

The Role of *GABRA2* in Risk for Conduct Disorder and Alcohol and Drug Dependence across Developmental Stages

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We use findings from the behavior genetics literature about how genetic factors (latently) influence alcohol dependence and related disorders to develop and test hypotheses about the risk associated with a specific gene, *GABRA2*, across different developmental stages. This gene has previously been associated with adult alcohol dependence in the Collaborative Study of the Genetics of Alcoholism (COGA) sample [Edenberg, H. J., Dick, D. M., Xuei, X., Tian, H., Almasy, L., Bauer, L. O., Crowe, R., Goate, A., Hesselbrock, V., Jones, K. A., Kwon, J., Li, T. K., Nurnberger Jr., J. I., O'Connor, S. J., Reich, T., Rice, J., Schuckit, M., Porjesz, B., Foroud, T., and Begleiter, H. (2004). *Am. J. Hum. Genet.* **74**:705–714] and other studies [Covault, J., Gelernter, J., Hesselbrock, V., Nellissery, M., and Kranzler, H. R. (2004). *Am. J. Med. Genet. B Neuropsychiatr. Genet.* **129B**:104–109; Lappalainen, J., Krupitsky, E., Remizov, M., Pchelina, S., Taraskina, A., Zvartau, E., Somberg, L. K., Covault, J., Kranzler, H. R., Krystal, J., and Gelernter, J. (2005). *Alcohol. Clin. Exp. Res.* **29**:493–498]. In a sample of children and adolescents ascertained as part of the COGA project, we find that *GABRA2* is significantly associated with childhood conduct disorder symptoms, but not with childhood alcohol dependence symptoms. A consistent elevation in risk for alcohol dependence associated with *GABRA2* is not evident until the mid-20s and then remains throughout adulthood. *GABRA2* is also associated with other drug dependence in our sample, both in adolescence and adulthood.

KEY WORDS: Alcohol; conduct disorder; dependence; drug; GABA; substance use.

INTRODUCTION

The field of behavior genetics has made great progress over the last several decades by convincingly demonstrating that genetic variation contributes to individual differences in virtually all behavioral domains (McGue and Bouchard, 1998; McGuffin *et al.*, 2001; Rose, 1995). Traditional behavior genetic analyses now have been expanded to address more complex and sophisticated questions about *how* genetic influences act. Areas currently being investigated include developmental changes in the nature and magnitude of genetic and environmental effects; the extent to which different behaviors are influenced

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by shared genes; and different forms of gene–environment correlation and interaction.

Parallel to the evolution of behavior genetics, advances in statistical and molecular genetics are making it possible to identify specific genes involved in behavioral predispositions. Most gene identification efforts to date have focused on specific disorders; however, as susceptibility genes are identified, the next step will be to characterize the risk associated with specific genetic variants, in order to better understand how these genes are involved in the pathways leading to illness. Substance use problems provide a particularly rich area of study in this respect, as their onset is rarely sudden, but rather, is usually preceded by a trajectory of risk-related behavior. This creates an exciting opportunity to use findings emerging from behavior genetics about how genetic influences act, to develop and test hypotheses about the developmental trajectories of risk associated with specific genes.

Twin studies suggest that there are several interesting developmental changes associated with genetic influences on alcohol use and dependence. The magnitude of importance of genetic effects appears to vary across stages of alcohol use. The initiation of alcohol use is largely influenced by environmental factors (Rhee *et al.*, 2003; Rose *et al.*, 2001b); however, as individuals progress from initial experimentation to more established, regular patterns of use, genetic influences become increasingly important. Data from the Finnish Twin Studies illustrate this dynamic change in the relative importance of genetic and common environmental influences on alcohol use across 14, 16, 17, and 18.5-year-olds. Genetic influences were negligible at 14 years, accounting for only 18% of the variance in drinking initiation at age 14, and this was significant only in girls (Rose *et al.*, 2001b). However, longitudinal data on drinking frequency across ages 16, 17, and 18.5 years demonstrate a steady increase in the relevance of genetic factors, with genes accounting for a third of the variation in drinking patterns in both sexes by age 16, and half of the variation by age 18 (Rose *et al.*, 2001a). Thus, alcohol initiation and use early in adolescence appears to be almost entirely influenced by family, school, and neighborhood influences; however, as drinking patterns develop, differentiate, and stabilize across adolescence, genetic factors assume increasing importance on drinking patterns (Rose *et al.*, 2003). This may in part reflect increasing independence from parents and family, providing opportunity for expression of genetic variation.

Alcohol dependence symptoms also appear to be influenced by different factors at different developmental stages. Although many studies have demonstrated that alcohol dependence in adult samples is significantly influenced by genetic factors (Heath *et al.*, 1997; McGue, 1999), alcohol dependence symptoms in early adolescence appear to be largely influenced by environmental factors. In *FinnTwin12*, 12% of the adolescents manifested some alcohol problems by age 14 (as indicated by the endorsement of DSM alcohol dependence symptoms); however, genetic analyses of alcohol dependence symptoms found no evidence of genetic effects in either males or females at age 14 (Rose *et al.*, 2004). Alcohol dependence symptoms were entirely environmentally influenced at this age. Data from the Missouri Adolescent Female Twin Study show a similar pattern of results, with alcohol dependence symptoms in adolescence largely influenced by environmental factors (Knopik, 2005). Lack of evidence for genetic influence on alcohol dependence in early adolescence also has been reported in the COGA sample (Kuperman *et al.*, 2001b). These findings may seem counter-intuitive, as early onset alcoholism is often cited as a more heritable form of the disorder (Cloninger *et al.*, 1981), as are early-onset cases of many disorders (e.g., breast cancer, Claus *et al.*, 1990); however, early onset alcoholism often refers to onset in the early 20s as compared to later in life, and the studies cited above showing little genetic influence refer to alcohol dependence symptoms in early adolescence.

