Gene × Environment Interaction Studies Have Not Properly Controlled for Potential Confounders: The Problem and the (Simple) Solution

Matthew C. Keller

Candidate gene × environment (G × E) interaction research tests the hypothesis that the effects of some environmental variable (e.g., childhood maltreatment) on some outcome measure (e.g., depression) depend on a particular genetic polymorphism. Because this research is inherently nonexperimental, investigators have been rightly concerned that detected interactions could be driven by confounders (e.g., ethnicity, gender, age, socioeconomic status) rather than by the specified genetic or environmental variables per se. In an attempt to eliminate such alternative explanations for detected G × E interactions, investigators routinely enter the potential confounders as covariates in general linear models. However, this practice does not control for the effects these variables might have on the G × E interaction. Rather, to properly control for confounders, researchers need to enter the covariate × environment and the covariate × gene interaction terms in the same model that tests the G × E term. In this manuscript, I demonstrate this point analytically and show that the practice of improperly controlling for covariates is the norm in the G × E interaction literature to date. Thus, many alternative explanations for G × E findings that investigators had thought were eliminated have not been.

Key Words: Adjustment, confounders, covariates, false positive rate, gene-by-environment interactions, gene × environment interactions, multiple regression, replication

Candidate gene × environment interaction (G × E) studies test the hypothesis that the effect of some environmental variable (e.g., childhood maltreatment) on some outcome measure (e.g., depression) depends on a particular ("candidate") genetic polymorphism. This research area has been a hot topic in genetics, with hundreds of publications reporting positive G × E discoveries over the last 15 years, but there has been increasing skepticism about the validity of many of these findings (1–6). This skepticism is based on a number of substantive and statistical concerns: 1) a low replication rate among attempted direct replications of G × E findings; 2) the possibility that G × E findings capitalized on chance from among many unreported analyses; 3) a publication bias toward positive findings; 4) small sample sizes that exacerbate the already-low statistical power for detecting interactions (7), which counter-intuitively increases the false positive rate; and 5) the low prior probability that a specified environmental variable interacts with a specified candidate gene polymorphism. These concerns have led some researchers to suggest that the false positive rate (8)—the proportion of significant "discoveries" that are actually false—in the G × E literature is very high, well above the nominal type-I error rate of .05 (1,6). In essence, skeptics are concerned that the lessons learned from high-powered genome-wide association studies, which failed to corroborate previous candidate gene findings (9–13), will apply equally to G × E findings once large genome-wide interaction studies (14) are performed. In response to such concerns, at least two journals, *Behavior Genetics* (15) and *Journal of Abnormal Child Psychology* (16), have recently published policies outlining stricter criteria that must be met before manuscripts reporting candidate gene main effects or interactions will be considered for review.

The current review focuses on an additional statistical problem that seems pervasive in the G × E literature, namely, potential confounders have not been properly controlled for in the statistical models used to test G × E effects. Typically, G × E studies enter three variables—the genetic polymorphism (e.g., using a dummy or effects coding), the environmental variable, and the product of these two variables (testing the G × E effect)—into a regression equation to predict some outcome measure. However, there are often variables such as ethnicity, gender, age, socioeconomic status, education, IQ, and so forth that investigators wish to eliminate as possible alternative explanations for any G × E finding. Investigators typically enter these variables into the regression equation as covariates to "control" for their potential confounding effects on the interaction of interest. However, although entering these covariates does control for their potentially confounding influences on the main effects of the genotype and the environment, it does nothing to control for the potential confounding influences these variables might have on the interaction term. Rather, to properly control for potential confounders, investigators need to enter all the covariate × environment and the covariate × gene interaction terms in the same model that tests the gene × environment interaction term. Note that all simple effects and interaction effects between the covariates and the genetic and environmental variables must be entered. So, for example, to control for ethnicity and gender, investigators need to enter six terms (ethnicity, gender, ethnicity × gene, ethnicity × environment, gender × gene, and gender × environment) along with the original terms (gene, environment, and G × E). The G × E term would then be properly adjusted for the potential confounding effects of these covariates.

