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Co-Use of Tobacco and Alcohol, the School-Specific Tobacco-Alcohol Use Association, and the *5HTTLPR* Gene

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ABSTRACT

Background. Previous research shows that school health behavioral environments and genetic influences are important determinants of tobacco and alcohol use separately. Very little is known about patterns of tobacco and alcohol co-use in adolescence and young adulthood and how genetic and environmental influences interact to predict risk. This is particularly important because co-use has substantially greater negative health effects than tobacco or alcohol use alone.

Methods. We use four waves of data from the National Longitudinal Study of Adolescent Health (Add Health; n = 10,034) to describe patterns of tobacco and alcohol co-use from adolescence through adulthood. We predict co-use profiles by combining rich information about the co-use environment (school-specific co-use) with genetic information from the respondents ((*5HTTLPR).* Pooled analyses of the odds of co- use were modeled using logistic regression as an interactive function of the school health behavioral environment and genotype. Finally, sibling fixed effects models of a continuous measure of co-use were used to provide a maximally stringent test of this hypothesis.

Results. The interaction of *5HTTLPR* and school-level tobacco and alcohol use association is positively associated with the odds of tobacco and alcohol co-use in longitudinal analyses. In sibling fixed effects models, this finding is confirmed.

Conclusion. The prediction of the differential susceptibility hypothesis of interactions between the *5HTTLPR* gene and the school health behavioral environment is confirmed.

Word Count: 2,832
Keywords: Tobacco and alcohol co-use, gene-environment interactions, health behaviors, schools

Introduction

Tobacco use is one of the leading causes of death worldwide, responsible for up to 6 million deaths per year (World Health Organization, 2012). Because 9 out of 10 cigarette smokers begin by age 18 (U.S. Department of Health and Human Services, 2007) and because tobacco use in adolescence and young adulthood is highly predictive of later life use, many prevention efforts are focused on adolescence and school-based settings.

Despite wide-spread scholarly interest, a number of facets of tobacco use are under-recognized in current research. First, moderate alcohol use interacts with tobacco use to predict deleterious outcomes well above those of tobacco use alone (Castellsague et al., 1999; Kalman et al., 2010). Yet comparatively little research has focused on tobacco and alcohol co-use during adolescence, with some exceptions (Jackson et al., 2005; Jackson et al., 2000; Kozlowski et al., 1993; Madden et al., 2000; Torabi et al., 1993). Previous research has found that the proportion of U.S. 12th graders who co-use tobacco and alcohol has been declining over time even as the association between the two behaviors has been increasing (*identifying cite omitted*). Furthermore, the school-specific association between tobacco and alcohol use is a key predictor of long-term patterns of tobacco and alcohol co-use from adolescence through the early 30s (*identifying cite omitted*). Yet much remains unknown about these behavioral patterns during adolescence.

Second, relatively little research incorporates information on both genetic influences on tobacco and alcohol use and the social environment to understand these patterns. *Gene-environment interactions* (GxEs) hold the potential to elucidate how combinations of social circumstances and genetic variation interact to predict important behaviors. Although a large number of genes (McHugh et al., 2010; Munafo et al., 2004; Serretti et al., 2006; Young-Wolff et al., 2011) and social influences have been linked to tobacco and alcohol use, relatively little research investigates multilevel gene-environment interactions for these outcomes, and none have taken such an

approach to studying tobacco and alcohol co-use. One study demonstrates that the relationship between school-level rates of alcohol and tobacco use differentially predict individual-level use as a function of the number of short alleles in the serotonin transporter gene *5HTTLPR* (*identifying cite omitted*). Specifically, persons with no copies of the *5HTTLPR**S' allele do not necessarily react to the social patterns of substance use in their schools, while persons with two copies react very strongly to these social forces; the reactive group has lower-than-expected tobacco/alcohol use in low-use schools and higher expected use in high-use schools. This is consistent with the predictions of the *differential susceptibility hypothesis* (Belsky and Pluess, 2009a, b; Conley et al., 2011; Ellis and Boyce, 2008) that the direction of genetic effects fundamentally depend on the social environment. Specifically, this hypothesis predicts that genotypes with lower average health outcomes in unhealthy environments will have higher average outcomes in healthy environments.

Together, these findings suggest the need for research incorporating information on individual tobacco and alcohol co-use, school-level indicators of the health behavioral environment, and the *5HTTLPR* and gene to test the differential susceptibility hypothesis. Specifically, we hypothesize that school-specific tobacco and alcohol co-use will predict individual tobacco and alcohol co-use interactively with *5HTTLPR* in a manner consistent with the differential susceptibility hypothesis.