Thus, one developmental change that can occur is that the magnitude of genetic influence on a trait may vary across time. Another developmental change involves genetic influences being expressed as different phenotypes at different developmental stages. Alcohol dependence has been shown to be highly comorbid with many psychiatric problems. Conduct disorder is one such related behavioral syndrome that is a robust predictor of both concurrent and future alcohol problems (Kuperman *et al.*, 2001a, b; Molina *et al.*, 2002; Moss and Lynch, 2001; White *et al.*, 2001). Twin studies have demonstrated that the correlation between adult alcohol dependence and childhood conduct disorder is due, in large part, to shared genetic factors (Kendler *et al.*, 2003; Slutske *et al.*, 1998). Further support for this idea can be found in a genome scan of retrospectively reported childhood conduct disorder that identified linkage to a chromosomal region (2p) that also showed linkage to adult alcohol dependence (Dick *et al.*, 2003).

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These findings are particularly interesting because childhood conduct disorder shows significant evidence of genetic influence (Goldstein *et al.*, 2001; Rose *et al.*, 2004; Simonoff *et al.*, 1998; Slutske *et al.*, 1997), in contrast to early adolescent alcohol symptoms. This, in combination with studies showing a shared genetic basis for conduct disorder and alcohol dependence, suggests that conduct disorder may be an adolescent manifestation of genes that later predispose to certain forms of adult alcohol dependence. And further, these studies suggest that genes impacting adult alcohol dependence may be more closely related to conduct disorder in adolescence than to early adolescent alcohol dependence symptoms, which appear to be largely caused by environmental factors, as reviewed above.

The recent identification of a gene influencing alcohol dependence in the Collaborative Study of the Genetics of Alcoholism (COGA) sample allowed us an opportunity to explore questions related to the risk associated with a specific genetic variant across development. In a 2004 report, Edenberg and colleagues reported that *GABRA2* was significantly associated with DSMIV alcohol dependence and a related EEG endophenotype, using a family-based association design in a sample of adult individuals (Edenberg *et al.*, 2004). Significant association ($p \leq 0.05$) was observed with 31 SNPs tested across *GABRA2*, and haplotype analyses were also highly significant. The association between *GABRA2* and alcohol dependence has subsequently been replicated by other independent research groups (Covault *et al.*, 2004; Lappalainen *et al.*, 2005).

In the current analyses, we use findings emerging from the behavior genetics literature about genetic influences (measured latently) to formulate developmental hypotheses about the effect associated with this specific gene identified as influencing adult alcohol dependence. In order to do this, we genotyped an additional sample of 860 children/adolescents, interviewed between the ages of 7 and 17 as part of the COGA project. We investigated a series of questions about the influence of *GABRA2* at different developmental stages. Based on the existing twin literature, we hypothesized that *GABRA2* would show a significant relationship with conduct disorder symptoms, but not with alcohol dependence symptoms, in the child/adolescent sample. Accordingly, we first tested the relationship between *GABRA2* and conduct disorder symptoms and alcohol dependence symptoms in our child/adolescent sample. Based on the twin literature suggesting that alcohol use and

dependence are more heritable as individuals move from adolescence to young adulthood, we hypothesized that the influence of the genotype on substance use would not emerge until later in adolescence/young adulthood. Thus, we conducted survival analyses in order to more thoroughly explore the influence of the genotype on substance use and dependence from childhood to young adulthood, a period that epidemiological studies suggest is particularly important in the development of substance use. Finally, the results from survival analyses of the influence of *GABRA2* across adolescence and into young adulthood led us to expand our analyses and examine survival curves of alcohol dependence across the lifespan, incorporating data from the new child sample reported here with the COGA adult sample. In addition to exploring the association between *GABRA2* and alcohol outcomes, we hypothesized that *GABRA2* may also be related to other drug dependence, as twin studies have found that alcohol and other drug dependence problems share common genetic factors (Kendler *et al.*, 2003), and previous data from COGA provide evidence of a common "addictive" factor for substance use transmitted in these families (Bierut *et al.*, 1998). Thus, illicit drug use and dependence were also included in survival analyses as outcomes of interest.

METHODS

Sample

The Collaborative Study on the Genetics of Alcoholism (COGA) is a multi-site project, in which families were collected at six centers across the United States: Indiana University, State University of New York Health Science Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St. Louis. Probands identified through inpatient or outpatient alcohol treatment programs at these six sites were invited to participate if they had a sufficiently large family (usually sibships > 3 with parents available) with two or more members in any COGA catchment area (Reich, 1996). The institutional review boards of all participating institutions approved the study. Multiplex alcoholic families that had at least two biological first degree relatives affected with alcohol dependence in addition to the proband were invited to participate in the more intensive stage of the study, which included obtaining blood for genetic analyses. Second and third

degree relatives in the families were assessed when they were considered to be informative for the genetic linkage studies. A total of 1227 families of alcohol dependent probands were recruited for the first stage of the study. Additionally, a sample of control families, obtained through random sources such as driver's license registries and dental clinics, was assessed. These families consisted of two parents and at least 3 children over the age of 14. Alcohol dependence and other psychiatric disorders were not exclusionary criteria for the control families. All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz *et al.*, 1994; Hesselbrock *et al.*, 1999). When possible, children between the ages of 7 and 17, from both proband and control families, were also assessed as part of the initial stage of the study. These children were interviewed using the child version of the SSAGA (available in two formats, for children aged 7–12 and 13–17, with subtle wording changes between the two versions to make the items more appropriate for each age group). When possible, a corroborative interview also was obtained from one of the child's parents (usually the mother) using the parent version of the SSAGA. Comparisons of self- and parent-interview reports of child/adolescent behavior in the COGA sample have suggested that children are more likely to report externalizing and substance use problems that go unreported by their parents (Fisher *et al.*, 2005, under review); accordingly, we have focused on child self-reports for the phenotypes analyzed in this paper. A ~5 year follow-up of family members has recently been completed of members from the severely affected families who originally participated in the study, and all children aged 7–17 years. Accordingly, a subset of the children analyzed here had follow-up data. Some of these children completed an adult interview if they were aged 18 or older at the time of follow-up.

