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Differential Susceptibility to Prevention: GABAergic, Dopaminergic, and Multilocus Effects

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Abstract

Background—Randomized prevention trials provide a unique opportunity to test hypotheses about the interaction of genetic predispositions with contextual processes to create variations in phenotypes over time.

Methods—Using two longitudinal, randomized prevention trials, molecular genetic and alcohol use outcome data were gathered from more than 900 youths to determine whether prevention program participation would, across 2 years, moderate genetic risk for increased alcohol use conferred by the dopaminergic and GABAergic systems.

Results—We found that (a) variance in dopaminergic (*DRD2*, *DRD4*, *ANKK1*) and GABAergic (*GABRG1*, *GABRA2*) genes forecast increases in alcohol use across 2 years, and (b) youths at genetic risk who were assigned to the control condition displayed greater increases in alcohol use across 2 years than did youths at genetic risk who were assigned to the prevention condition or youths without genetic risk who were assigned to either condition.

Conclusions—This study is unique in combining data from two large prevention trials to test hypotheses regarding genetic main effects and gene $\times$ prevention interactions. Focusing on gene systems purported to confer risk for alcohol use and abuse, the study demonstrated that participation in efficacious prevention programs can moderate genetic risk. The results also support the differential susceptibility hypothesis that some youths, for genetic reasons, are more susceptible than others to both positive and negative contextual influences.

Keywords

Alcohol use; African American; genetics; prevention; risk

Historically, residing in rural communities in the southern United States has protected African American preadolescents and adolescents from alcohol use. Recent epidemiological data, however, indicate that African American youths in rural areas use alcohol at rates equal to or exceeding those of youths in densely populated inner cities (National Center on Addiction and Substance Abuse, 2000). Escalation of alcohol use has prognostic significance for rural African American youths' educational and occupational opportunities and attainment, involvement with the criminal justice system, and physical health (Centers for Disease Control and Prevention, 2000). Prevention programs designed to deter alcohol use are scarce in the rural South. These circumstances and the resulting need for prevention programming led to the development of the Strong African American Families (SAAF) program for preadolescents and the Strong African American Families–Teen (SAAF–T)

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program for adolescents. Both family-centered prevention programs were developed, implemented, and tested in longitudinal, randomized efficacy trials. Evaluations of both programs confirmed their efficacy in preventing alcohol use across 2 years (Brody, Chen, Kogan, et al., 2012; Brody et al., 2006). As part of these evaluations, candidate genes that have been implicated in alcohol use and dependence patterns were genotyped from saliva samples.

Randomized prevention trials provide a unique opportunity to test hypotheses about the interaction of genetic predispositions with contextual processes to create variations in phenotypes over time. Through the implementation of randomized prevention trials, a causal relationship between an environmental manipulation and the alteration of a phenotype can be identified, and the likelihood that the environment can moderate genetic risk can be determined. An advantage of this approach is that, because the environmental variable is randomized, prevention trials control for gene-environment correlations that can be mistaken for G×E interactions (Shanahan & Hofer, 2005). Several studies have provided initial evidence of the utility of randomized trials in testing G×E hypotheses. These studies have shown intervention efficacy to moderate risk conferred by a 7-repeat allele of the dopamine receptor-4 gene (*DRD4*; see Brody, Beach, et al., in press, for a review). Although these effects are based on rather small samples (*N*s ranged from 157 to 400), the results supported experimentally the importance of G×E interactions while suggesting etiological hypotheses and demonstrating powerful strategies for examination of candidate G×E effects. These studies also indicate that, for genetic reasons, individuals differ in the extent to which they are affected by exposure to environmental influences (Belsky et al., 2009).

The present research used data pooled from the SAAF and SAAF-T trials to create a more powerful sample with which to extend knowledge about G×E hypotheses. The study was designed to answer three questions. First, do common variations in the dopaminergic genes (*DRD2*, *DRD2/ANKK1*, *TaqI A*, and *DRD4*) and GABAergic genes (*GABRG1* and *GABRA2*) forecast increases in alcohol use for youths assigned randomly to the pooled control condition? Presumably, youths in the control condition represent normative alcohol use patterns and therefore provide an opportunity to determine whether putative risk genes for alcohol use are actually associated with increases in use across 2 years. Second, can participation in efficacious prevention programming moderate the risk that dopaminergic and GABAergic genes confer for increases in youths' alcohol use? Third, can participation in efficacious prevention programming moderate the risk that the presence of multiple risk genes in the dopaminergic and GABAergic systems confers? To date, evaluations of prevention program efficacy in moderating genetic risk have been limited by their reliance on single genetic loci (predominantly *DRD4*), despite the likelihood that many youths carry multiple risk-conferring loci (Rutter, 2005).

