

# Gene–Lifecourse Interaction for Alcohol Consumption in Adolescence and Young Adulthood: Five Monoamine Genes

Guang Guo,<sup>1\*</sup> Kirk Wilhelmsen,<sup>2</sup> and Nathan Hamilton<sup>1</sup>

<sup>1</sup>Carolina Population Center, University of North Carolina at Chapel Hill, North Carolina

<sup>2</sup>Department of Genetics and Neurology, University of North Carolina at Chapel Hill, North Carolina

**Association analysis has suggested that common sequence variants of genes that affect monoamine function can affect substance use and abuse. Demonstration of these associations has been inconsistent because of limited sample sizes and phenotype definition. Drawing on the life course perspective, we predicted a stronger association between the polymorphisms in *5HTT*, *DAT1*, *DRD4*, *DRD2*, and *MAOA* and alcohol consumption in young adulthood than adolescence. This analysis tested for the gene–lifecourse interaction for the frequency of alcohol consumption in a nationally representative non-alcohol-dependent sample of 2,466 individuals that were visited during adolescence and young adulthood for four times between 1994 and 2002. All five genes are significantly associated with the frequency of alcohol consumption, with the genotype effects ranging 7%–20% of the mean score of alcohol consumption and their *P* values being 0.014, 0.0003, 0.003, 0.007, 0.005, and 0.003, respectively. The association is only observed in the life stage of young adulthood and not in adolescence. This analysis has demonstrated the potential usefulness of the life course perspective in genetic studies of human behaviors such as alcohol consumption.**

© 2007 Wiley-Liss, Inc.

**KEY WORDS:** alcohol use; *DRD4*; *DRD2*; *5HTT*; *DAT1*; *MAOA*; life course

Please cite this article as follows: Guo G, Wilhelmsen K, Hamilton N. 2007. Gene–Lifecourse Interaction for Alcohol Consumption in Adolescence and Young Adulthood: Five Monoamine Genes. *Am J Med Genet Part B* 144B:417–423.

## INTRODUCTION

The life course approach attempts to understand human behavior in a social context that changes continually throughout one's life span [Elder, 1974, 1999, 1998]. The changing social context can result from either a regular life-cycle

transition such as leaving home and entering college or an irregular historical event such as the Great Depression. Peer influence is considered among the most important forces in shaping adolescents' behavior [Harris, 1995]. Adolescents spend twice as much of their time with peers outside the family as do they with parents [Brown, 1990]. Peer influence was often found critical for the initiation and maintenance of risk behavior such as substance use, drinking, and smoking [Billy et al., 1984; Billy and Udry, 1985; Yamaguchi and Kandel, 1987; Ennett and Bauman, 1994; Rose, 1998; Brown and Theobald, 1999]. The intensity of peer influence seems to change over the life course. The presence of strong peer influence in adolescence contrasts with relatively weakened peer influence in young adulthood. The critical importance of peer influence in adolescence suggests that genotype effects on alcohol use in adolescence may be less prominent than in young adulthood.

Twin studies have also lent support for the gene–lifecourse hypothesis. Twin studies carried out in Australia, Finland, the Netherlands, and the United States consistently demonstrate that the initiation of alcohol use in mid-adolescence is associated predominantly with environmental rather than genetic factors and that genetic influences on alcohol use are increasing in importance starting in late adolescence [Heath and Martin, 1988; Prescott et al., 1994; Koopmans and Boomsma, 1996; Rose et al., 1999, 2001].

Monoamine neurotransmitters are released by a small fraction of neurons that typically have processes that extend throughout the nervous system. Monoamines act on receptors through complex signal transduction processes to modulate the function of neurons both transiently and through long-term changes in gene expression. Many genes code for proteins that directly and indirectly play a role in monoamine neurotransmission, including synthetic enzymes, receptors, and transporters. The drugs that bind to some of these gene products modulate behavior and are frequently abused. Implicit in this work and for many other studies is the hypothesis that the polymorphisms in genes that play a role in monoamine neurotransmission could affect behavior including drinking. Table I lists five genes and one polymorphism for each of the five genes that have been previously implicated as affecting alcohol use [e.g., Noble et al., 1994; Hutchison et al., 2002; Limosin et al., 2004; Herman et al., 2005; Munafó et al., 2005] and other risky behaviors and that were genotyped for this study. These polymorphisms are thought to affect gene expression or be in disequilibrium with variation that affects gene expression [*DAT1*, Mill et al., 2002; *MAOA*, Sabol et al., 1998; *5HTT*, Lesch et al., 1996; *DRD2*, Noble et al., 1994].

The serotonin transporter (*5HTT*, locus symbol *SLC6A4*) moves released serotonin back into presynaptic terminals and is the site of action of selective serotonin reuptake inhibitors (SSRIs). The association of *5HTT* promoter VNTR alleles with alcohol and abuse of other drugs has been inconsistent [Dick and Foroud, 2003; Herman et al., 2003; Munafó et al., 2004; Hopfer et al., 2005; Nilsson et al., 2005]. The dopamine

Grant sponsor: NIH; Grant number: P01-HD31921; R03 HD042490-02; Grant sponsor: NSF; Grant number: SES-0210389; Grant sponsor: National Institute of Child Health and Human Development; Grant number: P01-HD31921.

\*Correspondence to: Guang Guo, CB#3210, University of North Carolina, Chapel Hill, NC 27599-3210.

E-mail: guang\_guo@unc.edu.

