Marital Status, Alcohol Dependence, and *GABRA2:* Evidence for Gene-Environment Correlation and Interaction*

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ABSTRACT. Objective: The gene *GABRA2* has been associated with the risk for alcohol dependence in independent samples. This article explores how this genetic risk factor interacts with marital status, another factor previously shown to be associated with the risk for alcohol dependence. **Method:** Data from more than 1,900 male and female subjects from the Collaborative Study of the Genetics of Alcoholism (COGA) sample were analyzed. Subjects were recruited based on membership in a family with multiple individuals with alcoholism. A series of analyses was performed to evaluate the relationship between the following: (1) *GABRA2* and alcohol dependence, (2) marital status and alcohol dependence, (3) *GABRA2* and marital status, and (4) interactions between *GABRA2* and marital status on the development of alcohol dependence in the high-risk COGA sample. Additional analyses were carried out in a sample of ~900 individuals from control families to test the generalizability of results. **Results:** Both *GABRA2* and marital status contributed independently to the development of alcohol dependence in the COGA sample. The high-risk genotype at *GABRA2* was also related to a decreased likelihood of marrying and an increased likelihood of divorce, which appeared to be mediated in part by personality characteristics. There was also differential risk associated with the *GABRA2* genotype according to marital status. **Conclusions:** These analyses provide evidence of both gene-environment correlation and gene-environment interaction associated with *GABRA2,* marital status, and alcohol dependence. They illustrate the complex pathways by which genotype and environmental risk factors act and interact to influence alcohol dependence and challenge traditional conceptualizations of "environmental" risk factors. (*J. Stud. Alcohol* **67:** 185-194, 2006)

DESPITE THE DIFFICULTIES INVOLVED in identifying genes influencing complex behavioral disorders, an increasing number of genes are now being identified as contributing to an individual's risk for alcohol dependence (Dick and Foroud, 2003). An important next step is to characterize the risk associated with identified genes. This must involve integrating new genetic findings with the epidemiological literature on environmental risk factors, in order to delineate how specific genes act and interact with other epidemiological risk factors. This will allow us to better understand the relative importance of various risk factors and to determine whether and how the importance of some risk factors may be moderated by the presence or absence of other risk factors. For example, we can examine whether the importance of a particular genetic risk factor varies according to environmental context. Studies on twins have demonstrated dramatic changes in the relative importance of genetic influences across environments. Factors such as regional residency (Dick et al., 2001; Rose et al., 2001), religiosity (Koopmans et al., 1999), and marital status (Heath et al., 1989) have been shown to moderate the impact of genetic influences on alcohol consumption. However, genetic factors have been measured latently in these studies, inferred via comparisons between monozygotic and dizygotic twins. More recently, it has been possible to demonstrate that individuals who carry specific genetic variants differ in their response to environmental stressors (Caspi et al., 2002; Caspi et al., 2003).

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The behavior genetics literature has also challenged traditional conceptualizations of the environment (Plomin and Daniels, 1987), suggesting that it is a mistake to assume that genetic and environmental effects are independent sources of influence. Much of an individual's environment is not random in nature; rather, genes can influence an individual's exposure to certain environments, how that individual experiences the environment, and the degree of influence that certain environments exert over the individual (Kendler, 1995; Rutter, 1997). This phenomenon has been termed "gene-environment correlation" and refers to the fact that an individual's environment can be influenced by his or her genetic predispositions (as reviewed in Dick, 2005).

These inter-relationships make it very difficult to tease apart genetic and environmental influences into independent risk factors, and it would be overly simplistic to assume that an individual's risk for developing alcohol dependence is simply the sum of their accumulated genetic and environmental risk factors. Accordingly, the goal of this article is to explore the relationships between a specific genetic risk factor that has recently been identified as contributing to the risk for alcohol dependence, *GABRA2,* and an epidemiological risk factor that has received considerable attention in relation to alcohol use and problems, marital status. An association between *GABRA2* and alcohol dependence was previously reported using family-based association analyses in the Collaborative Study on the Genetics of Alcoholism (COGA) sample (Edenberg et al., 2004) and has subsequently been replicated by independent groups (Covault et al., 2004; Lappalainen et al., 2005). Marital status has been consistently related to alcohol use in cross-sectional studies, with married individuals consuming less alcohol than single or divorced individuals (Power et al., 1999; Temple et al., 1991; Wang and El-Guebaly, 2004). There is evidence from longitudinal studies that decreases in drinking follow the act of getting married and that increases in drinking are found after a divorce (Curran et al., 1998; Power et al., 1999; Temple et al., 1991).