This general point concerning proper covariate adjustment for interactions has been made before with respect to personality (17) and social psychological (18) research, but it does not seem to be in circulation in the genetics field, as evident from the literature review in the following text. Here, I demonstrate this problem analytically, discuss three example studies that have not properly controlled for covariates and how the conclusions of these studies might be misleading, and show that improper control for covariates is widespread in the G × E literature.

From the Department of Psychology and Neuroscience, and the Institute for Behavioral Genetics, University of Colorado at Boulder, Colorado.

Address correspondence to Matthew C. Keller, Ph.D., University of Colorado at Boulder, Department of Psychology and Neuroscience, 1480 30th Street, Muenzinger Hall, 345 UCB, Boulder, CO, 80309; E-mail: matthew.c.keller@gmail.com.

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Quantification of Bias When Improperly Controlling for Covariates in G × E Studies

The quantification of the bias that occurs in the interaction term in the presence of improperly modeled covariates has been derived under simplifying assumptions by Yzerbyt *et al.* (18), and so here I merely translate their conclusions to a G × E framework and refer the interested reader to their article. For simplicity, let G_i be the effects-coded (−1, 0, +1 for the *aa*, *Aa*, and *AA* alleles, arbitrarily coded) genetic variable where $p(a) = p(A) = .5$; E_i be a normally distributed, standardized environmental variable; and $C1_i$ be a mean centered covariate of interest (e.g., an ancestry score from a principal components analysis of the identity × state matrix) that is correlated (confounded) with either G_i or E_i. The substantive conclusions of what follows do not depend on these distributional assumptions, but the assumptions simplify the math. For the derivations to follow, let us first assume that $C1_i$ is confounded with G_i. In a properly specified model, the dependent variable, Y_i, is therefore a function of these variables and error:

$$Y_i = \beta_0 + \beta_G G_i + \beta_E E_i + \beta_{C1} C1_i + \beta_{G\times E} G_i E_i + \beta_{C1\times E} C1_i E_i + \varepsilon_i \quad (1)$$

where G_iE_i is the product of the genetic and environmental term and $C1_iE_i$ is the product of the covariate and environmental term.

Notice that when $C1_i$ is confounded with G_i, either G_i or $C1_i$ might interact with *Ei*, and thus both the G_iE_i and the $C1_iE_i$ term must be included in the properly specified model. This allows for the possibility that it is the covariate that interacts with the hypothesized environmental moderator rather than or in addition to the genetic polymorphism interacting with the hypothesized environmental moderator. If *C*1 is ethnicity, for example, one can imagine that individuals of a certain ethnic background are more sensitive to the environmental variable than individuals of another ethnic background. This could easily occur due to, for example, cultural differences in reporting of environmental adversity, such that the more "sensitive" ethnicity only reports environmental adversity when it is more severe and harmful. To the degree that there are genotype frequency differences between ethnicities, G_iE_i will be confounded with $C1_iE_i$. Alternatively, if *C*1 is socioeconomic status, subtle stratification not captured by self-report ethnicity or a gene–environment correlation might cause a relationship between the genetic polymorphism and *C*1, again leading to the G_iE_i term being confounded with the $C1_iE_i$ term. In either case, the model in Equation 1 will properly control for such alternative explanations, and $\beta_{G\times E}$ will be estimated correctly in the presence of $\beta_{C1\times E}$

However, assume that the investigators control for the covariate in the typical way by estimating only its main effect, and fit the following model:

$$Y_i = \beta_{0*} + \beta_{G*} G_i + \beta_{E*} E_i + \beta_{C1*} C1_i + \beta_{G\times E*} G_i E_i + \varepsilon_i \quad (2)$$