Methods

Data

Data for this study come from the National Longitudinal Study of Adolescent Health (Add Health), a nationally representative dataset on secondary school attendees in 132 schools in 1994. There are currently five waves of Add Health data: the in-school survey, and four waves of in-home interviews with a subset of the attendees of those schools. The in-school survey offers far less depth than the in-home surveys, but has the benefit of surveying everyone at each of these schools on a

moderate range of behavioral, attitudinal, and other social measures. The in-school survey asked students to report their cigarette smoking and alcohol drinking, producing data on the health behaviors of more than 90,000 students in 132 schools, which we used to measure the school-specific tobacco and alcohol use association. Furthermore, the longitudinal follow ups of a subset of these students (more than 15,000 in waves 3 and 4) permit study of the long-term influences of this association on tobacco and alcohol co-use.

In wave 4, genetic data was collected on all consenting participants using Oragene genetic data collection kits, and genotype calls were made for the *5HTTLPR* gene. See Harris and colleagues (2009) for more details on the Add Health design and data.

Measures

School-specific tobacco and alcohol use association

The school-specific tobacco and alcohol use association is measured employing an approach inspired by chi-squared tests. Briefly, tobacco and alcohol co-use can arise in one of two ways: either by chance due to the marginal probabilities of tobacco and alcohol use, or due to an association between them. For example, suppose that ¼ of the students at a school use alcohol and ½ use tobacco. If there were no association between the two, we would expect that ⅛ of students would engage in tobacco and alcohol co-use, simply by chance. As in a chi-square test, proportions of students engaging in both behaviors above (or below) the proportion expected by chance are indicative of an association between the two behaviors. Accordingly, we measure the school-specific tobacco and alcohol use association as the *excess proportion* of students at a school that engage in tobacco and alcohol co-use above the expected proportion based on the marginal distributions of each behavior at that school. Formally, this is calculated as

$$P_{ej} = p_{cj} - p_{sj}p_{dj}\,, \tag{1}$$

where P_{ej} is the excess proportion of students at school j who engage in co-use above the proportion expected under independence, p_{cj} is the proportion of students at school j who engage in co-use, p_{sj} is the proportion engaging in tobacco use, and p_{dj} is the proportion engaging in alcohol use. To better capture the community's health behavioral environment, middle school students are assigned the measure calculated at the high school into which their school feeds. This measure is separately calculated for any- and heavy-use of tobacco and alcohol, as is described presently.

In-school tobacco and alcohol use measures

Measures of tobacco and alcohol use from the in-school survey in Add Health are used when constructing this measure, because the in-school survey captures data from nearly all attendees at a school. The in-school tobacco use measure uses the question, "During the past twelve months, how often did you smoke cigarettes?" Responses were measured ordinally on a 0–6 scale. Any tobacco use is indicated by a value of "once or twice" (the lowest non-zero response category) or higher; heavy tobacco use is indicated by a response of "nearly everyday," which is the highest response category.

Alcohol use in the in-school sample is measured using the question, "During the past twelve months, how often did you drink beer, wine, or liquor?" Any alcohol use is indicated by a report of "once or twice" or higher; heavy alcohol use is indicated by values of "once or twice a week" or higher. The any-co-use and heavy-co-use dependent variables are then indicated by the combination of any tobacco and alcohol use or heavy tobacco and alcohol use, respectively.

In-home tobacco and alcohol use measures

More detailed measures were available in the in-home surveys, which we employ as the dependent variables in this analysis. Tobacco use frequency is measured using the question, "During the past 30 days, on how many days did you smoke cigarettes?" Tobacco use intensity is measured

by the question, "During the past 30 days, on the days you smoked, how many cigarettes did you smoke each day?" The product of these responses estimates number of cigarettes smoked over the previous month.

Alcohol use frequency is measured ordinally by the question, "During the past 12 months, on how many days did you drink alcohol?" Responses were measured identically to the equivalent measure in the in-school sample. Alcohol use intensity was then measured using the question, "Think of all the times you have had a drink during the past 12 months. How many drinks did you usually have each time? (A 'drink' is a glass of wine, a can of beer, a wine cooler, a shot glass of liquor, or a mixed drink.)" Legitimate skips were recoded to 0. Multiplying these values produces an interval-level estimate of the number of alcoholic drinks consumed over the last month.

Tobacco and alcohol co-use is then measured through two different means. First, two dichotomous indicators of co-use are constructed: any co-use is measured as whether any tobacco use *and* any alcohol use is reported using the above measures; heavy co-use is measured as whether above-median tobacco use and above-median alcohol use is reported. Additionally, a continuous measure of tobacco and alcohol co-use is used in the sibling fixed effects models described below, which simply multiplies the measures of number of cigarettes smoked and number of alcoholic drinks consumed described above together.