All loci included in this study were carefully selected on the basis of their demonstrated links with alcohol use and abuse. The 7-repeat allele of *DRD4* is associated with reduced gene expression (Schoots & Van Tol, 2003) and altered functioning (Asghari et al., 1995; Asghari et al., 1994). This gene is also associated with behavioral self-control problems such as alcoholism (Laucht, Becker, Blomeyer, & Schmidt, 2007), alcohol and other drug use (Brody, Chen, Yu, et al., 2012), and impulsivity (Eisenberg et al., 2007). Furthermore, evidence is accumulating to indicate that carriers of at least one allele of *DRD4* with 7 or more repeats experience heightened susceptibility to the influences of both positive and negative environmental circumstances (Belsky et al., 2009). Despite mixed results, several meta-analyses have demonstrated a positive association between alcohol dependence and the genes *DRD2* and *ANKK1* (Munafò, Johnstone, Welsh, & Walton, 2005; Noble, 1998). In a few studies, environmental interactions with these genes have been evaluated. These studies suggested that carriers experience greater alcohol-related problems in the presence of stress

(Madrid, MacMurray, Lee, Anderson, & Comings, 2001) and demonstrate greater novelty seeking when reared in environments assessed as punitive (Keltikangas-Järvinen & Salo, 2009). Thus, in addition to being linked with alcohol use and abuse, these genes also function to increase sensitivity to environmental events.

Another group of genes related to alcohol use and dependence includes those that encode many of the subunits of a receptor for the brain signaling molecule (i.e., neurotransmitter) - aminobutyric acid (GABA). Research with this group of genes has shown that certain alleles of several subunit genes, such as *GABRA1* and *GABRA2*, appear to contribute to risk for alcoholism, other addictions, and conduct disorder symptomatology, as well as modifying electrophysiological traits related to these disorders (Borghese & Harris, 2007; Edenberg, 2012). Both physiological and molecular evidence indicates that GABA_A receptors are affected by alcohol and are involved in many processes relevant to addiction, such as heightened subjective responses to alcohol and alcohol odors (Edenberg et al., 2004). Thus, the *GABRA1* and *GABRA2* variants seem to modify the activation of neural reward-related areas after exposure to alcohol-associated cues. Animal research indicates that *GABRA1* and *GABRA2* are expressed primarily in the amygdala and areas receiving innervation from the striatum, such as the substantia nigra (Schwarzer et al., 2001). The latter regions are involved with individual differences in impulsivity, reward, and addiction. These *GABRA* genes, however, are also likely to increase sensitivity to context. That they are expressed in the amygdala as well as in the substantia nigra suggests that they influence thresholds for sensitivity to both constructed and naturally occurring environments (Belsky & Pluess, 2009). In sum, GABAergic and dopaminergic genes were hypothesized both to forecast increases in alcohol use and to interact with prevention conditions to influence the development of alcohol use.

The environmental moderation hypotheses tested in this study were informed by differential susceptibility theory (Belsky & Pluess, 2009). Theorists espousing this view contend that persons who are most vulnerable to risk mechanisms also reap the most benefits from protective environments, including “constructed environments” such as prevention programs (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007). Because Belsky and colleagues (Belsky et al., 2007; Belsky et al., 2009) conjectured that dopaminergic and GABAergic systems function as environmental susceptibility genes, we hypothesized that (a) youths carrying at least one susceptibility variation of dopaminergic or GABAergic genes who were assigned randomly to the control condition would display greater increases in alcohol use than would (b) those assigned to a prevention condition who carried a dopaminergic or GABAergic susceptibility gene or (c) those assigned to either the prevention or control condition who carried no environmental susceptibility genes. By applying the differential susceptibility framework to a multilocus index, we expect to find a susceptibility cascade such that youths carrying more susceptibility genes will benefit most from participation in prevention programs.

Summary

This study is unique in combining data from two large prevention trials involving more than 900 youths to test hypotheses about gene $\times$ prevention interactions. Focusing on gene systems purported to confer risk for youth alcohol use and abuse, we tested hypotheses to determine whether prevention program participation could moderate risk that dopaminergic and GABAergic genes confer for increases in youth alcohol use across 2 years.