Thus far, most genetic analyses in the COGA sample have been limited to the sample of ~2300 individuals from 262 families densely affected with alcohol dependence that were considered to be most informative for genetic linkage analyses. This was the sample used to detect association between *GABRA2* and alcohol dependence (Edenberg *et al.*, 2004). In order to test developmental hypotheses about the effect associated with this gene earlier in childhood, we genotyped additional individuals who were between the ages of 7 and 17 at the time of their initial interview. There were 860 individuals in

this age range with genotypic and phenotypic data available for analysis. Of these individuals, 201 (23%) came from control families, and the remainder came from proband families. There were 516 children who were interviewed twice: 228 children with child SSAGAs at both time-points, and 288 individuals who were interviewed first using the child SSAGA, and, upon follow-up, using the adult SSAGA. The mean age at the initial interview was 12.11, SD = 3.28. The mean age at follow-up for the 516 children who had a second interview was 17.75, SD = 3.56.

Outcome Measures and Statistical Analyses

All data processing and analyses were performed using the statistical analysis packages SAS version 8.0 (Institute, 2001) and STATA (StataCorp, 2003), version 8.1. STATA was used to conduct regression and survival analyses. SAS was used to produce the survival graphs using the output from STATA.

Regression Analyses of Childhood Conduct Disorder Symptoms and Alcohol Dependence Symptoms

Conduct disorder symptoms and alcohol dependence symptoms were assessed in the SSAGA according to DSMIII-R criteria (1987). As our first hypothesis dealt with the presence of conduct disorder symptoms and alcohol dependence symptoms in childhood, we used data from child SSAGA reports available at times 1 or 2. For children with child SSAGAs at both assessments, a summary variable was created for analysis that indicated the maximal symptom score reported at either time-point. We ran linear regression analyses in STATA on symptom counts, and we also dichotomized the variables according to thresholds of theoretical interest and ran logistic regression, in order to compute odds ratios and to provide an indication of the effect size associated with the genotype. We compared children who reported three or more conduct disorder symptoms, the threshold used for diagnoses, with those who reported less than three. We also compared children who reported at least one alcohol dependence symptom, as an indicator of the presence of some early alcohol problems, with those who reported none. STATA's robust variance estimation for clustered data was used to take into account the fact that some members of the sample were related family members. Age and sex were used as covariates in all analyses.

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Survival Analyses of Substance Use/Dependence

To more fully explore the development of substance use disorders across different developmental stages, survival analyses were conducted in STATA (Cleves *et al.*, 2004) to test for differences in failure events (e.g., onset of the disorder) as a function of genotype. Kaplan–Meier survivor functions were computed, and the equality of survival curves across individuals with different genotypes was tested using the logrank test (Mantel and Haenszel, 1959). Firstly, we were interested in testing the age span ranging from childhood to young adulthood using our COGA child sample. Accordingly, information from all available interviews was combined to span early childhood into young adulthood. This included data from follow-up adult SSAGAs for the subset of children who received an adult SSAGA at time 2. Thus, the survival analyses incorporate data from assessments made when the subjects were between the ages of 7 and 28. Due to the small number of individuals older than 25 at the second assessment (based on the assessment strategy of ~5 year follow-up and the requirement that all children in the sample analyzed here had a child SSAGA at time 1), individuals over age 25 are collapsed on the survival curves presented here. As before, when an individual had multiple interview assessments, a summary variable was created, incorporating information from all available data.

In the survival analyses spanning childhood to young adulthood, we examined several outcome variables related to the age of onset of regular use and dependence. These included: regular use of alcohol, age at first intoxication, and age at which the individual first tried various illicit drugs. Use/dependence of both marijuana and street drugs were assessed. The street drugs queried fell into five categories: cocaine, stimulants, sedatives, opioids, and “other”. Marijuana and all street drugs were combined for analysis. The definition of “regular use of alcohol” differed for children assessed at different times. At time 1, regular drinking was assessed in the child SSAGA as drinking once or twice a week for at least 2 months; for individuals who received a time 2 SSAGA, regular drinking was defined as drinking at least once a month for 6 months or more. Age of onset of DSMIII-R alcohol dependence and DSMIII-R drug dependence were analyzed. Dependence on marijuana and/or other street drugs were again combined for analysis of illicit drugs, as virtually all individuals who met

dependence criteria for street drugs also were dependent on marijuana, with a small number of additional individuals meeting criteria solely for marijuana dependence. Accordingly, results were virtually identical when marijuana and other drug dependence were analyzed separately (results available by request). Age of onset of dependence was assessed for individuals who surpassed the symptom threshold by asking those individuals the age at which they first experienced the specific symptoms they had endorsed.

Our initial analyses examining childhood to young adulthood led us to examine trajectories of substance dependence across the lifespan. For these analyses, we incorporated all available information on interviewed COGA participants with genotypic data, combining the child sample described above with adult COGA participants. The mean age of individuals from the adult sample was 40.69 (SD=15.21), range 18–91. There were 4014 individuals with genotypic and phenotypic data included in these joint analyses. These include individuals from both proband ($N=2876$) and control ($N=1138$, 28%) families. We examined the age of onset of DSMIII-R alcohol dependence and DSMIII-R drug dependence (marijuana or other illicit drugs). Because of the small number of individuals with an onset of substance dependence over age 60, these individuals are collapsed on the survival graphs shown here.

DNA Analyses

Details about SNP genotyping were described in Edenberg *et al.* (2004). Briefly, genotyping was conducted using a modified single nucleotide extension reaction, with allele detection by mass spectrometry (Sequenom MassArray system; Sequenom, San Diego, CA). All genotypic data were checked for Mendelian inheritance of marker alleles with the USERM13 (Boehnke, 1991) option of the MENDEL linkage computer programs, which was then used to estimate marker allele frequencies. The SNP with the single most significant association with alcohol dependence, rs279871, was used to represent the risk-increasing haplotype for *GABRA2* in the analyses presented here. At rs279871 the A allele is over-transmitted to individuals with alcohol dependence (and is referred to as the “risk-increasing allele” in this paper). This SNP was associated with DSMIV alcohol dependence at $p=0.0004$ (Edenberg *et al.*,

2004), and subsequent analyses suggest that this SNP is largely responsible for the significance of the haplotype reported in the paper.