Analysis

The differential susceptibility hypotheses are tested using two different regression models. First, logistic regression models are employed to predict any co-use and heavy co-use outcomes as a function of the *5HTTLPR* gene, the school-specific tobacco and alcohol use association, and controls for school mean tobacco use and alcohol use, age, gender, and race/ethnicity. One-tailed tests are used to test interactive hypotheses because we have a directional (positive) hypothesis for these relationships; two-tailed tests are used to evaluate other relationships. Second, sibling fixed

effects models compare siblings using the continuous co-use measure as a dependent variable. This model drops the main effects of the school health behavioral environment from the model because this does not vary among siblings who attend the same school. However, the interactive effect of these measures and genotypes is retained because the interaction between a characteristic that varies within sibling pairs (genotype) and one that does not (school environment) does vary within sibling pairs (Allison, 2005). Simulation analyses previous conducted by one of the authors (*identifying cite omitted*) confirms that this model can estimate these types of interactive effects without bias. Non-independence of outcomes (via clustering in the same schools and repeated measures of the same individuals) is accounted for using the sandwich estimator (Rogers, 1993).

Results

Descriptive Statistics

13,773 unique individuals were conserved for this analysis, restricted to those who were in the normal age ranges for their grades in the early waves of Add Health and had valid survey responses for waves 1-4 of the dataset. This number was further restricted to the 10,034 individuals in this number with valid genetic data from wave 4 of Add Health. For these analyses all unique person*wave observations are employed, for an effective samples size of 40,136.

Table 1 provides descriptive statistics on the main variables of the present analysis. Across all four waves, 22.6% of individuals reported any tobacco and alcohol co-use, whereas only 8.8% of respondents reported heavy co-use. Heterozygotes for *5HTTLPR* were most common in this sample with 48.3%, followed by homozygotes for the S' allele (28%) and the L' allele (23.6%).

The average school has an any-use excess proportion of 9.08 (indicating that in a typical school, about 9.08% more students co-use than would be expected from the marginal distributions of tobacco and alcohol any-use), with a standard deviation of 2.49. The average school has a heavy-use excess proportion of 2.75, with a standard deviation of 1.22.

Logistic Regressions

Any Co-Use

The first column of Table 2 shows the results of a series of logistic regression models predicting any co-use. The key coefficients for present purposes are the interaction terms between *5HTTLPR* and the excess proportion (denoted EP). These findings offer support for the differential susceptibility hypothesis for the interaction of *5HTTLPR* and the excess proportion of co-users at a school. There is a positive and marginally statistically significant interaction (p=0.059) between the latter and the number of *5HTTLPR**S' alleles, as predicted by this hypothesis. Furthermore, as shown in Figure 1, there is a crossover in the predicted probability of any co-use as a function of these two variables. In this figure, we see that those with *5HTTLPR**S'/S' genotypes have lower predicted probabilities thereof than those with L'/L' genotypes at low excess-proportion schools, but that the opposite is true in high excess-proportion schools. The main effect of the excess proportion on co-use is not statistically significant, meaning that there is no significant effect among those with the *5HTTLPR**L'/L' genotype. This is consistent with the predictions of the differential susceptibility hypothesis. However, the difference in predicted outcomes is not statistically significant at the 1st decile, which is not consistent with this hypothesis.

Heavy Co-Use

The second column of Table 2 shows parallel results for heavy co-use. Once more, there is support for the differential susceptibility hypothesis for the interaction between *5HTTLPR* and the excess proportion measure, and this interaction is fully statistically significant. Furthermore, as seen in Figure 2, the plot of predicted probabilities of heavy co-use outcomes results in a statistically

significant crossover.[1] Therefore the results for heavy co-use are more strongly consistent with the predictions of the differential susceptibility hypothesis than is the case for any co-use outcomes.

Sibling Fixed Effects

Sibling fixed effects models can shed additional light on these hypotheses because they control for all family-level sources of confounding that operate on all members of a sibship equally. In addition to typical confounders such as parental socioeconomic status, neighborhood factors, or unmeasured school-level variables, this model also eliminates population stratification concerns.[2]

These results offer strong evidence in favor of the differential susceptibility hypothesis by *5HTTLPR*. As predicted by the differential susceptibility hypothesis, the interaction of *5HTTLPR* with the any-use excess proportion and heavy-use excess proportion is positive, large, and statistically significant. We interpret the results of these models as clear evidence in favor of the differential susceptibility hypothesis for the interaction of *5HTTLPR* and the school-specific tobacco-alcohol use association.