Method

Overview and Study Population

Data reported in this study were collected from youths assigned randomly to prevention and control conditions in SAAF and SAAF-T. Participants in each trial resided in rural counties in Georgia; these counties are representative of the southern “Black Belt” (Wimberly & Morris, 1997), a geographic concentration of rural poverty that coincides with the nation’s worst economic and health disparities by race (Hartley, 2004). For SAAF, alcohol use was assessed when the youths were 11 (pretest) and 14 (long-term follow-up) years of age; for SAAF-T, use was assessed when youths were ages 16 years (pretest) and 17 years, 10 months (long-term follow-up). Participants in each study provided saliva samples from which DNA was genotyped. Participant recruitment, enrollment, and retention were described extensively in earlier reports on SAAF (Brody et al., 2004) and SAAF-T (Brody, Chen, Kogan, et al., 2012; Kogan et al., 2012); due to space limitations, readers are referred to these sources of information. In the following sections, we describe the sample, intervention implementation, and data collection procedures for each program.

The demographic characteristics of both samples indicate that slightly more participants in each trial were female, and the SAAF-T primary caregivers were an average of 6+ years older than the SAAF primary caregivers. Households in the SAAF and SAAF-T trials included means of 2.7 and 2.5 children, respectively. More than 56% of the households in each trial were headed by married couples, and a majority of the primary caregivers were employed. The lack of employment opportunities in rural Georgia is particularly consequential for African Americans. According to an index described in the Measures section, the families experienced moderate levels of socioeconomic risk; more than 40% of the families in each trial lived at or below federal poverty guidelines.

Participants and Description of SAAF and SAAF-T

SAAF—At the pretest assessment, 667 African American families living in rural Georgia participated in SAAF. The preadolescents were 11 years of age; 53% were female. The intervention group included 369 families and the control group included 298 families. Of the families who provided data at pretest, 597 (90%) provided data 29 months later at long-term follow-up. More than 95% of these participants provided saliva samples for DNA genotyping. The trial data reported here were collected from 2002 to 2004. No demographic differences or differences in alcohol use emerged between families of youths who did ($n = 571$) or did not ($n = 26$) provide DNA. Depending on the specific SNPs and variable number tandem repeats (VNTRs) under study, DNA was available for 489 to 563 youths.

SAAF consists of seven group meetings held once a week. Each group was composed of approximately 10 preadolescents and their mothers. Each group meeting included separate, concurrent 1-hour skill-building sessions for caregivers and youths followed by a 1-hour joint caregiver-youth session that focused on family communication and support. Content of the program has been described extensively in earlier publications (e.g., Brody et al., 2004). While the SAAF program was being implemented, control families received information via postal mail about preadolescent and adolescent development. Complete implementation details are available from the authors.

SAAF-T—At pretest, 502 African American families living in rural Georgia took part in the study. The adolescents were 16 years of age; 56% were female. Families were assigned randomly to either the intervention group ($n = 252$) or an attention control group ($n = 250$). Of the families who provided data at pretest, 478 (95%) provided data 22 months later at long-term follow-up. The data were collected from 2007 to 2010. DNA samples were

collected from 94% of the adolescents ($n = 474$); no demographic differences or differences in alcohol use emerged between youths who did or did not provide DNA. Depending on the specific SNPs and VNTRs under study, DNA was available for 474 to 571 youths.

The SAAF-T program consists of five group meetings held once a week. Each group included approximately 10 adolescents and their mothers. The meeting structure and pedagogical format were identical to those of SAAF. The attention control group took part in a five-session family-centered intervention that was identical in structure to SAAF-T; its curriculum was designed to promote good nutrition and exercise.

Data Collection Procedures for SAAF and SAAF-T

African American university students and community members served as field researchers to collect pretest and long-term follow-up data. At each data collection point, the field researchers, who were blind to the families' group assignments, made one 2-hour home visit to each family. The time from pretest to long-term follow-up averaged 29 months for SAAF and 22 months for SAAF-T. Informed consent forms were completed at all data collection points. Interviews were administered via a self-paced computer program that the authors have used successfully with African American youths in epidemiological and prevention research.