Here we report further analyses of the influence of *GABRA2* in the COGA sample, characterizing the risk associated with *GABRA2* and how this gene acts and interacts with marital status to contribute to the development of alcohol problems. Both *GABRA2* and marital status contribute independently to the development of alcohol dependence, and, interestingly, we find evidence for both gene-environment correlation and gene-environment interaction.

Method

Sample

COGA is a multisite project in which families were collected at six centers across the United States (see Reich, 1996). The institutional review boards of all participating institutions approved the study. Probands were identified through inpatient or outpatient alcohol treatment programs at each site. Multiplex alcoholic families that had at least two biological first-degree relatives with alcohol dependence in addition to the proband were invited to participate in the genetic study. 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 2,282 individuals from 262 multiplex alcoholic families were included in COGA's genetic analysis sample. Of the total sample, 83% of these families reported their race as white, 13% as black, and 4% as other descent. The mean (SD) age of the sample was 39.5 (14.8) (range: 17-91). The sample includes slightly more women (54%) than men, although men were more likely to be affected (46% vs 20%). The rate of alcohol dependence as defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV; American Psychiatric Association, 1994), among the genotyped individuals in the sample was 41%. The mean age at interview of the alcohol-dependent individuals was 37.1 (12.3) years, and the mean age at interview of the nonalcoholic individuals in the sample was 40.6 (15.7). The average age at onset of alcohol dependence per DSM-IV criteria was 23.6 (8.4). Table 1 shows the breakdown of individuals by gender, affection status, and marital status.

The exact number of individuals included in analyses varied as a function of missing genotypic or phenotypic data: 1,916 individuals from 261 independent families had complete data on the variables analyzed here and were included in the regression analyses testing for interaction between genotype and marital status in predicting alcohol dependence. The average family size in this sample was 7.34 individuals; 23% of the families consisted of 4 or fewer members, 39% consisted of 5-7 members, 23% consisted of 8-10 members, and 15% consisted of more than 10 members.

Additionally, a sample of 234 control families was assessed, in which each family consisted of two parents and at least three children over the age of 14. These families

TABLE 1. Age and percentage of individuals in the COGA sample, broken down by affection status (DSM-IV alcohol dependence), gender, and marital status

	Women		Men	
Variable	Unaffected	Affected (20%)	Unaffected	Affected (46%)
Age, mean (SD)	40.8 (15.3)	34.4 (10.5)	40.1 (16.2)	38.3 (13.0)
Currently married, % yes	62%	41%	60%	41%
Stable marriage, % yes	53%	30%	52%	32%

Notes: COGA = Collaborative Study of the Genetics of Alcoholism; DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition.

were obtained through random sources such as driver's license registries and dental clinics. Because the COGA families represent a unique, high-risk population, we used the control families to test the replicability of results in an unselected population. There were 915 individuals with genotypic and phenotypic information used in the analyses of controls reported here. Although this sample is considerably smaller in size than the COGA sample, it allowed us to test whether there were trends consistent with those found in the COGA sample.

Measures

All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz et al., 1994; Hesselbrock et al., 1999). For this study, lifetime diagnoses of alcohol dependence were made using DSM-IV criteria, as this was the phenotype associated with *GABRA2* in the original publication (Edenberg et al., 2004). All other disorders were diagnosed in the SSAGA-I using criteria from the DSM, Third Edition, Revised (DSM-III-R; American Psychiatric Association, 1987), as DSM-IV was under development at the time that interviewing began. Current marital status was assessed in the SSAGA by asking individuals whether they were presently married, widowed, separated, divorced, or never married. To assess the influence of having a current spouse, individuals who reported currently being married were compared with individuals whose responses fell in all of the other categories; this variable is referred to as "marital status." Individuals were also asked how many times they have been legally married. This item was combined with the current marital status question to create a marital index (referred to as "marital index") in which individuals were classified as never married, stably married (reported being currently married and only having one marriage), or married and subsequently divorced (hereafter referred to as "married and divorced"). Individuals who reported being separated were also classified in the latter category. Widowed individuals were omitted from the marital status index, as the death of one's spouse might affect the surviving spouse's alcohol consumption through processes that differ from the influence of marital status not related to loss. In the COGA sample, 47% of individuals reported that they were not currently married at the time of interview, and 53% reported being married at the time of interview. For the marital index, 26% of individuals reported never being married, 32% reported being married and divorced, and 42% reported one, stable marriage.