Received 4 October 2005; Accepted 21 March 2006

DOI 10.1002/ajmg.b.30340

TABLE I. Five Genetic Polymorphisms Analyzed in the Project

Gene	Description	Polymorphism	Association with alcohol-related and other behaviors	Selected references
<i>5HTT</i> (17q11.1.1-12)	Member of the 6 serotonin transporter family; limit level and duration of serotonin receptor activation	44 bp VNTR in 5' regulatory region	Association with alcohol use	Herman et al. [2003]; Munafó et al. [2005]
<i>DAT1</i> (5p15.3)	Mediates uptake of dopamine into neurons; site of action of methylphenidate	3'UTR 40bp VNTR	Association with alcohol use	Sander et al. [1997]; Schmidt et al. [1998]
<i>DRD4</i> (11p15.5)	Member of the D2 receptor family; inhibits adenylylate cyclase, decreases C-AMP and is inhibitory	Exon 3 48-bp VNTR	Association with ADHD and substance abuse	Paterson et al. [1999]; Grady et al. [2003]
<i>DRD2</i> (11q23)	Inhibits adenylyl cyclase, thereby downregulating cAMP	3' TaqIA	Association with alcohol use	Noble [2003]; Hopfer et al. [2005]
<i>MAOA-uVNTR</i> (Xp11.3-11.4)	Responsible for degradation of dopamine, serotonin, and norepinephrine	30 bp VNTR in 5' regulatory region	Association with alcohol use	Parsian et al. [2003]; Herman et al. [2005]

transporter (*DAT1* known by the locus symbol *SLC6A3*) acts to take released dopamine back up into presynaptic terminals and is inhibited by cocaine. Polymorphisms in this gene have been associated with alcohol withdrawal seizures or delirium [Sander et al., 1997] and may lead to more severe withdrawal symptoms in chronically intoxicated alcoholics [Schmidt et al., 1998]. The 48-base-pair exon-3 dopamine-D4-receptor (*DRD4*) polymorphism affects binding properties for ligands for the receptor [Van Tol et al., 1992]. The *DRD4* coding sequence has been extensively examined since the studies by Benjamin et al. [1996] and Ebstein et al. [1996] who reported the association with novelty seeking behavior. Dozens of papers have reported associations and non-replications with aspects of personality, psychiatric diseases [Lopez et al., 2005], attention deficit disorder [Faraone et al., 2001], and substance abuse [reviewed in Paterson et al., 1999]. The dopamine D2 receptor (*DRD2*) is a G-coupled receptor. Blum et al. [1990] reported an association between a 3' non-coding sequence polymorphism, called TaqIA (dbSNP ID rs112594) and alcoholism. Others have not been able to replicate these results [Gelernter et al., 1993; Buckland, 2001]. The X-linked Monoamine Oxidase A (*MAOA*) is an important enzyme in the monoamine degradation pathway. Several investigators have found suggestive associations with alcohol use disorders [Parsian et al., 1995, 2003; Samochowiec et al., 1999; Saito et al., 2002; Herman et al., 2005].

The goal of this study is to examine the genes by life-course interactions for alcohol use in adolescence and young adulthood, using the DNA sample (N = 2,466) of the National Longitudinal Study of Adolescent Health (Add Health). We hypothesize that the associations between the genetic variants and alcohol consumption tend to be stronger in young adulthood than in adolescence among the general or non-alcohol-dependent population. Figure 1 illustrates our hypothesis. The curves in Figure 1 describe the typical age pattern of alcohol consumption in adolescence and young adulthood in the United States [Kandel and Logan, 1984; Chen and Kandel, 1995]. The hypothesis of gene-lifecourse interaction suggests that the differential use of alcohol by genotype is likely to appear starting from early young adulthood.

## MATERIALS AND METHODS

### Subject

The data source for our analysis is the sibling sub-sample of about 2,466 participants in Add Health, a school-based study of the health-related behaviors of adolescents in grades 7–12 (aged 12–18) in 1994–1995 [Harris et al., 2003]. Add Health is

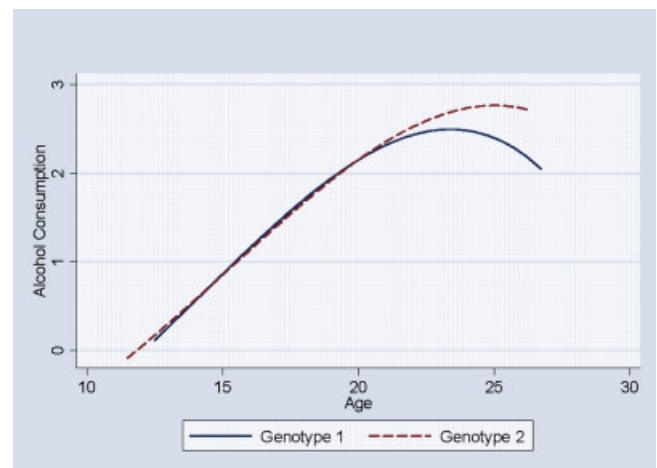


Fig. 1. Age pattern of alcohol consumption in adolescence and young adulthood by genotype.

based on a stratified sample of 132 high schools and middle schools in the United States; the schools were stratified by region, urban/rural, school type (public, private, parochial), ethnic mix, and size. The Add Health participants were interviewed four times during an 8-year period in 1994 (In-School), 1994–1995 (Wave I In-Home), 1995–1996 (Wave II In-Home), and 2001–2002 (Wave III In-Home). Data on alcohol consumption were collected on each of the four occasions. Our analysis uses the sibling sample of Add Health because DNA measures are currently only available for this subset of the Add Health respondents. Our sibling sample is composed of monozygotic twins, dizygotic twins, and full biological siblings.