Because it remains unknown how this gene is involved in the predisposition to alcohol dependence at a biological level, it was not clear, *a priori*, in what manner this genetic risk factor might act. This becomes a particular concern since power is limited to detect genes of small effect, furthering the need to minimize multiple testing. Accordingly, when we initiated follow-up analyses of *GABRA2* aimed at further characterization of risk, we conducted initial exploratory analyses for a small number of primary phenotypes comparing risk for alcohol dependence among individuals who carried 0, 1, or 2 copies of the A allele in the primary COGA sample. These analyses suggested that the risk associated with *GABRA2* was only evident among individuals homozygous for the A allele; thus, all subsequent analyses have compared individuals carrying 2 copies of the risk allele with those carrying 0 or 1 (Dick *et al.*, 2005, in press). We employed a parallel strategy here in the child/adolescent sample, testing whether there were distinct levels of risk associated with genotype, i.e., whether significant differences existed between individuals who carried 0 vs. 1, and 1 vs. 2 copies of the A allele, for a small number of key childhood behavioral and substance use outcomes. In the child dataset, there was no difference in outcome between individuals carrying 1 or 2 copies of the A allele (e.g., of individuals who carried no copies of the risk-increasing allele, 10% surpassed the conduct disorder threshold of 3+ symptoms; of individuals with 1 copy, 17% surpassed the threshold; and of the individuals with 2 copies, 18% did). Similarly, age of onset curves differed between individuals carrying 0 vs. 1 allele for drug dependence ($p=0.01$) and alcohol dependence ($p=0.08$)¹, but did not differ significantly between individuals carrying 1 vs. 2 alleles for drug dependence ($p=0.48$) or alcohol dependence ($p=0.25$).

In order to examine substance use outcomes across the lifespan, we combined data from the child sample and the adult sample. Because a different pattern of genetic effects had been detected in the child and adult sample, we conducted initial parallel analyses testing for differences between individuals carrying 0 vs. 1 and 1 vs. 2 alleles. In the combined sample, there was not a significant difference between

Table I. Number of Individuals by *GABRA2* Genotype in the Child/Adolescent Sample and the Combined Child/Adult Sample

Genotype ^a	Child sample	Combined sample
0	109	584 ^c
1	423 ^b	2009 ^c
2	328 ^b	1421
Total <i>N</i>	860	4014

^aNumber of copies of the A allele at the marker rs279871.

^bNo significant difference between these groups, combined for genetic analysis.

^cNo significant difference between these groups, combined for genetic analysis.

individuals carrying 0 vs. 1 copies of the A allele for drug dependence ($p=0.69$) or alcohol dependence ($p=0.08$)², but there were highly significant differences between individuals carrying 1 vs. 2 copies of the allele (drug dependence, $p=0.0003$; alcohol dependence, $p=0.0009$). The (different) patterns found in the child and adult/combined datasets were consistent across additional SNPs that also showed significant association with alcohol dependence in the adult sample. It was also consistent across COGA and control individuals. Accordingly, genotypes that did not differ significantly were grouped for all subsequent analyses, as reported in this paper and consistent with other papers conducting further phenotypic analyses of this gene (Dick *et al.*, 2005, in press). Table I shows the breakdown of individuals by genotype for the child and combined child/adult samples.

RESULTS

Regression Analyses of Childhood Conduct Disorder Symptoms and Alcohol Dependence Symptoms

The regression equation using the *GABRA2* genotype, age, and sex to predict number of childhood conduct disorder symptoms was highly significant ($F(3, 318) = 38.23, p < 0.001$). Total R^2 for the equation was 0.105, indicating that just over 10% of the variance in conduct disorder symptoms was accounted for by the *GABRA2* genotype, age, and sex. The regression coefficient for each of the variables was also significant. Table II shows the beta coefficients, standard errors (SE) and p -values for each of the variables included in the equation. *GABRA2* was

¹Although this p -value is not statistically significant at $p < 0.05$, there is a considerably smaller number of subjects ($N=532$, see Table I) used in this analysis.

²Although this p -value approaches significance, it appears to be due to the large number of subjects available for this analysis ($N=2593$, Table I).

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Table II. Results from Regression Analyses of Conduct Disorder Symptoms and Alcohol Dependence Symptoms in the Child Sample ($N=860$)

	Conduct disorder symptoms			Alcohol dependence symptoms		
	β	SE	p -value	β	SE	p -value
GABRA2 genotype (0 vs. 1, 2 copies)	0.30	0.16	0.05	0.09	0.08	0.28
Age	0.13	0.01	<0.001	0.10	0.01	<0.001
Sex	0.58	0.10	<0.001	-0.09	0.06	0.14

significantly related to conduct disorder symptoms ($p=0.05$); the change in R^2 associated with adding genotype to the model was 0.0055. Age and sex were highly significant ($p<0.001$). The model predicting children with 3+ symptoms was also highly significant (Wald $\chi^2(3)=53.33$, $p<0.001$). *GABRA2* was significantly related to conduct disorder outcomes of 3+ symptoms (OR=2.00, 95% CI=1.02–3.90, $p=0.04$). Only 10.00% of individuals with no copies of the risk-increasing allele at rs279871, in contrast to 17.47% of individuals who carried one or more copies of the risk-increasing allele, reported three or more conduct disorder symptoms.

The regression equation using the *GABRA2* genotype, age, and sex to predict alcohol dependence symptoms was highly significant, $F(3, 317)=s32.89$, $p<0.001$; however, inspection of the regression coefficients suggested that this was due to the highly significant relationship between age and alcohol dependence symptoms (See Table II). Neither *GABRA2* genotypes ($p=0.28$), nor sex ($p=0.14$), was significantly related to alcohol dependence symptoms in the child sample. In addition, there was no significant relationship between the genotype and reporting any (*versus* no) alcohol dependence symptoms (OR=1.31, 95% CI=0.66–2.51, $p=0.44$).

Survival Analyses of Substance Use Outcomes from Childhood to Young Adulthood

There was no significant difference in survival curves by *GABRA2* genotype for age of onset of regular alcohol use ($p=0.32$), age at first intoxication ($p=0.48$), or age at which individuals tried illicit drugs ($p=0.20$). The survival curves for age of onset of alcohol dependence did not differ significantly by genotype across this age range ($p=0.14$); however, individuals who were between ages 15 and 20 who were carrying at least one copy of the risk-increasing allele had a consistently elevated incidence of alcohol dependence (Fig. 1a). This difference only emerged

around age 15 and disappeared at age 21. There was a significant difference in survival curves for age of onset of illicit drug dependence by genotype ($p=0.02$, Fig. 1b), with individuals carrying one or more copies of the risk-increasing allele evidencing higher rates of drug dependence.