DNA analyses

Details about single nucleotide polymorphism (SNP) genotyping and association analyses with alcohol dependence were described in Edenberg et al. (2004). Briefly, SNPs were chosen across *GABRA2* from public databases. Locations were in most cases determined from the annotations in the National Center for Biotechnology Information human genome assembly. Genotyping was done 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. There were 49 SNPs genotyped in *GABRA2,* of which 31 showed significant association with alcohol dependence ($p < .05$) using the average Pedigree Disequilibrium Test (Martin et al., 2000). The associated SNPs formed a haplotype block toward the 3' end of the gene and were in high linkage disequilibrium. The SNP with the single most significant association with alcohol dependence, rs279871, was used to represent the high-risk haplotype for *GABRA2* in the analyses presented here. In our dataset, the A allele at rs279871 is overtransmitted to individuals with alcohol dependence. This SNP was associated with alcohol dependence as judged by DSM-IV criteria ($p = .0004$; Edenberg et al., 2004), and subsequent analyses suggest that this SNP is largely responsible for the significance of the haplotype reported in the article. Exploratory analyses in our dataset suggest that the increase in risk was associated with being homozygous for the risk allele; there was not a significant increase in risk associated with carrying a single copy of the A allele. Accordingly, all individuals were classified as to whether they were homozygous for the allele that conferred risk. Individuals who were homozygous for the A allele at rs279871 are referred to as carrying the high-risk genotype; 35% of genotyped individuals in the COGA sample carried the high-risk genotype. The remaining individuals, who were homozygous for the other variant at rs279871 or who were heterozygous, are referred to as carrying the low-risk genotype.

Statistical analyses

A series of analyses was performed to evaluate the relationship between the following: (1) *GABRA2* and alcohol dependence, (2) marital status and alcohol dependence, (3) *GABRA2* and marital status, and (4) interactions between *GABRA2* and marital status on the development of alcohol dependence. The individual and joint effects of covariates on outcome measures were analyzed using generalized linear mixed models (GLMM; Byrk and Raudenbush, 1992; Goldstein, 1995; Kreft and De Leeuw, 1988; McCullagh and Nelder, 1989). We chose to analyze the data using a regression-based approach because it is a flexible analytic strategy with multiple advantages, including the ability to

examine both categorical and continuous traits, to test multiple categorical or continuous explanatory variables, to obtain fit statistics for individual variables as well as the overall model, and to test hypotheses relevant to mediation and moderation. Advantages of studying genetic associations within the context of regression-based approaches have been detailed elsewhere (Waldman et al., 1999). Although family-based methods provide protection against possible population stratification effects (Spielman and Ewens, 1996), power is generally considerably reduced. Many traditional family-based tests, such as the Transmission Disequilibrium Test and its extensions, test for overtransmission of an allele from heterozygous parents to affected offspring, limiting information to families with a heterozygous parent. We used family-based methods to establish the original association between *GABRA2* and alcohol dependence (Edenberg et al., 2004) and thus feel comfortable that population stratification is not a significant factor in the association. However, to test more complex questions about interactive effects associated with the genotype, which are generally more difficult to detect than main effects, we chose to use regression-based tests that allow us to make use of all individuals in the sample (while also correcting for correlated family observations, as detailed below), rather than limiting information to the subset used in family-based analyses.

The link function in the GLMM depended on the distribution of the given outcome measure: logit links for binomial outcomes (i.e., alcohol dependence), identity links for normally distributed outcomes (i.e., dimensional personality scales), and log links for Poisson-distributed outcomes (i.e., alcohol symptom counts). For example, the effects of *GABRA2* and marital status on the risk of alcohol dependence were analyzed using univariate and multivariate logistic regression by employing logit links. Gene-environment interactions were further tested hierarchically by first including the main effects (e.g., marital status and *GABRA2*) in the GLMM and subsequently testing for the statistical interaction between those effects (van der Tweel and Schipper, 2004). Because the data from individuals in the same family were nested within that family, they were, in some way, correlated with each other and so could not be considered independent. This familial clustering was accounted for by the flexible covariance structure of the GLMM that incorporated a population-average component (assessing, for example, the average influence of a gene on the progression to alcohol dependence) as well as subject-specific components (i.e., modeling individual variation about this population average) through the use of random-effect terms. Different types of covariance structures (unstructured, variance components, and compound symmetry) were compared for each model using the likelihood-ratio test and the AIC goodness-of-fit test (Akaike, 1974). Compound symmetry most adequately described the data for all models and can be assumed for all models reported herein. All models were fit with the %GLIMMIX macro (Wolfinger and O'Connell, 1993), using SAS software, version 8 (SAS Institute Inc., Cary, NC). All p values were two-sided. Mean comparisons between groups for quantitative variables were made with t tests or analysis of variance (ANOVA), where appropriate. Associations between categorical variables were compared using chi-square tests.