Measures

Socioeconomic risk index—A socioeconomic risk index was formed using demographic data. This index, used in prior research (Brody, Chen, Kogan, et al., 2012), was included as a control in all data analyses. A score of 1 was assigned to each of the following variables: family poverty based on federal guidelines, caregiver unemployment, receipt of Temporary Assistance for Needy Families, caregiver single parenthood, caregiver education of less than high school graduation, and caregiver-reported inadequacy of family income.

Intervention status and gender—Intervention status and gender were dummy coded. SAAF and SAAF-T participants were coded 1 and control participants were coded 0. Male youths were coded 1 and female youths were coded 0.

Alcohol use—Two widely used items in the public health literature were used to assess alcohol use (Johnston, O'Malley, & Bachman, 2000). SAAF and SAAF-T participants indicated how often during the past month they consumed a drink of alcohol (beer, wine, wine cooler, whiskey, gin, or other liquor) and how often they consumed more than four drinks on any single occasion. Summation of these items has commonly been used to characterize alcohol use in evaluations of prevention programs (Brody et al., 2006; Spoth, Redmond, Shin, & Azevedo, 2004). SAAF participants responded to these items in an open format, and SAAF-T participants fit their responses into six categories that ranged from 0 (*never*) to 6 (*30 or more times*). To produce a common metric across samples, SAAF participants' responses were recoded into the SAAF-T response categories.

Genotyping—Youths' DNA was obtained using Oragene DNA kits (DNA Genotek; Kanata, Ontario, Canada). Youths rinsed their mouths with tap water and then deposited 4 ml of saliva in the Oragene sample vial. The vials were sealed, inverted, and shipped via courier to the Psychiatric Genetics Laboratory in Iowa City, Iowa, where samples were prepared according to the manufacturer's instructions. Genotyping was performed for genes in the dopaminergic system—*DRD4* 48 base pair VNTR; *DRD2* (rs6279, rs6277, rs6275); *ANKK1* (rs1711539, rs4938015, rs7118900, rs11604671); and *TaqI* (rs1800497)—and the GABAergic system—*GABRG1* (rs497565, rs1497571) and *GABRA2* (rs567926, rs534459,

rs529826, rs279858). Frequency distributions for all genes conformed to Hardy-Weinberg equilibrium. Detailed information about extraction and genotyping procedures is available from the authors. The designation of risk statuses for *DRD4* (Brody, Chen, et al., in press; Ray et al., 2008) and *TaqI* (Yang et al., 2008) were based on the empirical literature. The risk statuses for the *GABRG1*, *GABRA2*, *ANKK1*, and *DRD2* haplotypes were based on the association of each haplotype with alcohol use among youths in the study sample. This strategy was used because no empirical literature was available on the associations of the SNPs that comprise these haplotypes with alcohol use among African American community samples.

Linkage disequilibrium (LD) was also calculated among SNPs within genes using Haploview 4.2 (Barrett, Fry, Maller, & Daly, 2005). SNPs with high LD were assigned into block groups, resulting in two block groups for *GABRG1* and one block group each for *GABRA2*, *ANKK1*, and *DRD2* (information on LD plots for each block group is available upon request from the authors). We estimated haplotype frequencies in each block group using PHASE 2.11 (Stephens & Donnelly, 2003); haplotypes with less than .05 expected frequencies (i.e., rare haplotypes) were excluded from all analyses. Table 1 presents the dopaminergic and GABAergic genes that were hypothesized to confer risk for increases in alcohol use. Because only 12.8% of the sample carried the *TaqI* risk gene, it was excluded from the data analyses for lack of power.

Statistical Analysis

Poisson regression models (Cameron & Trivedi, 1998) were used to test the study hypotheses because the alcohol use data were positively skewed. Poisson models allow for skewness in dependent measures, rendering them appropriate for regression analysis. Three sets of analyses were executed. First, the contributions that dopaminergic and GABAergic candidate genes made to increases in alcohol use was determined by regressing the logarithm of mean count [$\log(u)$] of alcohol use at the long-term follow-up assessment separately on each candidate gene and three control variables (i.e., alcohol use at pretest, participant gender, and socioeconomic risk). Second, the capacity of participation in prevention programming to moderate genetic risk was examined. Candidate genes that significantly forecast alcohol use in the aforementioned main effect analysis were included, separately, in analyses in which alcohol use at the long-term follow-up assessment was regressed on prevention condition, dopaminergic or GABAergic gene, the prevention condition $\times$ dopaminergic or GABAergic gene interaction, and the three control variables. Third, the preceding gene $\times$ prevention program analysis was reexecuted using a multilocus genetic index (described in the Results section) to detect the potential of participation in prevention programming to moderate risk conferred by multiple genes. Finally, additional analyses were executed with prevention program added as a moderator of two interactions, gene $\times$ prevention condition and multilocus condition $\times$ prevention program, to determine whether any of the results obtained from the aforementioned analyses were more evident for youths in either SAAF or SAAF-T. Because *no* such interactions were detected, all analyses reported are for results obtained with the pooled sample.