A total of 2,466 individuals contributed 1–4 observations of alcohol consumption. Of the 2,466 individuals, 1,527 contributed one observation at the In-School Wave; 2,430, 2,298, and 2,434 contributed one observation at the Waves I, II, and III surveys, respectively. Of the 2,466 individuals, about 60%, 18%, 14.3%, and 7.6% are White, African, Hispanic, and Asian Americans, respectively. Our data have measures on alcohol use taken at ages from 13 to 26 covering the life stages of adolescence and young adulthood. The Add Health Study has received written informed consent from all participants and the institutional IRB at the University of North Carolina, Chapel Hill, has approved this study.

### Measures of Adolescent Alcohol Use

At the In-School as well as the three subsequent Home interviews, respondents were asked about their frequency of alcohol use. At the In-School interview, respondents were asked, “During the past 12 months, how often did you drink beer, wine, or liquor?” In the Home interviews, respondents answered the question, “During the past 12 months, on how many days did you drink alcohol?” In all Waves, respondents’ answers were recorded as 0, never; 1, 1 or 2 days in the past 12 months; 2, once a month or less; 3, 2 or 3 days a month; 4, 1 or 2 days a week; 5, 3 to 5 days a week; or 6, everyday or almost everyday. This seven-point scale is simplified in the analysis to a six-point scale in which categories 1 and 2 of the original measure are collapsed. Thus, the drinking score of an individual is recorded as 0, 1, 2, 3, 4, or 5 for never, once in a month or less, two to three times in a month, one to two times a week, three to five times a week, or almost everyday, respectively. At the In-School Wave, the responses were obtained via self-administered paper-and-pencil survey. To protect confidentiality and reduce non-responses, this section of the interview was self-administered by audio-CASI (Computer Assisted Self Interview) at the three Home interviews. The sensitive questions were read to respondents by means of audio headphones. Respondents were given instructions on how to complete their answers on the computer.

### DNA Preparation and Genotyping

At Wave III in 2002, in collaboration with the Institute for Behavioral Genetics (IBG) in Boulder, Colorado, Add Health collected, extracted, and quantified DNA samples from the sibling sub-sample. Genomic DNA was isolated from buccal cells using a modification of published methods [Lench et al., 1988; Meulenbelt et al., 1995; Spitz et al., 1996; Freeman et al., 1997]. All of the methods employed Applied Biosystem’s instruments and reagents. Microsatellite and VNTR polymorphisms were done using fluorescent primers that were analyzed on an ABI capillary electrophoresis instrument. Single nucleotide polymorphisms were analyzed using an ABI Sequence Detection System and 5'-nuclease (Taqman<sup>®</sup>) methodology. The additional details on DNA collection and genotyping are at Add Health website (Smolen and Hewitt, <http://www.cpc.unc.edu/projects/addhealth/>).

A series of Chi square tests for each polymorphism and for each self-reported ethnic group (European, African American, Hispanic, and Asian) reveals no deviation from the Hardy-Weinberg equilibrium.

### Analytical Strategies

To test the hypothesis of gene–lifecourse interaction for alcohol consumption in adolescence and young adulthood, a three-step analytical strategy is adopted. The first step is a contingency table analysis in which the means of alcohol consumption across genotypes and life stages (adolescence vs. young adulthood) were compared. The second step is a mixed regression model [Searle, 1971; Searle et al., 1992] that adjusts for race/ethnicity and gender and for correlation among siblings and repeated measures in the data. The third step addresses potential bias from population stratification.

In the rest of this section, we elaborate on the second and third steps. Our sample consists of twins and siblings as well as the repeated observations of the same individual over different Add Health Waves and these observations are not independent. The mixed model has long been established in the statistical literature for the analysis of data that are not independent. Several major statistical packages (e.g., SAS and STATA) include the mixed model as a standard procedure. The following equation describes the mixed model of alcohol consumption

$$\text{Alcohol}_{jit(s)} = \beta'X_{ji} + \beta_1\text{genotype}_{ji} + \beta_2\text{adult}_{jit} + \beta_3(\text{genotype}_{ji} \times \text{adult}_{jit}) + u_{j0(s)} + v_{ji} + e_{jit(s)} \quad (1)$$

where  $j$ ,  $i$ , and  $t$  index sibling cluster or pair, individual, and Add Health Waves, respectively;  $s = m, d, \text{ or } f$  indicates whether the sibling cluster or pair are MZ twins, DZ twins, or full biological siblings;  $adult$  is a dummy variable taking the value of 1 when the measure of alcohol consumption is taken at age 19 or older and the value of 0 when the measure is taken at age younger than 19; and  $(\text{genotype}_{ji} \times \text{adult}_{jit})$  is an interaction term between genotype and life stage. The size and significance level of the coefficient for the interaction term is a direct test of the gene–lifecourse hypothesis. The model allows the random effect at the sibling cluster level and the measure level to vary by type of sibling cluster because the strength of the correlation varies considerably by type of sibling clusters ( $s$ ). Conditional on the three random intercepts at the level of sibling clusters and one random intercept at the individual level, the siblings and repeated measures are assumed to be independent. The models were estimated by SAS [SAS Institute Inc, 1965–2005].

We used three strategies to address the potential impact of population structure. First, we adjusted for self-reported race/ethnicity in all regression analysis so that comparisons across genotypes are made within each race/ethnicity. Tang et al. [2005] showed a near-perfect correspondence between the four self-reported ethnic categories (European Americans, African Americans, East Asians, and Hispanics) and the categories determined by 326 microsatellite markers.