Results

GABRA2 and alcohol dependence

In the overall COGA sample, 41% of individuals met criteria for lifetime alcohol dependence as defined by the DSM-IV. Among those individuals who carried the high-risk genotype, 47% met DSM-IV criteria for alcohol dependence in the COGA sample, compared with 39% of individuals who did not carry the high-risk genotype. Regression models demonstrated a significant association between the high-risk *GABRA2* genotype and alcohol dependence (odds ratio [OR] = 1.40, 95% confidence interval [CI]: 1.17-1.67, $p = .0003$).

Marital status and alcohol dependence

Marital status was significantly associated with alcohol dependence in the COGA sample. Of the total sample, 51% of individuals who reported that they were not currently married met criteria for alcohol dependence, whereas only 32% of individuals who were currently married met criteria for alcohol dependence. Alcohol dependence was strongly associated with being unmarried (OR = 2.16, 95% CI: 1.83-2.56, $p < .0001$). Table 2 shows the percentage of individuals who met DSM-IV criteria for alcohol dependence for the marital index (along with corresponding ORs), using data from all individuals with information on these variables in the COGA sample ($N = 2,127$). This relationship was also highly significant ($p < .0001$). A gradient of risk was observed whereby individuals who were stably married had the lowest rates of alcohol dependence, followed by individuals who reported being married and di-

TABLE 2. Number and percentage of individuals meeting DSM-IV alcohol-dependence diagnoses by marital status; associated ORs with 95% CIs are also shown

	Alcohol dependent				
	Yes		No		
Marital index	*n*	%	*n*	%	OR (95% CI)
Stable marriage	284	32	610	68	–
Married and divorced	304	45	376	55	1.54 (1.32-1.76)
Never married	307	56	246	45	2.67 (2.46-2.88)

Notes: DSM-IV = Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; OR = odds ratio; CI = confidence interval.

vorced. The highest risk was observed among individuals who had never been married.

As part of the SSAGA, individuals reported on the year of their marriages and divorces. Using self-reported information on year of birth, we were able to calculate their age at marriage and divorce and combine this with information about the age of onset of alcohol dependence to construct a timeline to better understand the relationship between these variables. Of the 601 individuals who reported being married and affected with alcohol dependence as defined by DSM-IV criteria, 49% had an onset of alcohol dependence before they were married, 45% were married before the onset of alcohol dependence, and 6% reported that both events occurred in the same year. Of these 601 individuals, 44% also reported that they had divorced. Of the 264 individuals who reported divorce, 71% reported an onset of alcohol dependence preceding their divorce, 25% reported divorce preceding alcohol dependence, and 3% reported the onset of these events in the same year. Thus, in the COGA sample, alcohol dependence was more likely to precede divorce than to occur after the divorce.

To further investigate the timeline between marital status and alcohol dependence in our sample, we examined follow-up data that were available on a subset of the COGA participants who completed interviews approximately 5 years after their initial interview. We compared rates of alcohol dependence at Time 1 and Time 2 between individuals who remained married over the two interviews (n = 88) and individuals who divorced between Time 1 and Time 2 (n = 606). Rates of alcohol dependence at Time 1 were higher among the group that subsequently divorced (35.2%) compared with the group that remained married (21.1%; p = .003). Rates of alcohol dependence increased in both groups at Time 2 (presumably due to new-onset cases), but the escalation was not considerably higher among the group that divorced (39.8%, an increase of 4.6%) compared with the group that stayed married (24.4%, an increase of 3.3%). In addition, we compared individuals who were unmarried at Time 1 and married at Time 2 (n = 77) with those individuals who remained unmarried (n = 230). Rates of alcohol dependence at Time 1 were higher among the group that remained unmarried (46.5%) compared with the group that subsequently married (33.8%, p = .05). Again, the increase in alcohol dependence at Time 2 was not disproportionate in one group compared with the other (52.6% among those who remained unmarried, an increase of 6.1%, compared with 40.3% among those who married, an increase of 6.5%). These findings suggest that in the COGA sample, differences in rates of alcohol dependence are more evident *prior* to a change in marital status. These results are in accord with a previous longitudinal study of twins that found that women who later divorced drank more than women who remained married, and their results suggested that this effect may be mediated by familial processes, as there was also higher alcohol consumption among identical twins whose co-twin had divorced (Prescott and Kendler, 2001).