Power analyses—Power analyses were conducted for the genetic main effect and the gene $\times$ prevention interaction models using Monte Carlo simulation analyses. The parameters used in the simulation were based on the allele distributions presented in Table 1. For the genetic main effect model, the simulation analysis indicated that the study has more than 90% power to detect a small effect size for the lowest frequency risk allele (.31). For the gene $\times$ prevention interaction analyses, the study also has 90% power to detect gene $\times$ prevention interaction for a small effect size for the lowest frequency allele (.31). Thus, this

study is adequately powered to detect small genetic main effects and gene $\times$ prevention interactions.

Results

Do Dopaminergic and GABAergic Genes Forecast Increases in Alcohol Use?

The Poisson regression models that included each of the dopaminergic and GABAergic genes included in the study—listed in Table 1—along with the control variables—past-month alcohol use at pretest, gender, and socioeconomic risk at pretest—were executed to forecast increases in past-month alcohol use for youths in the control group. Each gene forecast increases in alcohol use: *DRD4*, $\beta = .30$, $p = .04$; *GABRG1*, Block 1, $\beta = 1.04$, $p = .001$; *GABRG1*, Block 2, $\beta = .36$, $p < .05$; *GABRA2*, Block 1, $\beta = .38$, $p < .01$; *DRD2*, $\beta = .61$, $p < .001$, and *ANKK1*, $\beta = .62$, $p < .01$.

The following procedure was used to specify further the contribution of carrying a risk allele to increases in past-month alcohol use across 2 years. Exponentiation of the coefficients (e^{β}) provides the incident rate ratios (IRRs), which represent the increase in alcohol use as a function of carrying a risk allele. For interpretation purposes, percentage of change in IRR for carriers of a risk allele is calculated by first subtracting the IRR from 1 and then multiplying it by 100 [$100 \times (1 - e^{\beta})$]. Accordingly, youths who carried at least one allele of *DRD4* with 7 or more repeats evinced a 35% greater increase [$100 \times (1 - e^{-.30})$] in alcohol use than did youths who carried two alleles with 6 or fewer repeats. Similarly, youths who carried the risk allele of *GABRG1* in Block 1 evinced a 183% greater increase [$100 \times (1 - e^{-1.04})$] in alcohol use than did youths who did not carry the risk allele. Youths who carried the risk allele of *GABRG1* in Block 2 demonstrated a 43% greater increase [$100 \times (1 - e^{-.36})$] in alcohol use than did youths who did not carry the risk allele. Youths who carried the risk allele of *GABRA2* in Block 2 showed a 46% greater increase [$100 \times (1 - e^{-.38})$] in alcohol use than did youths who did not carry the risk allele. Youths who carried one or two copies of the risk allele of *DRD2* evinced an 84% greater increase [$100 \times (1 - e^{-.61})$] in alcohol use than did youths who did not carry the risk allele. Finally, youths who carried one or two copies of the risk allele of *ANKK1* evinced an 86% greater increase [$100 \times (1 - e^{-.62})$] in alcohol use than did youths who did not carry the risk allele.

Can Participation in Prevention Programming Moderate Genetic Risks?

Table 2 presents tests of hypothesized gene $\times$ prevention program interaction effects on increases in alcohol use. Prevention effects were not detected in the presence of genetic status. The hypothesized prevention moderation effects emerged for *DRD4*; *GABRG1*, Block 1; *GABRG1*, Block 2; *GABRA2*, Block 1; *DRD2*; and *ANKK1*. Consistent with the standards of genetic association studies (Dahlman et al., 2002), we applied the false discovery rate (FDR; Benjamini & Hochberg, 1995) to the significance levels of each of the significant gene $\times$ prevention program interactions presented in Table 2 to preclude the reporting of false positive findings. Of the six significant gene $\times$ prevention interactions, three remained significant ($p < .05$) after significance levels were adjusted for multiple comparisons: *GABRG1*, Block 2; *GABRA2*, Block 1; and *DRD2*. These interactions are depicted in Figure 1. As hypothesized, environmental moderation was evident for each type of genetic risk. Specifically, youths carrying a risk gene who were assigned randomly to the control condition evinced larger increases in alcohol use than did their counterparts who were assigned to the prevention condition or youths assigned to either condition who carried the non-risk variants. These results lead to the last research question.