The second strategy addressed this concern by re-estimating the models after eliminating individuals who reported belonging to more than one racial category. In keeping with the new Census policy, Add Health respondents were allowed to mark as many ethnicity categories as they felt applied to them [Harris et al., 2003]. About 7.5%, 3.5%, and 0% of Black, Hispanic, and White participants marked more than one category, respectively. Of those who marked more than one ethnic category, the large majority (86%) marked two ethnic categories and 12% marked three ethnic categories.

As a third strategy, we applied Allison et al.'s [1999] procedure to test for possible population stratification. Following the idea used in the development of sibship tests of linkage and association [Curtis, 1997; Boehnke and Langefeld, 1998; Spielman and Ewens, 1998], Allison et al. reasoned that the probabilities of genotypes of siblings depended entirely on parental genotypes and that controlling for the effects of sibship would be equivalent to controlling for parental genotypes. Indexing sibships by  $j$ , individuals by  $k$ , and genotypes by  $i$ , they proposed a procedure that can be written as a mixed model

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk} \quad (2)$$

Where  $\alpha_i$ , or the effect of genotype  $i$  is assumed to be fixed;  $\beta_j$ , or the effect of sibship  $j$  is assumed to be random; and  $(\alpha\beta)_{ij}$  is an interaction term specifying the dependence of the random effect of sibship on genotype. The conditioning on sibship in the model eliminates the possible confounding of population stratification. This model is a special case of the mixed model [Searle, 1971; Searle et al., 1992].

**RESULTS**

**Contingency Table Analysis**

Table II shows the mean score of alcohol use by genotype and life stage. The two life stages of adolescence and young adulthood are defined as the age range of 13–18 and 19–26, respectively. Table II provides comparisons of mean alcohol consumption across genotypes within each life stage. The table also gives genotype frequency in proportion for each polymorphism examined and number of observations used for each comparison. Alcohol consumption appears to be associated with all the five monoamine genes considered in this analysis; however, the association consumption seems to mainly exist in young adulthood and not in adolescence.

In adolescence, of the six comparisons across genotypes, only the 3R allele of the *DRD4* gene seems to be associated with alcohol use (1.236 vs. 1.038). In young adulthood, the mean alcohol use seems to differ by genotype in all comparisons except the one concerning *MAOA* among the females. The comparison with regards to *MAOA* was carried out separately for males and females because of the X-linked *MAOA*. The genotype effect on alcohol consumption ranges from 0.104 for serotonin transporter (*5HTT*) to 0.414 for the dopamine D4 receptor (*DRD4*), representing about 5%–20% of the mean score of alcohol use. When a rare genotype was not the focus of comparison, it would be included in the category that was used as the reference category in the regression analysis. Because of

the sibling clustering and repeated observations in the data, standard  $t$  tests are not valid for these comparisons. In the next section, results are presented from significance tests conducted within the mixed-model framework that has taken into consideration the correlation in the data.

**Regression Analysis**

Table III presents the coefficients and their  $P$  values in eight separate regression models. Except for the first “No genes” model and the last “All genes” model, each of these models includes a main genotype effect and an interaction term between genotype and the dummy variable (age  $\geq 19$ ) for young adulthood. *MAOA* is again analyzed separately for males and females separately. The last “All genes” model includes simultaneously the main effects and the interaction effects of all the polymorphisms under consideration in this analysis except *MAOA*.

The “No genes” model shows that the alcohol consumption in the life stage of young adulthood is up 1.09 relative to that in adolescence, that the males tend to consume more alcohol than the females, and that African, Hispanic, and Asian Americans consume considerably less alcohol than White Americans. These non-gene effects generally hold in the “genetic” models. In the “*5HTT*” model, those with one or two copies of the short allele (Any S) do not differ ( $P = 0.22$ ) in alcohol consumption from those with the L/L (long/long) genotype in adolescence; in contrast, in young adulthood, those with the Any S genotype score 0.144 points higher than those with the L/L genotype ( $P = 0.014$ ). This pattern of gene–lifecourse interaction is observed in every polymorphism examined in this analysis.

In the “*DAT1*” model, only in young adulthood do individuals with the Any 9R genotype score significantly higher (0.206;  $P = 0.0003$ ) than individuals with the 10R/10R genotype. The “*DRD4*” model compares two relatively rare genotypes (Any 3R and 7R/7R) against the by-far the most frequent genotype of Any 4R; both Any 3R and 7R/7R consume more alcohol than Any 4R only in young adulthood (0.47,  $P = 0.003$ ; 0.46,  $P = 0.007$ , respectively). The models of “*DRD2*” and “*MAOA* (male)” reveal the same pattern of genotype effects. In the model of “*MAOA* (female),” Any 3R is not related to alcohol use in either adolescence or young adulthood. These regression results are remarkably consistent with those from the contingency table analysis in Table II. In the “All-genes” model in which the four polymorphisms are considered simultaneously, all the interaction coefficients and their  $P$  values remain similar to those in the models that consider only one gene at a time, suggesting little correlation among these genetic polymorphisms with respect to alcohol use. To test for

TABLE II. Comparing Mean Score of Alcohol Use Across Genotypes and Life Course Stages

Gene	Genotype	Genotype frequency	Mean (age 13–18)	Number of observations	Mean (age 19–26)	Number of observations
<i>5HTT</i>	Any S	0.66	1.052	3,942	2.184	1,835
	L/L	0.34	1.050	2,020	2.08	941
<i>DAT1</i>	Any 9R	0.38	1.036	2,256	2.269	1,051
	10R/10R	0.62	1.061	3,706	2.07	1,725
<i>DRD4</i>	7R/7R	0.043	1.004	265	2.306	121
	Any 3R	0.060	1.236	365	2.548	157
<i>DRD2</i>	Any 4R	0.83	1.038	4,960	2.134	2,318
	Any A1	0.45	1.055	2,664	2.07	1,266
	A2/A2	0.55	1.049	3,298	2.214	1,510
<i>MAOA (m)</i>	3R/others	0.41	1.166	1,125	2.367	545
	All else	0.59	1.122	1,649	2.54	779
<i>MAOA (f)</i>	Any 3R	0.60	0.98	1,894	1.864	889
	All else	0.40	0.96	1,295	1.837	563