GABRA2 and marital status

The *GABRA2* high-risk genotype was associated with current marital status as well as the marital index. Among individuals with the low-risk genotype, 54% reported being currently married, whereas only 48% of individuals with the high-risk genotype reported being currently married (χ^2 = 6.62, 1 df, p = .01). This association also existed for the marital stability index: Individuals with the high-risk genotype at *GABRA2* were more likely to report never being married and less likely to report stable marriages than individuals with a low-risk genotype (Table 3). Because *GABRA2* was also associated with alcohol dependence, it raised the possibility that the association between the genotype and marital status simply was a reflection of the association of *GABRA2* with alcohol dependence. To the extent that *GABRA2* influenced alcohol dependence and alcohol dependence led to marital problems and divorce, this may have caused the association between *GABRA2* and marital status. To minimize this possibility, we examined the association between *GABRA2* and marital status only among the individuals without alcohol dependence in the sample. Interestingly, the association between *GABRA2* and marital status was more prominent among nonalcoholic individuals. Among nonalcoholic individuals with the low-risk geno-

TABLE 3. Number and percentage of individuals within each genotype category of *GABRA2* by marital index

	COGA sample				Nonalcoholics in COGA				Control sample				Nonalcoholic controls			
	Low risk		High risk		Low risk		High risk		Low risk		High risk		Low risk		High risk	
Marital index	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Never married	310	25	204	31	135	18	93	27	227	37	122	43	191	34	107	42
Married and divorced	403	32	203	30	232	31	104	30	50	8	26	9	42	8	23	9
Stable marriage	536	43	260	39	388	51	153	44	341	55	139	48	326	58	127	49
	χ^2 = 7.47, 2 df, p = .02				χ^2 = 11.71, 2 df, p = .003				χ^2 = 3.59, 2 df, p = .17				χ^2 = 6.58, 2 df, p = .04			

Notes: The high-risk group includes individuals who were homozygous for the allele at rs279871 that was overtransmitted to alcohol-dependent individuals in family-based analyses (Edenberg et al., 2004). The low-risk group includes individuals who were heterozygous or homozygous for the other variant at rs279871. COGA = Collaborative Study of the Genetics of Alcoholism.

type, 63% reported that they were currently married, whereas only 53% of nonalcoholic individuals with the high-risk genotype reported currently being married (χ^2 = 10.21, 1 df, p = .001). The association between genotype and the marital stability index was also enhanced (Table 3).

To better understand the association between *GABRA2* and marital status among individuals without alcohol dependence, we tested whether the *GABRA2* high-risk genotype was associated with a variety of other variables among nonalcoholic individuals in our sample. We tested for differences in rates of other psychiatric disorders (anxiety disorders, other drug dependence, eating disorders, or lifetime major depressive episodes); differences in personality (Novelty Seeking, Harm Avoidance, Reward Dependence [RD], and Persistence, as measured by the Tridimensional Personality Questionnaire [TPQ]; Cloninger, 1987) and rates of diagnoses of antisocial personality disorder (ASPD), as assessed by the SSAGA; differences in Wechler Adult Intelligence Scale (WAIS) scores; and differences in alcohol use and problems (maximum number of drinks in a 24-hour period, number of symptoms of alcohol dependence as defined by DSM-IV criteria) that might be associated with the *GABRA2* genotype among non-alcohol-dependent individuals. We found that RD, as measured by the TPQ, was significantly lower among individuals with the high-risk genotype (mean = 12.95 [3.86]) as compared with individuals with the low-risk genotype (mean = 13.64 [3.38]; p = .004). In addition, the rates of ASPD were higher among individuals with the high-risk genotype (5.3%) as compared with individuals with the low-risk genotype (2.5%; χ^2 = 5.62, 1 df, p = .02). These were the only comparisons significant at $p < .05$. RD was also significantly associated with current marital status (12.90 vs 13.93 between individuals who were unmarried and married, respectively, $p <$ .0001), as was ASPD (6% vs 3% among unmarried and married individuals; $p < .0001$). These analyses suggest that RD and ASPD may mediate the relationship between *GABRA2* and marital status. We subsequently tested the effect of *GABRA2,* RD, and ASPD on current marital status in a regression analysis. The effect of *GABRA2* on current marital status remained significant after entering RD and ASPD into the equation, suggesting that the relationship between *GABRA2* and current marital status is not completely mediated by RD and ASPD (Baron and Kenny, 1986).