Discussion

This paper examines the differential susceptibility hypothesis that the effect of a key school-level indicator of the health behavioral environment (the school-specific tobacco-alcohol use association) on individual tobacco and alcohol co-use will vary by *5HTTLPR* genotype such that those with more S' alleles respectively will show stronger effects. We found strong support for this hypothesis: the interaction of *5HTTLPR* and the excess proportion is statistically significant in all regression models (albeit only borderline significant for any-use). Furthermore, models that compare full siblings with divergent *5HTTLPR* genotypes in common school environments also support the

[1] Although the predicted outcomes at the 9th decile are not quite statistically significant, beyond this value they are statistically significantly different.

[2] Population stratification occurs when genetic ancestry is associated both with genotype frequencies and the distribution of an outcome, which without appropriate measures would create the appearance of an association between genotype and outcomes when in fact none existed.

predictions of this hypothesis. This finding is extremely important because sibling fixed effects models such as this one control for population stratification and many other sibling-level forms of confounding. Together, this evidence confirms the potential generality of the differential susceptibility hypothesis, as well as the joint importance of both genetic and environmental influences on health behaviors. Focusing on environments like schools is also important for GxE research because selection into neighborhoods and schools is likely exogenous to genetic factors related to drinking or smoking. This reduces the likelihood of gene-environment correlation which can complicate the interpretation of GxE associations (Jaffee and Price, 2007).

In sum, we believe that our results are important because they continue to expand upon our understanding of the environment in GxE studies. For instance, the most highly cited GxE study is the work of Caspi and colleagues (2003) who show that the effects of stressful life events on depression is significantly stronger for carriers of short allele in the *5HTTLPR* gene. In this formulation, the environment is posited to be a characteristic of the individual; individuals are exposed to different levels of stress. However, as we show, the same individuals who may be sensitive to stress and report elevated symptoms of depression, may also be those who are *the most* likely to co-use alcohol and tobacco in the most unhealthy environments and *the least* likely to co-use in the most healthy environments. Because behaviors like smoking, drinking, and co-use cluster differently within and across social contexts, it is important to prioritize the focus on these environments. In other words, focusing exclusively on each behavior loses sight of the fact that they are often derived from the same environmental source. While it is difficult to assess the social mechanisms driving excess comorbidity, we believe that this measure captures something unique about schools that is not seen from either behavior independently. These results are consistent with a key role for normative environments that describe the expected behavior regarding the way in which substances are used together. Adolescence is a time when individuals learn whether, how,

and when to engage in tobacco and alcohol use. These results suggest that both an individual's genes and the type of health behavior environment in which they experience adolescence is an important determinant of this important process.

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Table 1: Descriptive Statistics

Individual Measures	**Mean**
Any Co-Use	0.226
High Co-Use	0.088
5HTTLPR	
L'/L'	0.236
S'/L'	0.483
S'/S'	0.280
Age	20.259
Female	0.520
Race	
White	0.530
Black	0.219
Hispanic	0.160
Asian	0.075
Other	0.015
School Measures	**Mean (SD)**
Any-Use	9.076
Excess Proportion	(2.491)
Heavy-Use	2.746
Excess Proportion	(1.218)

Table 2: Logistic Regression Models of Tobacco and Alcohol Co-use

	(1)	(2)
Excess Proportion (EP)	0.989	1.033
	(0.431)	(0.530)
5HTTLPR*S'	0.891+	0.834+
	(0.105)	(0.096)
5HTTLPR*S' x EP	1.018+	1.059*
	(0.059)	(0.049)
School Mean Tobacco Use	20.44*	189.0*
	(0.000)	(0.000)
School Mean Alcohol Use	1.302	0.589
	(0.335)	(0.509)
Age	1.034*	1.067*
	(0.000)	(0.000)
Female	0.909*	0.637*
	(0.002)	(0.000)
Black	0.394*	0.336*
	(0.000)	(0.000)
Hispanic	0.691*	0.483*
	(0.000)	(0.000)
Asian	0.710*	0.611*
	(0.000)	(0.000)
Other	0.705*	0.939
	(0.012)	(0.760)
Constant	0.063*	0.0219*
	(0.000)	(0.000)
Observations	35,514	35,514

NOTE: P-values in parentheses. *: p<.05; +: p<0.10. Two-tailed tests for all coefficients except interactions, which are one-tailed tests.