Can Participation in Efficacious Prevention Programming Moderate Risk Conferred by Multiple Genes?

A multilocus risk index was formed by summing *GABRG1*, Block 2; *GABRA2*, Block 1; and *DRD2*, which interacted with prevention condition and remained significant after adjustment for FDR. This yielded a distribution in which 21% of the sample carried no risk genes, 53% of the sample carried one risk gene, 23% of the sample carried two risk genes, and 3% carried three risk genes. The results are depicted in Figure 2. The data analysis revealed main effects for prevention condition, $\beta = -.39, p < .01$, and multilocus genetic score, $\beta = .40, p < .01$. Participants in the prevention condition evinced slower increases in alcohol use than did control youths. The main effect for the multilocus index indicated, as expected, that the presence of more risk genes was associated with greater increases in alcohol use across 2 years. A significant multilocus index $\times$ prevention interaction conditioned these main effects, $\beta = -.65, p < .001$. A perusal of Figure 2 reveals that youths in the control condition who carried two or three multilocus genes evinced substantially greater increases in alcohol use than did youths in the other multilocus $\times$ prevention conditions. Further examination of Figure 2 reveals that increases in alcohol use for participants within the *prevention* condition follows a differential susceptibility cascade. Youths carrying two or three multilocus genes evinced smaller increases in alcohol use, indicating greater responsiveness to prevention, than did carriers of zero or one ($p < .054$) multilocus gene. Increases in alcohol use were similarly low for carriers of two or three multilocus genes.

An additional analysis was executed in which the genes in the multilocus index were not constrained to those that retained their significance after adjustment for FDR. Instead, all genes in the study were included in the multilocus index. Main effects for the multilocus index and prevention condition again emerged, as did their interaction. The only new finding was that increases in past-month alcohol use were greater for youths in the control condition who carried three or more multilocus genes than for similar youths in the prevention condition. In the constrained multilocus analysis, presented previously, differences between the prevention and control groups emerged for carriers of two risk genes. Both analyses illustrate a differential susceptibility cascade.

Discussion

This study was designed to extend the gene $\times$ prevention program literature by answering three research questions. First, we established that dopaminergic and GABAergic candidate genes forecast increases in alcohol use across 2 years. This finding established the validity of these genes' designation as risk conferring because each gene forecast increases in alcohol use. Second, the risk conferred by the six genes was moderated by participation in a prevention program. Youths who carried the risk variation of the dopaminergic and GABAergic genes who were assigned to the control condition evinced more alcohol use over time than did (a) those who were assigned to the prevention condition or (b) youths assigned to either condition who carried the non-risk genetic variant. Finally, we demonstrated that participation in prevention moderated risk conferred by multiple genes, in a manner that reflected a differential susceptibility cascade.

The results support Belsky et al.'s (2007) differential susceptibility hypothesis in which variants of specific genes, including dopaminergic and GABAergic genes, are proposed to render individuals more susceptible to the surrounding environment whether it is characterized by high positivity or high risk. The finding that, after exposure to the protective processes that the prevention programs offered, carriers of risk/sensitivity genes evinced less alcohol use over time than did similar youths in the control condition supports differential susceptibility predictions. These results extend the existing literature to

previously unstudied genes. The finding that participation in prevention moderated genetic risk and the retention of significance by three of those genes after controls for FDR were applied support the importance of prevention efforts and the explanatory value of differential susceptibility theory.