Survival Analyses of Substance Dependence Across the Lifespan

Figure 2 shows the survival curves for age of onset of alcohol dependence in the combined sample. There was a highly significant difference in the cumulative risk for alcohol dependence by genotype ($p=0.0035$, Fig. 2a); individuals with 2 copies of the risk-increasing allele had a higher cumulative incidence of alcohol dependence. Figure 3 shows the survival curves for age of onset of illicit drug dependence in the combined sample. Parallel to the results for alcohol dependence, there was a highly significant difference in the cumulative risk for illicit drug dependence by genotype ($p=0.0002$, Fig. 3a).

There was some concern that the genotype–phenotype relationships may be affected by the unique nature of the highly selected, densely affected COGA families. Accordingly, panel (b) in Figures 2 and 3 show the survival curves for the individuals from control families ($N=1138$) only. As evidenced in the figures, although the overall rates of dependence are lower in the control families, the curves show parallel trends in control families. The cumulative incidence of alcohol dependence differed significantly by genotype in the control sample (Fig. 2b, $p=0.013$), as did the incidence of drug dependence (Fig. 3b, $p=0.009$). Expectedly, the p -values were not as significant in this reduced sample; however, the difference in the cumulative failure function by genotype (i.e., the spread between the curves) was of a similar magnitude in the control sample, as compared to the combined sample, for both alcohol

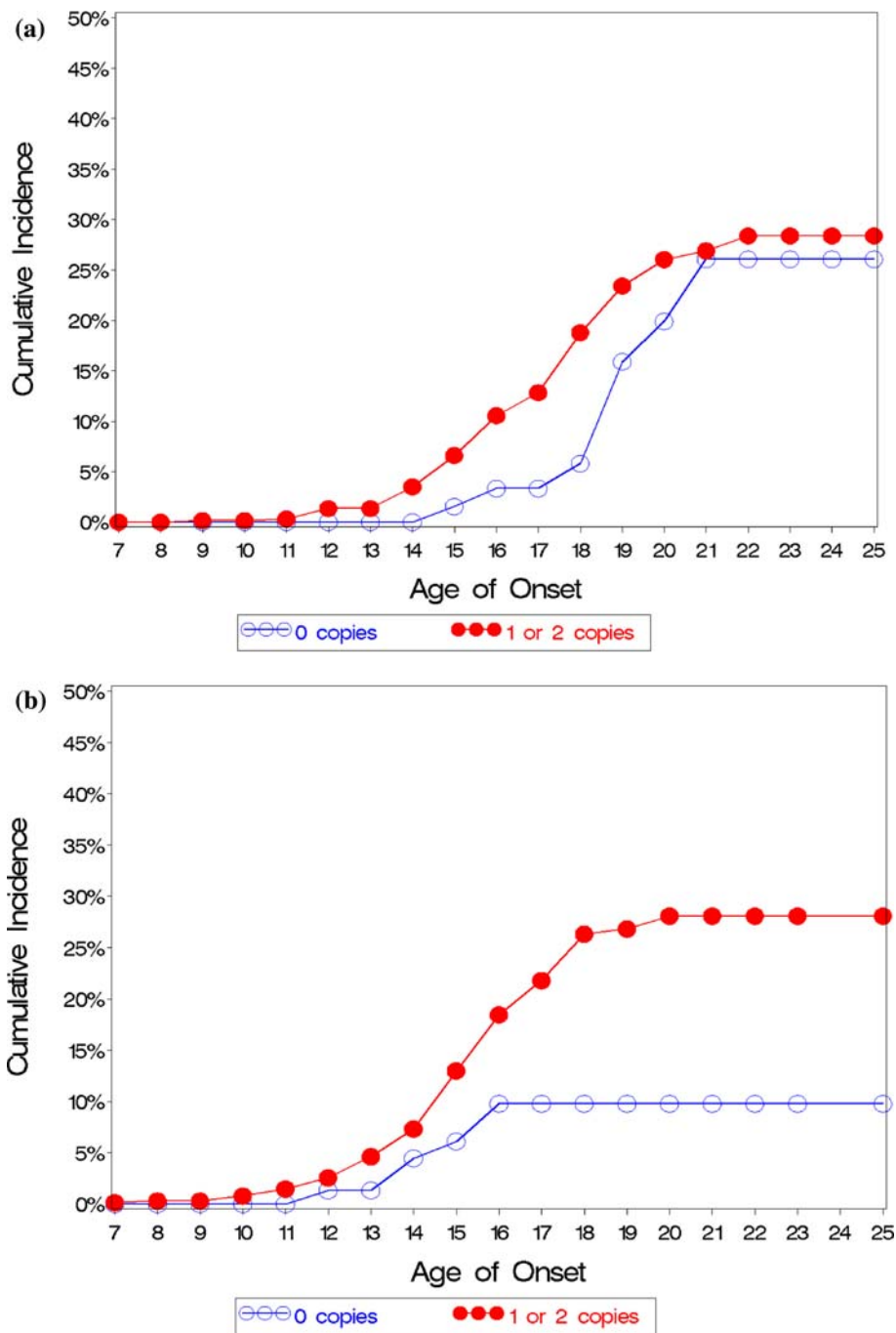


Fig. 1. Kaplan–Meier failure estimates for age of onset of (a) DSM-III-R Alcohol Dependence and (b) DSM-III-R Illicit Drug Dependence by GABRA2 genotype from childhood through young adulthood. Tests of group differences: (a) $p=0.14$, (b) $p=0.02$.

dependence (difference of 0.087 in controls vs. 0.076 in combined sample) and illicit drug dependence (difference of 0.050 in controls vs. 0.056 in combined sample).