There is concern about the generalizability of these findings, because the COGA sample represents a highly selected sample of multiplex alcoholic pedigrees. Accordingly, even the unaffected individuals in these families are not representative of the general population in the sense that they are likely to have experienced disrupted family relationships and other environmental disadvantages associated with having alcohol-dependent family members, and these factors may be related to marital choices. Accordingly, we tested whether these findings replicated in the sample of control families also collected as part of the COGA project. Although the rates of marriage and divorce were very different in the control families, in part attributable to the selection strategy used to be part of the control sample, a parallel trend toward association between *GABRA2* and marital status was evident (though not significant in this smaller sample), with 58% of individuals with the low-risk genotype reporting being married and 53% of individuals with the high-risk genotype being married (χ^2 = 2.10, 1 df, p = .15). Furthermore, as in the COGA sample, this association was accentuated among the nonalcoholic individuals (n = 816), with 61% of individuals with the low-risk genotype being married compared with 54% of those with the high-risk genotype (χ^2 = 3.70, 1 df, p = .05). The percentages for the marital index in these control samples are included in Table 3. Finally, in the control sample, genotype was also significantly associated with ASPD (1.1% among those with the low-risk genotype, 3.5% among the high-risk genotype, p = .015), and ASPD was also associated with marital status (1.0% among married individuals, 3.2% among unmarried individuals, p = .01). There were no significant differences in RD by genotype or by marital status in the control sample.

GABRA2, marital status, and alcohol dependence

There was a significant interaction between *GABRA2,* alcohol dependence, and both marital indices in the COGA sample. Figure 1 shows the rates of alcohol dependence for individuals with high- and low-risk *GABRA2* genotypes, separately for individuals who reported that they were not currently married and individuals who were currently married. Among individuals who were not married, there was virtually no difference in rates of alcohol dependence by genotype (51% alcohol dependence among the low-risk genotype, 52% among the high-risk genotype). However, among individuals who were married, differences in rates of alcohol dependence by *GABRA2* were evident, with only 29% of individuals with the low-risk genotype meeting criteria for alcohol dependence, but 41% of individuals with the high-risk genotype meeting alcohol dependence criteria (OR = 1.71, 95% CI: 1.19-2.46). In the regression equation including genotype, marital status, and their interaction, both genotype (p = .003) and marital status ($p < .0001$) each had highly significant main effects, and the interaction was significant (p = .004).

Figure 2 shows the interaction with the marital stability index. This interaction was also significant (p = .016), and a gradient of risk was observed. There was no change in rates of alcohol dependence by genotype among individuals who were never married (56% alcohol dependence among individuals with the low-risk genotype, 54% alcohol dependence among individuals with the high-risk genotype),

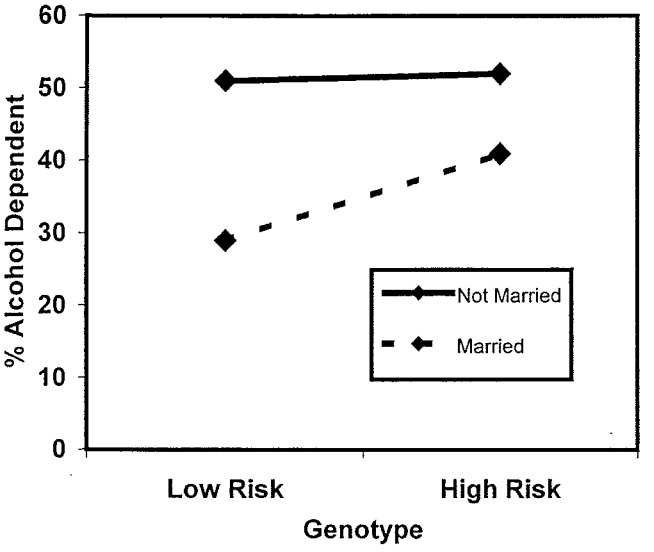

FIGURE 1. Rates of alcohol dependence as a function of genotype and current marital status

a modest association among individuals who had been married and divorced (42% vs 49% alcohol dependence among individuals with low- and high-risk genotype, respectively; OR = 1.39, 95% CI: 0.87-2.24), and a more pronounced relationship among individuals who reported a single stable marriage (28% vs 41% alcohol dependence among individuals with low- and high-risk genotype, respectively; OR = 1.98, 95% CI: 1.24-3.18).