The multilocus result has heuristic value for understanding gene $\times$ prevention/intervention interactions and, by extension, G $\times$ E interactions in general. Using dopaminergic and GABAergic genes that the literature suggested had functional significance and some predictive validity for alcohol use and abuse, we demonstrated that carrying two out of three genes from the multilocus index accounted for enhanced response to prevention programming. This finding constitutes novel evidence for the utility of multilocus profiles in understanding individual differences in response to prevention by demonstrating, for the gene systems in this study, that enhanced susceptibility effects become apparent for youths carrying two or more risk/susceptibility loci. Such findings go undetected when analyses focus only on single genetic loci. It should be noted, however, that the data presented in Figure 2 show that youths in the prevention group who carried no risk alleles increased their alcohol use more than did similar youths in the control group. We view this finding as anomalous and in need of replication because only 21% of the total sample—approximately 10% of each subsample, prevention and control—carried no risk alleles. Together, the multilocus results constitute a proof-of-principle for the utility of incorporating a multilocus genetic profile into gene $\times$ prevention hypothesis testing. Future research should refine genetic profile scores by assigning different weights to loci of potentially different effect sizes and examining their prevention enhancement effects. Although beyond the scope of this study, such an approach should be considered in future research.

Generalizability of the present research is limited because the prevention programs were designed to meet a need in rural Southern communities for efficacious prevention programming for African American adolescents. The findings' applicability with ethnically and socioeconomically diverse participants residing in urban or rural locations must be established empirically. Even though the distributions of the genes in this study have not been shown to vary between persons of African and European descent (Chang, Kidd, Livak, Pakstis, & Kidd, 1996; Chen, Burton, Greenberger, & Dmitrieva, 1999), in attempting future replications, researchers should note whether their study populations differ in the distribution of these genes. Such studies are important to an understanding of the etiology of alcohol use and abuse. These issues aside, the present study demonstrates the utility of using randomized prevention trials to test G $\times$ E hypotheses.

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Abbreviations

SAAF	Strong African American Families program
SAAF-T	Strong African American Families-Teen program
SNP	single nucleotide polymorphism
VNTR	variable number tandem repeat

LD linkage disequilibrium

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- Hypotheses were tested to determine whether prevention program participation would ameliorate the risk that dopaminergic and GABAergic genes confer for increases in youths' alcohol use across 2 years.
- Data were combined from two large alcohol use prevention trials involving more than 900 African American youths living in a rural region of the southeastern United States.
- Dopaminergic and GABAergic candidate genes, both singly and in combination, forecast
- Prevention program participation ameliorated the risk that the candidate genes conferred.
- Prevention program effects reflected a differential susceptibility cascade.

Figure 1. Gene $\times$ prevention interaction for dopaminergic and GABAergic genes, with standard error bars included. Alcohol use is defined as occasions on which alcohol was consumed during the past month, rated on a scale of 0 (*never*) to 6 (*30 or more times*).

Figure 2.

Multilocus genes $\times$ prevention interaction, with standard error bars included. Alcohol use is defined as occasions on which alcohol was consumed during the past month, rated on a scale of 0 (*never*) to 6 (*30 or more times*).

Table 1

Dopaminergic and GABAergic Single Nucleotide Polymorphisms, Variable Number Tandem Repeats, and Haplotypes

Gene	Risk Model	Percentage of Study Sample Carrying Risk Allele
<i>DRD4</i>	7 or more repeats	46.8
<i>DRD2</i>	CAG & CGG	35.5
<i>ANKK1</i>	TTAG	30.0
<i>GABRG1</i>		
Block 1	GT	62.4
Block 2	AGTC & AGTT	76.4
<i>GABRA2</i>		
Block 1	CA & CG	31.3

Table 2
Poisson Regression Analysis of Gene $\times$ Prevention Interaction on Change in Past-Month Alcohol Use

Predictors	DRD4		GABRG1 Block 1		GABRG1 Block 2		GABRA2 Block 1		DRD2		ANKK1	
	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
Alcohol use, pretest	.67***	.04	.69***	.04	.68***	.03	.68***	.04	.69***	.04	.67***	.04
Gene	.28*	.15	.88**	.26	.36**	.14	.38**	.14	.59**	.17	.60**	.19
Prevention	-.17	.15	.15	.34	-.19	.14	-.12	.15	-.19	.12	-.39***	.11
Gene $\times$ Prevention	-.52*	.22	-.76*	.36	-.86***	.22	-.67**	.21	-1.19***	.29	-.90**	.34
Gender, male	-.16	.11	.02	.10	-.00	.11	-.04	.10	.04	.11	.00	.10
Socioeconomic risk	.09*	.04	.08*	.03	.03	.04	.06	.03	.08*	.04	.06	.03

Note. The estimates in the models were in $\log(\mu)$. SE = standard error.

* $p < .05$.

** $p < .01$.

*** $p < .001$.