DISCUSSION

The goal of this paper was to explore the risk associated with *GABRA2*, a gene that has previously

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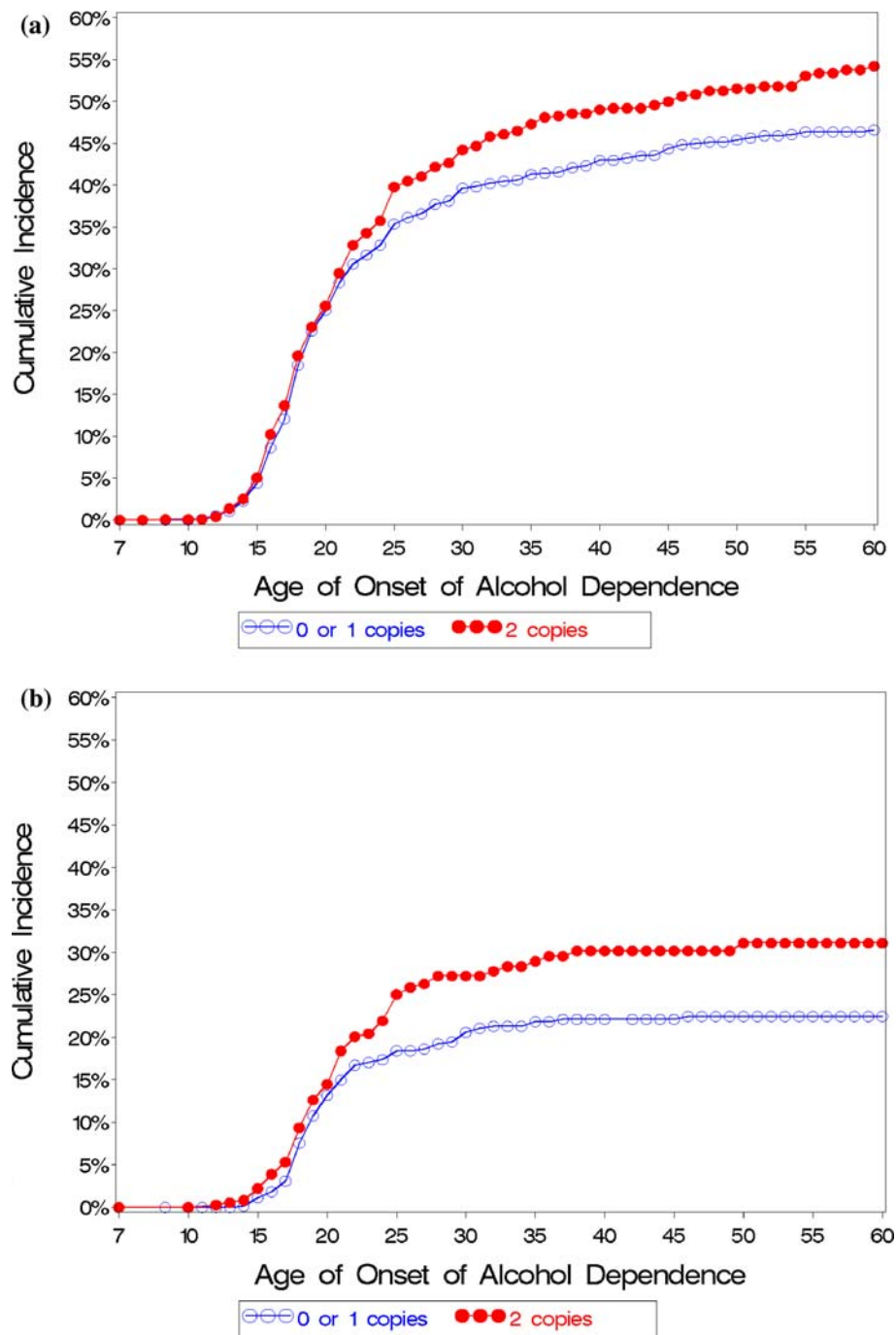


Fig. 2. Kaplan–Meier failure estimates for age of onset of DSM-III-R Alcohol Dependence across the lifespan for (a) the combined COGA/control sample and (b) individuals from control families only. Tests of group differences: (a) $p=0.0035$, (b) $p=0.013$.

been associated with adult alcohol dependence, across development. These analyses were guided by the behavior genetics literature suggesting that there are interesting developmental changes associated with genetic influences on alcohol use and

dependence. Namely, there has been suggestion that genetic factors influencing adult alcohol dependence are associated, at an earlier developmental stage, with childhood conduct disorder symptoms. Furthermore, the importance of genetic effects on alcohol use and