The interactions between genotype and current marital status (p = .06) and genotype and the marital index (p = .09) also showed trends toward significance in the control sample. Similar to findings in the COGA sample, an elevation in risk associated with the genotype was only evident among individuals who were currently married (5.3% alcohol dependence among those with the low-risk genotype vs 9.2% alcohol dependence among the high-risk genotype) and who reported stable marital relationships (4.4% alcohol dependence among the low-risk genotype vs 8.6% alcohol dependence among the high-risk genotype).

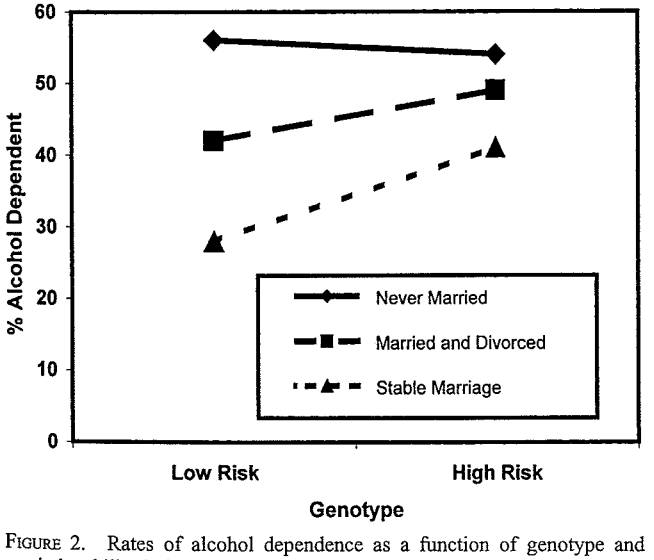

FIGURE 2. Rates of alcohol dependence as a function of genotype and marital stability index

Marital status is correlated with age; individuals who are not currently married and/or have never been married are significantly younger in our sample. The mean age of individuals who reported that they were not currently married was 36.3 (14.1), and the mean age of individuals who reported being presently married was 44.5 (14.3) ($p < .0001$). Similarly, individuals who reported never being married were significantly younger (mean = 28.4 [8.0]) than individuals who reported being married and divorced (mean = 43.3 [11.4]) and those who reported one stable marriage (mean = 44.4 [15.0]; $p < .0001$). Accordingly, we added age into the regression equation to verify that the interaction was not due to age artifacts. Age had a significant (p = .006) but small (β estimate = -.009) main effect when entered into the equation with genotype and current marital status. However, there was no significant interaction between genotype and age (p = .40), and the interaction between marital status and genotype remained significant (p = .003) even after controlling for age in the model. An identical pattern of results emerged for the marital index. As an additional precaution, we stratified the sample into age cohorts (30 years of age and below, 31-40 years, 41-55 years, and 56 years and older) and reran the associations between genotype and marital status. Although there were changes in the percentages of individuals who were married in each cohort (with higher rates of marriage with increasing age), across all cohorts there was an increased rate of being unmarried among those with the high-risk genotype (data available from the author upon request).

We also entered gender into the regression equations. There was a significant influence of gender on alcohol dependence, with males having higher rates of alcohol dependence than females ($p < .0001$), as previously described. However, there was no interaction between gender and current marital status (p = .62) or the marital stability index (p = .30). There was no interaction between gender and the *GABRA2* genotype (p = .42), and there was no evidence of any three-way interactions between *GABRA2*, gender, and current marital status (p = .33) or the marital stability index (p = .15). Furthermore, the interactions between *GABRA2* and marital status and marital stability remained significant after gender was entered into the models, suggesting that gender does not play a role in this relationship.