TABLE III. Gene-Lifecourse Interaction for Alcohol Use in Adolescence and Young Adulthood: Regression Estimates Using Five Monoamine Genes

Predictors	No genes		5HTT		DAT1		DRD4		DRD2		MAOA (male)		MAOA (female)		All genes	
	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>	$\beta$	<i>P</i>
Intercept	1.087	0.0001	1.129	0.0001	1.125	0.0001	1.081	0.0001	1.051	0.0001	1.277	0.0001	1.082	0.0001	1.125	0.0001
Age $\geq 19$	1.095	0.0001	0.999	0.0001	1.017	0.0001	1.086	0.0001	1.663	0.0001	1.455	0.0001	0.859	0.0001	0.985	0.0001
Male	0.319	0.0001	0.320	0.0001	0.321	0.0001	0.319	0.0001	0.320	0.0001	0.320	0.0001	0.320	0.0001	0.322	0.0001
Black	-0.522	0.0001	-0.527	0.0001	-0.525	0.0001	-0.514	0.0001	-0.53	0.0001	-0.588	0.0018	-0.183	0.028	-0.520	0.0002
Hispanic	-0.251	0.0019	-0.25	0.002	-0.253	0.0017	-0.242	0.0026	-0.259	0.0016	-0.245	0.0612	-0.232	0.131	-0.259	0.0017
Asian	-0.657	0.0001	-0.654	0.0001	-0.663	0.0001	-0.646	0.0001	-0.668	0.0001	-0.609	0.0057	-0.742	0.027	-0.660	0.0001
Any S			-0.063	0.2215											-0.062	0.22
Any S * Age $\geq 19$			0.144	0.0145											0.145	0.013
Any 9R					-0.099	0.0464									-0.098	0.047
Any 9R * Age $\geq 19$					0.206	0.0003									0.198	0.0006
Any 3R							0.063	0.642							0.066	0.62
Any 3R * Age $\geq 19$							0.470	0.003							0.456	0.0040
7R/7R							-0.106	0.470							-0.107	0.46
7R/7R * Age $\geq 19$							0.461	0.007							0.466	0.0068
Any A1									0.086	0.082					0.084	0.091
Any A1 * Age $\geq 19$									-0.156	0.005					-0.151	0.0069
Any 3R											0.114	0.128	0.114	0.079		
Any 3R * Age $\geq 19$											-0.255	0.0039	0.0383	0.596		
-2 Log likelihood	30173		30167		30160		30159		30165		14652		15314		30131	
Number of persons	2,466		2,466		2,466		2,466		2,466		1,169		1,297		2,466	
Number of observations	8,739		8,739		8,739		8,739		8,739		4,098		4,641		8,739	

the gene-gene interactions more formally, we estimated a number of models containing the two-way gene-gene interactions. These tests yielded a possible interactive effect between the 7R/7R of *DRD4* and Any A1 of *DRD2* ( $P = 0.02$ ). Given the large number of tests carried out, however, we consider this result suggestive.

To address population stratification, we carried out the three strategies described earlier. Race/ethnicity was added as a control in all regression models estimated (Table III). The set of models were re-estimated after eliminating individuals who designated themselves as multi-racial/ethnic. The results remained essentially unchanged (data not shown). We estimated Allison models (1999) that include one random effect at the sibling level and a second random effect at the individual level plus the required interaction parameters; our results in these models do not differ substantively from those presented in Table III (data not shown).

## DISCUSSION

The contingency table analysis has yielded exploratory evidence for the gene-lifecourse interaction hypothesis from all the five polymorphisms examined. The frequency of alcohol consumption seems to differ by genotype primarily in the life stage of young adulthood, in which all the five genetic variants appear to have an effect on alcohol consumption with the genotype effects ranging 5%–20% of the mean alcohol consumption. The mixed model regression analysis further tests the hypothesis after adjusting for the effects of life stage, gender, and race/ethnicity and after taking into account the correlation among the siblings and repeated observations in the sample. The regression results have proved to be quite consistent with those from the analysis of contingency table. While none of the genetic variants are related to alcohol consumption in adolescence, all of them are associated with alcohol consumption in young adulthood, with the genotype effects of 0.144, 0.20, 0.47, 0.46, -0.15, and -0.25, respectively, ranging 7%–20% of the mean score of alcohol consumption. The corresponding *P* values are 0.014, 0.0003, 0.003, 0.007, 0.005, and 0.003, respectively. These results remain essentially unchanged when all the polymorphisms (except *MAOA*) are included in one single model, implying little correlation among them. The last 'all genes' model suggests that genotypes can make substantial differences in alcohol consumption because a single individual can possess a number of higher-risk alleles. The effect of these higher-risk alleles can add up.

All the five polymorphisms in the five monoamine genes have been previously implicated in alcohol-use related measures. The studies of Herman et al. [2003] and Munafó et al. [2005] are two of the few that have investigated the role of the *5HTT* gene in alcohol consumption in non-alcohol dependent populations. Herman et al. reported an association between the short form of the *5HTT* gene and elevated alcohol consumption in a general college student population (mean age = 19.1). Using a general adult population (aged 33–73), Munafó et al. showed that the short allele of the *5HTT* gene was significantly associated with increased alcohol consumption. This analysis demonstrates an increase in alcohol consumption among young adults with at least one copy of the short allele of the *5HTT* gene.