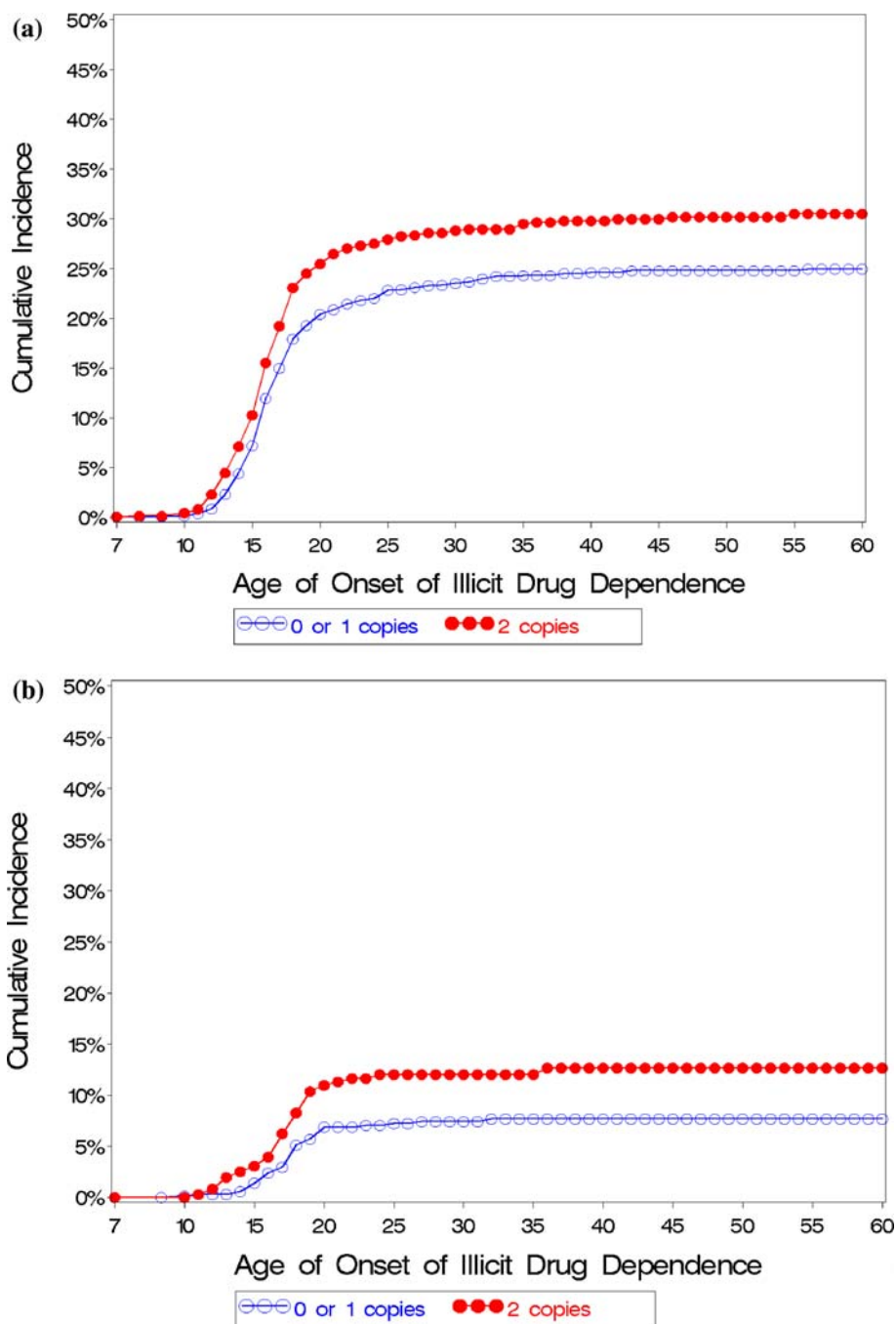


Fig. 3. Kaplan–Meier failure estimates for age of onset of DSM-IV Drug Dependence across the lifespan for (a) the combined COGA/control sample and (b) individuals from control families only. Tests of group differences: (a) $p = 0.0002$, (b) $p = 0.009$.

dependence symptoms has been shown to change across different developmental stages; specifically, genetic influences appear to increase in importance across adolescence and into young adulthood. Finally, because alcohol dependence and drug dependence have been demonstrated to share genetic

influences, we examined the possible role of *GABRA2* in the development of illicit drug use and dependence, in addition to alcohol dependence.

We found that *GABRA2* was associated with conduct disorder symptoms in a sample of children/adolescents, ranging in age from 7 to 17, supporting

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our initial hypothesis. Children carrying one or more copies of the risk allele that was overtransmitted to alcoholics in the adult sample had an increased number of conduct disorder symptoms. These children were twice as likely to surpass the threshold of 3 or more conduct disorder symptoms. It is interesting that the OR associated with *GABRA2* and childhood conduct disorder symptoms (OR = 2.0, 95% CI: 1.02–3.90) is larger than the OR associated with adult alcohol dependence in the COGA sample (OR = 1.40, 95% CI: 1.17–1.67) (Dick *et al.*, 2005, in press). However, we note that the CI for conduct disorder is broad in our child/adolescent sample, highlighting the large samples necessary to obtain sufficient numbers of affected children. Additionally, because our analyses were based on cross-sectional data, it is not possible to directly determine whether *GABRA2* has a stronger relationship with conduct disorder symptoms than with adult alcohol dependence. Longitudinal data will be necessary to better characterize these unfolding relationships and to elucidate the underlying pathways of risk. We note that in our adult COGA sample, we have also found a relationship between *GABRA2* and antisocial personality disorder (Dick *et al.*, 2005, in press). This does not appear to be attributable solely to comorbidity with alcohol dependence, as only ~25% of the alcohol dependent individuals meet criteria for antisocial personality disorder. In addition, the relationship between *GABRA2* and antisocial personality disorder is also observed among individuals who do not meet criteria for alcohol dependence (Dick *et al.*, 2005, in press). These findings may suggest that *GABRA2* contributes to a general predisposition toward disinhibited behavior, and that externalizing symptoms and substance use problems may be alternative manifestations of this predisposition. This would also be supported by the significant relationship observed between *GABRA2* and illicit drug dependence.

There was no significant association between *GABRA2* and alcohol dependence symptoms in the child/adolescent sample. However, inspection of the survival curves for age of onset of alcohol dependence across this age range was informative about the risk associated with the genotype. Few individuals (12% of those who had an onset of alcohol dependence in adolescence/young adulthood) had an onset of alcohol dependence prior to age 15. After age 15, the survival curves indicate that individuals with the risk-increasing genotype consistently had elevated rates of alcohol dependence through age 20. However, after age 21, there was a “catch-up” effect, whereby the

incidence of alcohol dependence among individuals carrying no copies of *GABRA2* very nearly approached that of individuals who did carry a copy. This pattern is interesting, as it suggests that *GABRA2* may be related to the onset of alcohol dependence, but this is not detectable until mid- to late-adolescence, and then the effect is obscured by a rise in the incidence of alcohol dependence that occurs as individuals reach the legal drinking age. It is possible that the removal of government sanctions against underage alcohol use acts as catalyst for increased alcohol use, which subsequently leads to a spike in the onset of problems around this age. Accordingly, laws regulating alcohol use may serve as an environmental influence that obscures differences associated with the genotype around this age. Interestingly, the elevation in incidence appeared to start after age 18, a time when many individuals are moving out of their childhood homes and beginning work or college. It is also of note that we found no evidence that the genotype was associated with patterns of initiation of alcohol/drug use (age at which regular alcohol use began, age at first intoxication, and age at trying illicit drugs). This is in line with twin studies suggesting that the initiation of substance use is largely influenced by environmental factors rather than genetic factors.