Table 3: Sibling Fixed Effects Results (Continuous Co-Use)

	(1)	(2)
5HTTLPR*S'	-954.3*	-798.6*
	(0.000)	(0.000)
5HTTLPR*S' x Any EP	108.7*	--
	(0.000)	
5HTTLPR*S' x Heavy EP	--	302.0*
		(0.000)
Age	38.90	28.15
	(0.612)	(0.713)
Female	-886.2*	-887.0*
	(0.000)	(0.000)
Constant	809.6	1,037
	(0.646)	(0.556)
R-squared	0.007	0.008
Number of Sibships*Waves	4,380	4,380

NOTE: P-values in parentheses. *: p<.05; +: p<0.10. Two-tailed tests for all coefficients except interactions, which are one-tailed tests.

Figure 1: Predicted Probabilities of Any Tobacco and Alcohol Co-Use

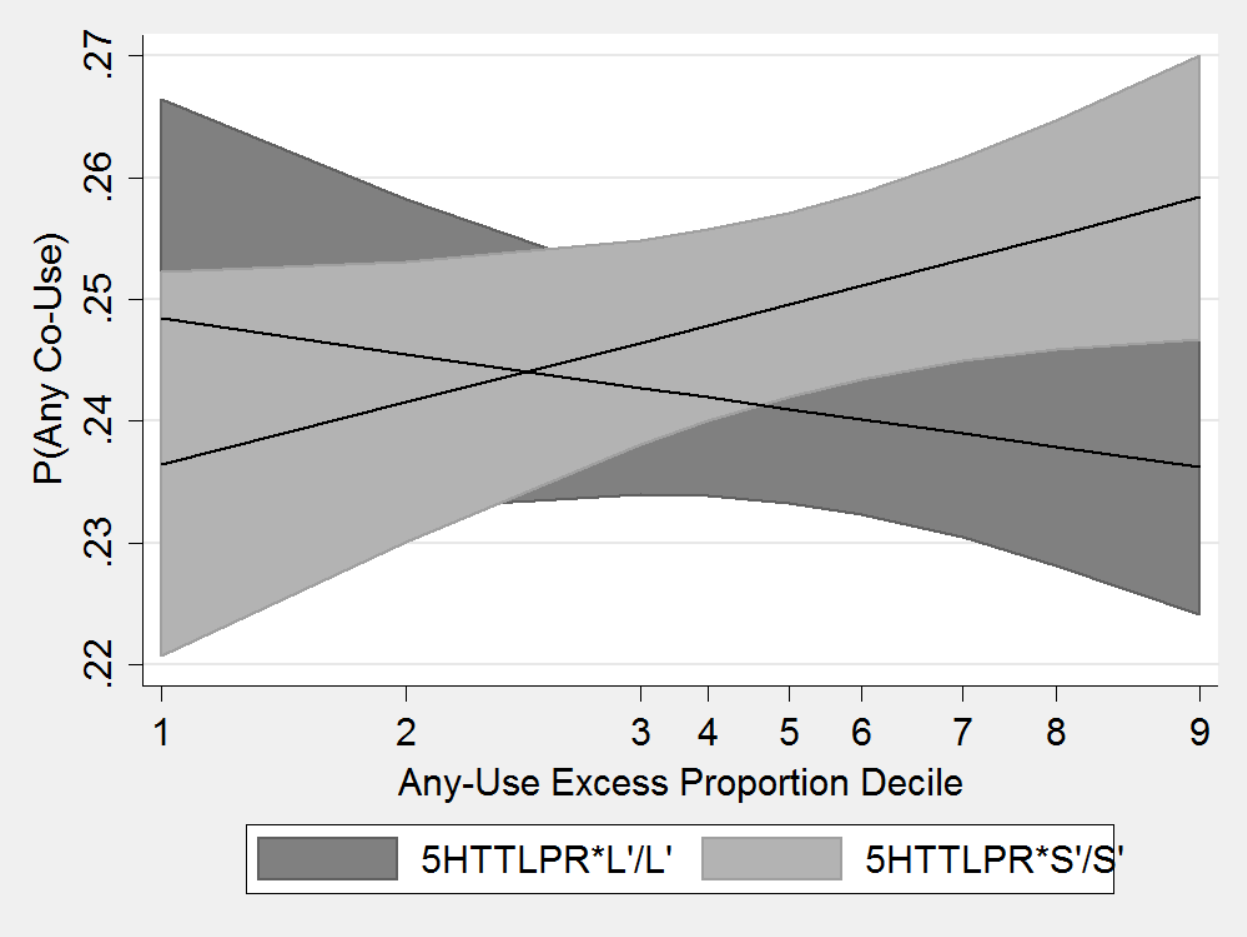

Figure 2: Predicted Probabilities of Heavy Tobacco and Alcohol Co-Use