Two studies [Sander et al., 1997; Schmidt et al., 1998] reported a significant higher prevalence of the 9-repeat (9R) of the *DAT1* gene among alcoholics displaying withdrawal syndromes than among matched non-alcoholics. This analysis shows an approximate 10% increase ( $P = 0.0004$ ) in the score of alcohol consumption among young adults with one or two copies of 9R allele of the *DAT1* gene.

The in vitro studies suggest that the exon III *DRD4* 7-repeat allele (7R) has decreased affinity for dopamine and transmits

weaker intracellular signals in comparison to the most common 4R exon III allele [Asghari et al., 1995]. A number of studies have reported the association of the 7R of *DRD4* with novelty seeking behavior, attention deficit disorder, and substance abuse. This analysis finds an approximate 20% increase in alcohol consumption among young adults with either two copies of 7R or one copy of 3R of the *DRD4* gene ( $P = 0.007$  or  $0.003$ , respectively) relative to the 4R allele.

The *DRD2* gene has long been implicated in alcoholism [Blum et al., 1990]. A meta-analysis of the published studies using Caucasians showed a significantly higher prevalence in the *DRD2* A1 allele among alcoholics than among the controls [Noble, 2003]. This analysis uses the A2/A2 genotype as the reference category and finds that young adults with the A1/A2 or A1/A1 genotype are associated with decreased alcohol consumption (about 8% reduction,  $P = 0.006$ ). Hopfer et al. [2005], using Add Health Waves I–III data, reported a significant association between the usual number of drinks consumed per drinking episode (vs. our measure of drinking frequency) and the same *DRD2* polymorphism at Wave III, but not at Waves I–II. The respondents were aged 12–18, 13–19, and 19–26 at Waves I–III, respectively.

Previous association studies concerning *MAOA* have produced inconsistent results regarding the direction of the associations. Samochowiec et al. [1999] reported a higher prevalence of the 3R allele (the *MAOA* low activity allele) among dissociated alcoholics. Saito et al. [2002], based on a Finnish male sample of alcoholics, detected no association between alcoholics and the *MAOA* alleles. Parsian et al. [2003] found a higher frequency of the 3R allele in their controls than alcoholics. The findings in this analysis are consistent with Parsian et al.'s. This analysis uses a sample from the general population and detects a decrease in alcohol consumption among young adults for those with the 3R allele ( $P = 0.0039$ ).

Our study has a number of limitations. Like gender and ethnicity, life course is a proxy variable under which a host of other factors could be operating. We briefly discussed the difference in the intensity of peer influence between adolescence and young adulthood as a possible explanation for the gene–lifecourse interactions. Other factors such as the degree of parental control and mental maturity across the two life stages could be important. Peer influence may not be as straightforward as it appears to be. Individuals may, to various extents, select friends; they and their friends may share certain genetic background [Guo, 2006].

This study is only able to investigate one polymorphism in each of the five genes. An alternative explanation to these findings is that other functional variants within the five genes or in other genes on the same chromosome are in linkage disequilibrium with the genetic variants evaluated herein. However, it is biologically plausible that some of these genetic variants directly affect alcohol consumption. Because actual alcohol consumption is difficult to observe, this study relies on self-reported information, which can be biased or imprecise. This study is also limited because some of the genetic variants such as the 7R/7R and Any 3R of the *DRD4* gene are quite rare in spite of the reasonably large sample. It is possible that some of the findings related to the rare variants are attributable to chance. A follow-up study for the Add Health project (Wave IV of Add Health) is scheduled to collect DNA data from its entire sample of about 17,000 individuals, creating an opportunity to replicate these findings in an even much larger population-based study.

In conclusion, our analysis of the 2,466 individuals has produced unambiguous evidence supporting the gene–lifecourse interaction hypothesis. Our data show that all the five genes examined (*5HTT*, *DAT1*, *DRD4*, *DRD2*, and *MAOA*) are significantly associated with the frequency of alcohol

consumption; the association is only observed in the life stage of young adulthood and not in adolescence. Our analysis has demonstrated the potential usefulness of the life course perspective in genetic studies of human behaviors such as alcohol consumption.

## ACKNOWLEDGMENTS

This research uses data from Add Health, a program project designed by J. Richard Udry, Peter S. Bearman, and Kathleen Mullan Harris, and funded by a grant P01-HD31921 from the National Institute of Child Health and Human Development, with cooperative funding from 17 other agencies ([www.cpc.unc.edu/addhealth/contract.html](http://www.cpc.unc.edu/addhealth/contract.html)). Special acknowledgment is due to Andrew Smolen and John K. Hewitt of the Institute for Behavior Genetics, University of Colorado for DNA isolation and genotyping. We gratefully acknowledge supports from NIH, P01-HD31921 to Add Health; R03 HD042490-02 to Guang Guo; from NSF, SES-0210389 to Guang Guo.

