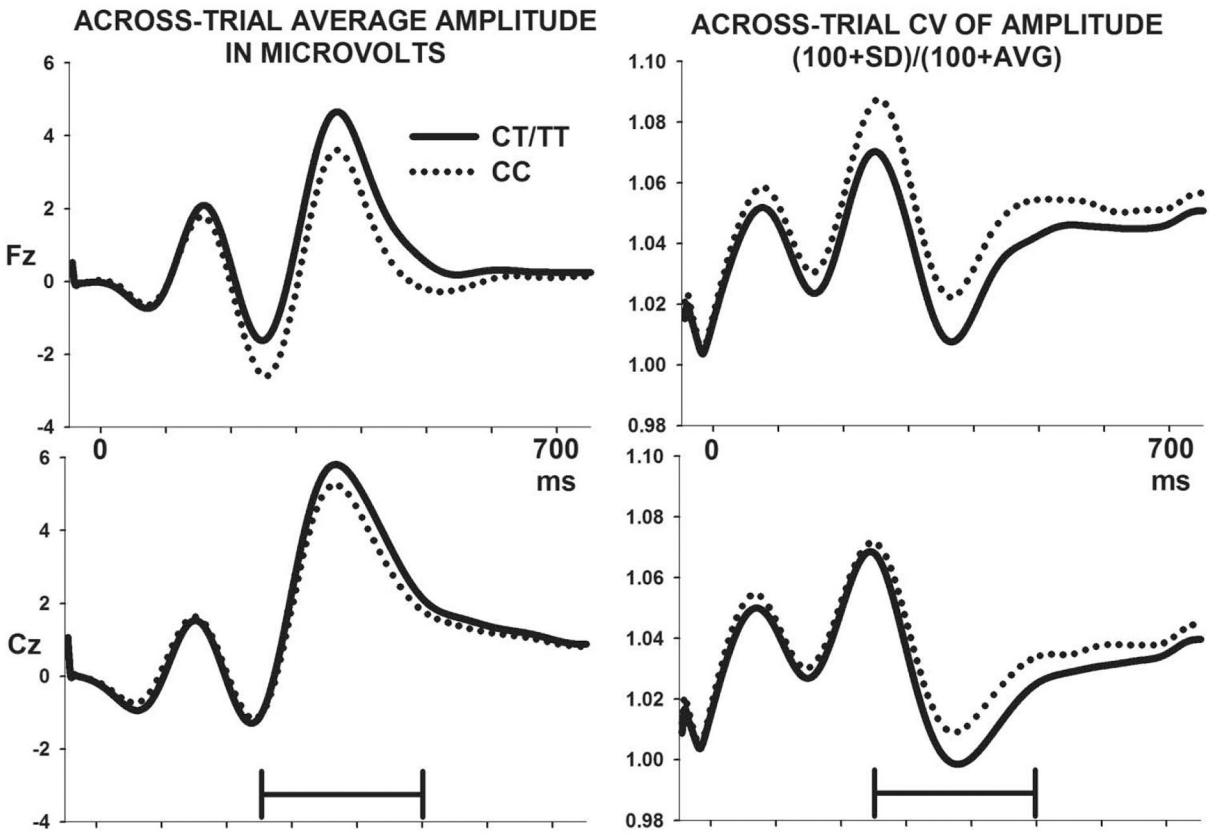

Figure 1.

Event-related EEG responses to No-Go stimuli recorded at Fz (top) and Cz (bottom) electrode sites for major allele homozygotes (CC) and minor allele carriers (CT/TT). The left panel of the figure shows the conventional summary in which responses are averaged across trials and voltage (in microvolts) is plotted relative to the average voltage during the pre-stimulus period. The right panel shows the variability across trials in response amplitude. The variability estimate was adjusted for the mean by the formula, (100+SD)/(100+AVG). Note in the figure that major allele homozygotes (dotted line) demonstrate greater inter-trial variability than minor allele carriers (solid line) within the 250–500 ms post-stimulus-onset window (hashmark) over which the data were summarized.

Table 1.

Background features by rs1361995 genotype

	Major Allele Homozygote (CC)	Minor Allele Carrier (CT/TT)
Age, yrs(SD)	36.9(9.4)	36.2(11.3)
% Female (n/total)	40% (20/50)	54.1% (39/72)
Education, yrs	13.1(1.9)	13.5(2.5)
KBIT Composite Standard Score	104.9(9.6)	103.8(12.4)
Wender Utah Rating Scale	79.3(37.0)	71.1(35.0)
Beck Depression Inventory-Version 2	11.7(9.0)	10.2(9.1)
Beck Anxiety Inventory	7.3(9.2)	7.2(6.6)
Fagerstrom Nicotine Dependence Scale	2.1(2.3)	1.5(1.9)
Michigan Alcoholism Screening Test *	8.9(6.9)	6.0(6.6)
Drug Abuse Screening Test *	12.9(7.4)	9.7(7.9)

* $p<0.02$

Table 2.

Associations of genotypes with diagnoses

	Major Allele Homozygote (CC)	**Minor Allele Carrier (CT/TT)**	**Unadjusted X^2**	**Logistic Regression Test Result (OR, 95% CI, and p-values adjusted for age and sex)**
No Alcohol Dependence Alcohol Dependence	33.7%(31) 63.3%(19)	66.3%(61) 36.6%(11)	X^2=8.2, p<0.01	OR=3.3, 1.3–8.1, p<0.01
No Cocaine Dependence Cocaine Dependence	27.2%(15) 52.2%(35)	72.8%(40) 47.8%(32)	X^2=7.7, p<0.01	OR=2.9, 1.3–6.6, p<0.01
No Conduct Disorder Conduct Disorder	30.6%(19) 51.7%(31)	69.4%(43) 48.3%(29)	X^2=5.5, p<0.01	OR=2.6, 1.2–5.6, p<0.02
No Antisocial Personality Disorder Antisocial Personality Disorder	33.3%(25) 53.2%(25)	66.7%(50) 46.8%(22)	X^2=4.7, p<0.03	OR=2.2, 1.1–5.2, p<0.05

Table 3.

No-Go P300 average amplitude, inter-trial variability in P300 amplitude, and task performance data [covariate-adjusted Mean(SE)]

	Major Allele Homozygote (CC)	Minor Allele Carrier (CT/TT)	Test result
Average amplitude in μV at Fz	1.41(0.23)	1.97(0.27)	F=1.79, p=0.18
Average amplitude in μV at Cz	2.95(0.26)	3.50(0.31)	F=0.70, p=0.40
CV of amplitude at Fz *	1.048(0.003)	1.034(0.003)	F=12.17, p<0.001
CV of amplitude at Cz *	1.030(0.003)	1.020(0.004)	F=4.37, p<0.04
Proportion of Go trials with correct response	0.97(0.006)	0.97(0.005)	F=0.02, p=0.86
Proportion of No-Go trials with incorrect response	0.16(0.013)	0.14(0.011)	F=1.41, p=0.23
Reaction time in ms	257(7.72)	270(6.40)	F=1.59, p=0.21

* p<0.05