Because we knew from previous analyses that *GABRA2* was associated with alcohol dependence in our adult sample (Edenberg *et al.*, 2004), but our analyses of childhood/adolescence did not yield significant association between *GABRA2* and alcohol dependence, we combined the child and adult samples to obtain a more global picture of risk across the lifespan. As Figure 2 illustrates, there are not consistent, maintained differences in the incidence of alcohol dependence by genotype until after the mid-20s. In contrast, we found that *GABRA2* was associated with illicit drug dependence in both the adolescent and combined child/adult samples. Differences by genotype emerged around age 15 and persisted through adulthood. This may be because drug experimentation is a more deviant behavior and there is less normative experimentation. Thus, with drug use, there is no societal “rite of passage” and accompanying spike in use associated with reaching the legal age limit for use, as there is with alcohol.

We note that across phenotypes, an elevation in risk in the child/adolescent sample was observed among individuals who carried one or more copies of the risk allele at *GABRA2*; however, when

incorporating data from the adult sample, consistent elevation in risk *across the lifespan* is associated with being homozygous for the risk-increasing allele. The consistency of this finding across alcohol and illicit drug dependence may suggest that one copy of the risk allele at *GABRA2* is sufficient to increase risk for some deleterious outcomes in childhood/adolescence, but two copies are necessary before an elevation in risk is detectable in adults. This may be consistent with the genetic variant being a regulatory, rather than a coding, polymorphism, a hypothesis we have proposed previously (Edenberg *et al.*, 2004). Carrying one copy of the mutation may cause differences in regulatory elements involved in neurotransmitter pathways that are a sufficient perturbation such as to have detectable involvement in childhood outcomes; however, more severe differences in regulation (as might be associated with carrying 2 copies of the variant) may be necessary before association with adult clinical disorders can be detected. Although developmental changes in *GABRA2* expression are not well-characterized in humans, there is some work from animals suggesting that the level of expression of GABA-A receptor subunits (including alpha 2) is highest in early periods of development (Chen *et al.*, 2001). This would be consistent with fewer risk alleles necessary to impact behavior in childhood as compared to later in life. Alternatively, it is also plausible that this difference is a statistical artifact of our sample, and may be related to power issues associated with studying a gene of small effect. We believe that additional analyses of the gene in other samples will be necessary to definitively address this question.

There are several other limitations that should be discussed in relation to the current study. The first is that all analyses reported here have been based on genotypic risk characterized by a single SNP, rs279871, in *GABRA2* that is associated with adult alcohol dependence. Although genotypic differences at this SNP are correlated with differences in risk for alcohol dependence, it is unknown how *GABRA2* is involved in the biological alteration of susceptibility to alcohol dependence (and related phenotypes). Rs279871 is intronic; thus, we know that it is not a coding SNP, but we have no additional data on possible other functions. It is unclear whether this particular SNP is involved in susceptibility to substance dependence and related outcomes, or whether the SNP is in linkage disequilibrium with the actual variant in *GABRA2* that causes differences in susceptibility. More accurate characterization of risk by genotype will be possible once the specific mutation

in *GABRA2* has been discovered; however, the process from detection of association to discovery of the genetic mutation is often difficult and time-consuming. At this time, it is unknown how long it will take to discover the mutation in *GABRA2* (and other associated genes) and understand how it contributes to risk at a molecular level. We believe our analyses demonstrate that interesting characterization of risk can be performed with associated SNPs prior to the detection of the specific mutation. Nonetheless, our use of the terminology “risk-increasing” should be interpreted cautiously, and with the knowledge that rs279871 may not be the variant that causes increases in risk, but rather, may be correlated with the actual risk-related variant.

The analyses reported here were exploratory in nature and must be replicated in other samples. The majority of the individuals included in our analyses come from families densely affected with alcohol dependence. Thus, they are not representative of the general population. However, the observation of parallel trends among individuals in control families suggests these effects may apply more broadly. Incorporating genetic analyses of the sort reported here into on-going, population-based, longitudinal studies will be a powerful method to test hypotheses about the risk associated with particular genes across development. The analyses presented here were all cross-sectional, in order to make use of data from all available COGA participants; however, longitudinal data will be necessary to more accurately test developmental changes associated with genotypes, particularly to assess intra-individual change. Because substance dependence outcomes are rare in adolescence, large samples will be necessary to detect the small effects that are likely to be associated with any single gene. We note that despite an interesting trend in differences in the incidence of alcohol dependence by genotype in mid- to late-adolescence in our sample of 860 individuals, this difference did not reach statistical significance ($p=0.14$). Finally, we note some limitations associated with the assessment procedures used in COGA. The age of onset reports were made retrospectively and may include recall bias. Although these reports appear to be fairly reliable (e.g., $r=0.75$ for age of onset of alcohol dependence for individuals with two assessments), this likely varies by disorder severity and age of the informant. This may contribute to the different effects associated with carrying one or two copies of the risk-increasing allele in childhood/adolescence versus adulthood. It is possible that adult reports were made at a more distant

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time from the occurrence of the event; thus, more severe episodes may be recalled by adults, contributing to the fact that these phenotypes are associated with carrying more copies of the risk-increasing allele. Additionally, the lack of association with regular drinking may be due to the changing way in which this phenotype was assessed in individuals interviewed at different points in the study.

In conclusion, this paper illustrates the strategy of using findings emerging from the behavior genetics literature to develop hypotheses to test about the risk associated with specific genes. We find that *GABRA2* is significantly associated with childhood conduct disorder symptoms in a sample of children/adolescents, but not with alcohol dependence symptoms. There is suggestion that the genotype may influence alcohol dependence onsets between ages 15 and 20, but that this association is diminished in the early 20s, perhaps reflecting an influx of alcohol use and problems surrounding the removal of legal sanctions against its use. A consistent elevation in risk for alcohol dependence associated with *GABRA2* is evident after the mid-20s and appears to continue throughout the lifespan. *GABRA2* is also associated with other illicit drug dependence in our sample, both in adolescence and across the lifespan. These findings are consistent with several interesting findings that have emerged from twin studies aimed at characterizing the manner in which genetic influences (latently) impact the development of trajectories of alcohol use and dependence, and related behavioral disorders. We believe that using these findings to characterize the risk associated with *specific* genes that are beginning to be identified for many behavioral disorders has the potential to dramatically enhance our understanding of the development of substance use disorders and related problems.

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