## REFERENCES

- Allison DB, Heo M, Kaplan N, Martin ER. 1999. Development of sibling-based tests of linkage in the presence of association for quantitative traits that do not require parental information. *Am J Hum Genet* 64: 1754–1764.
- Asghari V, Sanyal S, et al. 1995. Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. *J Neurochem* 65:1157–1165.
- Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. 1996. Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. *Nat Genet* 12:81–84.
- Billy JOG, Udry RJ. 1985. The influence of male and female best friends on adolescent sexual behavior. *Adolescence* 20:21–32.
- Billy JOG, Rodgers JL, Udry RJ. 1984. Adolescent sexual behavior and friendship choice. *Social Forces* 62:653–678.
- Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, Nogami H, Briggs AH, Cohn JB. 1990. Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA* 263:2055–2060.
- Boehnke M, Langefeld CD. 1998. Genetic association mapping based on discordant sib pairs: The discordant-alleles test. *Am J Hum Genet* 62: 950–961.
- Brown BB. 1990. Peer Groups. In: Feldman S, Elliot G, editors. *At the threshold: The developing adolescent*. Cambridge: Harvard University Press. pp 171–196.
- Brown BB, Theobald W. 1999. How peers matter: A research synthesis of peer influences on adolescent pregnancy. In *Peer potential: Making the most of how teens influence each other*. Washington, DC: National Campaign to Prevent Teen Pregnancy.
- Buckland PR. 2001. Genetic association studies of alcoholism—problems with the candidate gene approach. *Alcohol Alcohol* 36:99–103.
- Chen K, Kandel DB. 1995. The natural history of drug use from adolescence to the mid-thirties in a general population sample. *Am J Public Health* 85:41–47.
- Curtis D. 1997. Use of siblings as controls in case-control association studies. *Ann Hum Genet* 61:319–333.
- Dick DM, Foroud T. 2003. Candidate genes for alcohol dependence: A review of genetic evidence from human studies. *Alcohol Clin Exp Res* 27:868–879.
- Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennett ER, Nemanov L, Katz M, Belmaker RH. 1996. Dopamine D4 receptor (*DRD4*) exon III polymorphism associated with the human personality trait of Novelty Seeking. *Nat Genet* 12:78–80.
- Elder GH Jr. 1974, 1999. *Children of the great depression: Social change in life experience*. Chicago: University of Chicago Press.
- Elder GH Jr. 1998. The life course and human development. In: Lerner RM, editor. *Handbook of child psychology, Volume 1: Theoretical models of human development*, 5th edition. New York: Wiley. pp 939–991.
- Ennett ST, Bauman KE. 1994. The contribution of influence and selection to adolescent peer group homogeneity: The case of adolescent cigarette smoking. *J Pers Soc Psychol* 67:653–663.