Discussion

These analyses were undertaken to further explore the relationships among the *GABRA2* genotype, marital rela-

tionships, and alcohol dependence. We conducted a series of analyses that systematically evaluated the relationship between the following: (1) *GABRA2* and alcohol dependence, (2) marital status and alcohol dependence, (3) *GABRA2* and marital status, and (4) interactions between *GABRA2* and marital status on the development of alcohol dependence. We found that both *GABRA2* and marital status were independently related to the development of alcohol dependence in the COGA sample. The OR associated with the high-risk genotype at *GABRA2* in the COGA sample was 1.4. Being unmarried, and being married and divorced, were also associated with the development of alcohol dependence. Perhaps most interestingly, our analyses suggest complex inter-relationships between *GABRA2,* marital status, and alcohol dependence: Our data provide evidence of gene-environment correlation between *GABRA2* and marital status and evidence of gene-environment interaction between *GABRA2* and marital status in the development of alcohol dependence. These findings are present in our high-risk COGA sample, and they appear to generalize to an independent sample of control families.

Although gene-environment correlation has been widely discussed in the literature on twins, to our knowledge, this is the first documentation of a specific gene being associated with exposure to a particular environment. Individuals who carry the high-risk genotype at *GABRA2* are more likely never to have been married and more likely to experience marital instability (multiple marriages and/or divorce) than individuals with the low-risk genotype. Furthermore, this gene-environment correlation does not appear to result solely from the association between *GABRA2* and alcohol dependence. Among individuals without alcohol dependence, *GABRA2* is more significantly associated with marital status. Interestingly, even among individuals who have not developed alcohol dependence, *GABRA2* is associated with higher rates of ASPD and lower rates of RD (a trait characterized by items such as "I like to please other people as much as I can"; Cloninger, 2004). Although the association with RD did not replicate in the control sample, the associations between *GABRA2* and marital status, and with ASPD, were significant. These analyses suggest that, in addition to its influence on alcohol use, *GABRA2* also influences personality characteristics that contribute to the likelihood of getting and staying married. These findings serve as a reminder to avoid simple conceptualizations of "environmental" risk factors and challenge us to develop more complex etiological models that take into account gene-environment interplay.

Interestingly, the risk of alcohol dependence associated with the *GABRA2* genotype varies as a function of marital status in our sample. Among individuals who report that they are not currently married and/or have never been married, the influence of *GABRA2* on alcohol dependence is not detectable. However, among individuals who report a stable marriage, the influence of *GABRA2* is significant, and the OR between *GABRA2* and alcohol dependence is elevated among this group of individuals compared with the overall sample. This effect is likely influenced by the main effect observed between marital relationships and alcohol dependence. Rates of alcohol dependence are significantly elevated among individuals who have never been married or who have married and divorced: 56% of individuals who have never married in our high-risk COGA sample meet criteria for alcohol dependence. Thus, the rates of alcohol dependence among this group appear to be so high that the association between the *GABRA2* gene and alcohol dependence is no longer detectable. This may be due to other potent environmental risk factors, other genetic risk factors, or both that are operating in this group of individuals and that mask any influence of *GABRA2.* Among individuals who report only one, stable marriage, the overall rate of alcohol dependence is lower, and susceptibility associated with *GABRA2* and alcohol dependence is detectable. However, it is interesting to note that the risk associated with the high-risk genotype at *GABRA2* in the "low-risk environment" of being married is still considerably lower than the rate of alcohol dependence among individuals with the low-risk genotype who are in the "high-risk environment" of being unmarried. These analyses demonstrate that the risk associated with the *GABRA2* genotype for alcohol dependence varies as a function of the marital environment in our study; this was true both in the high-risk COGA sample and in the control sample.

In conclusion, this article builds upon previously published reports (Edenberg et al., 2004; Prescott and Kendler, 1996) and further details the relationship between *GABRA2* and alcohol dependence, and marital status and alcohol dependence. We find that *GABRA2* is associated with marital status, even after taking into account the relationship between *GABRA2* and alcohol dependence. This association appears to be mediated, in part, by the influence of the genotype on personality; individuals with the high-risk *GABRA2* genotype, but who are not alcoholic, are more likely to have increased rates of ASPD. This provides an interesting example of gene-environment correlation. In addition, the risk associated with *GABRA2* on alcohol dependence appears to vary according to marital status: There is no significant association of the genotype with alcohol dependence among individuals who are unmarried in our sample; the risk for alcohol dependence may be already so high among this group that the effect of this one particular gene cannot be detected. These analyses illustrate the complex pathways by which genotype and environmental risk factors likely act and interact to influence alcohol dependence. As we continue to identify genes contributing to the susceptibility for alcohol dependence, we will be able to better explore these kinds of complex interactive relationships.

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