- Faraone SV, Doyle AE, Mick E, Biederman J. 2001. Meta-analysis of the association between the 7-repeat allele of the dopamine D(4) receptor gene and attention deficit hyperactivity disorder. *Am J Psychiatry* 158:1052–1057.
- Freeman B, Powell J, Ball D, Hill L, Craig I, Plowmin R. 1997. DNA by mail: An inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behavior Genet* 27:251–257.
- Gelernter J, Goldman D, Risch NJ. 1993. The A1 allele at the D2 dopamine receptor gene and alcoholism. A reappraisal. *JAMA* 269:1673–1673.
- Grady DL, Chi H-C, Ding Y-C, Smith M, Wang E, Schuck S, et al. 2003. High prevalence of rare dopamine receptor D4 alleles in children diagnosed with attention-deficit hyperactivity disorder. *Mol Psychiatry* 8:536–545.
- Guo G. 2006. Genetic similarity shared by best friends among adolescents. *Twin Res Hum Genet* 9:1–9.
- Harris JR. 1995. Where is the Child's Environment? A Group Socialization Theory of Development. *Psychol Rev* 102:458–489.
- Harris KM, Florey F, Tabor J, Bearman PS, Jones J, Udry JR. 2003. The National Longitudinal Study of Adolescent Health: Research Design [WWW document]. URL: <http://www.cpc.unc.edu/projects/addhealth/design>.
- Heath AC, Martin NG. 1988. Teenage alcohol use in the Australian Twin Register: Genetic and social determinants of starting to drink. *Alcohol Clin Exp Res* 12:735–741.
- Herman AI, Philbeck JW, Vasilopoulos NL, DePetrillo PB. 2003. Serotonin transporter promoter polymorphism and differences in alcohol consumption behaviour in a college student population. *Alcohol Alcohol* 38:446–449.
- Herman AI, Kaiss KM, Ma R, Philbeck JW, Hasan A, Dasti H, DePetrillo PB. 2005. Serotonin transporter promoter polymorphism and monoamine oxidase type A VNTR allelic variants together influence alcohol binge drinking risk in young women. *Am J Med Genet Part B* 133B:74–78.
- Hopfer CJ, Timberlake D, Haberstick B, Lessem JM, Ehringer MA, Smolen A, Hewitt JK. 2005. Genetic influences on quantity of alcohol consumed by adolescents and young adults. *Drug Alcohol Depend* 78:187–193.
- Hutchison KE, McGeary J, Smolen A, Bryan A, Swift RM. 2002. The DRD4 VNTR polymorphism moderates craving after alcohol consumption. *Health Psychol* 21:139–146.
- Kandel DB, Logan JA. 1984. Patterns of drug use from adolescence to young adulthood: I. Periods of risk for initiation, continued use, and discontinuation. *Am J Public Health* 74:660–666.
- Koopmans JR, Boomsma DI. 1996. Familial resemblances in alcohol use: Genetic or cultural transmission? *J Stud Alcohol* 57:19–28.
- Lench N, Stanier P, Williamson R. 1988. Simple non-invasive method to obtain DNA for gene analysis. *Lancet* 1988:1356–1358.
- Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL. 1996. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274:1527–1531.
- Limosin F, Loze J-Y, Boni C, Fedeli L-P, Frederi Rouillon MH, Ades J, Gorwood P. 2004. The A9 allele of the dopamine transporter gene increases the risk of visual hallucinations during alcohol withdrawal in alcohol-dependent women. *Neurosci Lett* 362:91–94.
- Lopez LS, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM. 2005. The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. *Biol Psychiatry* 57:999–1003.
- Meulenbelt I, Droog S, Trommelen GJ, Boomsma DI, Slagboom PE. 1995. High-yield noninvasive human genomic DNA isolation method for genetic studies in geographically dispersed families and populations. *Am J Hum Genet* 57:1252–1254.
- Mill J, Asherson P, Browes C, D'Souza U, Craig I. 2002. Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. *Am J Med Genet* 114:975–979.
- Munafó M, Clark T, Johnstone E, Murphy M, Walton R. 2004. The genetic basis for smoking behavior: A systematic review and meta-analysis. *Nicotine Tob Res* 6:583–597.
- Munafó MR, Lingford-Hughes AR, Johnstone EC, Walton RT. 2005. Association between the serotonin transporter gene and alcohol consumption in social drinkers. *Am J Med Genet Part B* 135B:10–14.
- Nilsson KW, Sjöberg RL, Damberg M, Alm PO, Ohrvik J, Leppert J, Lindstrom L, Orelund L. 2005. Role of the serotonin transporter gene and family function in adolescent alcohol consumption. *Alcohol Clin Exp Res* 29:564–570.
- Noble EP. 2003. D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *Am J Med Genet Part B* 116B:103–125.
- Noble EP, Syndulko K, Fitch RJ, Ritchie T, Bohlman MC, Guth P, Sheridan PJ, Montgomery A, Heinzmann C, Sparkes RS, Blum K. 1994. D2 dopamine receptor TaqI A alleles in medically ill alcoholic and nonalcoholic patients. *Alcohol Alcohol* 29:729–744.
- Parsian A, Suarez BK, Tabakoff B, Hoffman P, Ovchinnikova L, Fisher L, Cloninger CR. 1995. Monoamine oxidases and alcoholism. I. Studies in unrelated alcoholics and normal controls. *Am J Med Genet* 60:409–416.
- Parsian A, Cloninger CR, Sinha R, Zhang ZH. 2003. Functional variation in promoter region of monoamine oxidase A and subtypes of alcoholism: Haplotype analysis. *Am J Med Genet Part B* 117B:46–50.
- Paterson AD, Sunohara GA, Kennedy JL. 1999. Dopamine D4 receptor gene: Novelty or nonsense? *Neuropsychopharmacology* 21:3–16.
- Prescott CA, Hewitt JK, Heath AC, Truett KR, Neale MC, Eaves LJ. 1994. Environmental and genetic influences on alcohol use in a volunteer sample of older twins. *J Stud Alcohol* 55:18–33.
- Rose RJ. 1998. A developmental behavior-genetic perspective on alcoholism risk. *Alcohol Health Res World* 22:131–143.
- Rose RJ, Kaprio J, Winter T, Koskenvuo M, Viken RJ. 1999. Familial and socioregional environmental effects on abstinence from alcohol at age sixteen. *J Stud Alcohol* 13:63–74.
- Rose RJ, Dick DM, Viken RJ, Kaprio J. 2001. Gene-environment interaction in patterns of adolescent drinking: Regional residency moderates longitudinal influences on alcohol use. *Alcohol Clin Exp Res* 25:637–643.
- Sabol SZ, Hu S, Hamer D. 1998. A functional polymorphism in the monoamine oxidase A gene promoter. *Hum Genet* 103:273–279.
- Saito T, Lachman HM, Diaz L, Hallikainen T, Kauhanen J, Salonen JT, Ryyanen OP, Karvonen MK, Syvalahti E, Pohjalainen T, Hietala J, Tiihonen J. 2002. Analysis of monoamine oxidase A (MAOA) promoter polymorphism in Finnish male alcoholics. *Psychiatry Res* 109:113–119.
- Samochowiec J, Lesch KP, Rottmann M, Smolka M, Sygailo YV, Okladnova O, Rommelspacher H, Winterer G, Schmidt LG, Sander T. 1999. Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial alcoholism. *Psychiatry Res* 86:67–72.
- Sander T, Harms H, Podschus J, Finckh U, Nickel B, Rolfs A, Rommelspacher H, Schmidt LG. 1997. Allelic association of a dopamine transporter gene polymorphism in alcohol dependence with withdrawal seizures or delirium. *Biol Psychiatry* 41:299–304.
- SAS Institute Inc. 1965–2005. WWW.SAS.COM.
- Schmidt LG, Harms H, Kuhn S, Rommelspacher H, Sander T. 1998. Modification of alcohol withdrawal by the A9 allele of the dopamine transporter gene. *Am J Psychiatry* 155:474–478.
- Searle SR. 1971. Linear models. New York: Wiley.
- Searle SR, Casella G, McCulloch C. 1992. Variance Components. New York: Wiley.
- Spielman RS, Ewens WJ. 1998. A sibship test for linkage in the presence of association: The sib transmission/disequilibrium test. *Am J Hum Genet* 62:450–458.
- Spitz E, Moutier R, Reed T, Busnel MC, Marchaland C, Roubertoux PL, Carlier M. 1996. Comparative diagnoses of twin zygosity by SSLP variant analysis, questionnaire, and dermatoglyphic analysis. *Behav Genet* 26:55–64.
- Tang H, Quertermous T, Rodriguez B, Kardia SLR, Zhu X, Brown A, Pankow JS, Province MA, Hunt SC, Boerwinkle E, Schork NJ, Risch NJ. 2005. Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. *Am J Hum Genet* 76:268–275.
- Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V. 1992. Multiple dopamine D4 receptor variants in the human population. *Nature* 358:149–152.
- Yamaguchi K, Kandel D. 1987. Drug use and other determinants of premarital pregnancy and its outcome: A dynamic analysis of competing life events. *J Marriage Fam* 49:257–270.