The bias in the G × E term can then be quantified as the difference between $\beta_{G\times E}$ (the unbiased estimate from Model 1) and $\beta_{G\times E*}$ (the biased estimate from Model 2). In this case:

$$\beta_{G\times E*} = \beta_{G\times E} + \beta_{C1\times E}\frac{\sigma_{C1,G}}{\sigma_G{}^2} \quad (3)$$

and $\beta_{G\times E*}$ is biased as a function of $\beta_{C1\times E}\frac{\sigma_{C1,G}}{\sigma_G{}^2}$. Note that β_{C1} does not affect the bias; controlling for the main effect of the covariate does nothing to control for the effect of the covariate on the interaction. It is therefore possible that some or all of the estimated G × E effect in a model that "controls" for only the main effect of a covariate is due to the interaction between the covariate and the environmental term rather than the G × E effect itself. A similar situation occurs if the covariate is correlated with the environmental variable and interacts with the genetic polymorphism. For example, the effect of the genetic polymorphism might depend on ethnic or socioeconomic background rather than on the hypothesized environmental moderator. In this case, $\beta_{G\times E*}$ is biased as a function of $\beta_{G\times C1}\frac{\sigma_{C1,E}}{\sigma_E{}^2}$. Thus, to properly control for all the potential ways *k* covariates might confound the G × E effect of interest, investigators should fit the following model:

$$Y_i = \beta_0 + \beta_G G_i + \beta_E E_i + \sum_k \beta_k C_{ki} + \sum_k \beta_{G\times Ck} G_i Ck_i + \sum_k \beta_{Ck\times E} Ck_i E_i + \beta_{G\times E} G_i E_i + \varepsilon_i \quad (4)$$

When the signs of the $\beta_{C1\times E}\frac{\sigma_{C1,G}}{\sigma_G{}^2}$ or the $\beta_{G\times C1}\frac{\sigma_{C1,E}}{\sigma_G{}^2}$ terms are opposite the sign of the $\beta_{G\times E}$ term, properly controlling for covariates can increase power to detect true G × E interactions. However, when those terms are of the same sign as the $\beta_{G\times E}$ term, properly controlling for covariates will weaken evidence for apparent G × E interactions.

The G × E term will be biased in Model 2 when: 1) the covariate is related to the genetic variable and the covariate × environment interaction coefficient is nonzero; or 2) the covariate is related to environmental variable and the covariate × gene interaction coefficient is nonzero. Nevertheless, the decision of whether to include or drop covariates along with their interaction terms in a model should be based on theory, not on statistical significance. As demonstrated via simulation by Yzerbyt *et al.* (18), dropping nonsignificant covariate interaction terms can seriously inflate the type-I error rate of the G × E term. Terms that are nonsignificant can still share enough variance with the G × E term to change conclusions about its significance.

Finally, it should be noted that, even if a G × E result "disappears" after properly controlling for covariates, this does not necessarily mean that the original G × E hypothesis was wrong. For example, the genetic polymorphism might cause changes in the covariate, which in turn moderates the environmental variable, in which case the covariate is a mediating mechanism by which the gene moderates the environmental variable (19). That said, this possibility applies to all models that statistically control for covariates in regression, and the traditional interpretation of "disappearing" effects after controlling for a covariate is that the true causal pathway is ambiguous and alternative (confounding) explanations cannot be ruled out. However, in some cases, a particular causal pathway can be discarded as impossible or unlikely. In such cases, investigators can be more definitive about ruling out certain hypotheses. For example, changes at a genetic polymorphism will not lead to changes in ethnicity, and so a G × E hypothesis can be safely discarded if it is mediated by an ethnicity × environment interaction.

Three Examples of Misspecified Models in the G × E Literature

I briefly review three highly cited examples from the G × E literature where investigators improperly attempted to control for covariates in their regression models. The purpose is not to draw attention to these studies per se or to suggest that they are particularly egregious examples of this practice; as shown below, no G × E study reviewed here properly controlled for covariates. Rather, the purpose is to better illustrate the problem with

examples representative of the field and to allow the reader to gauge the plausibility (or implausibility) of alternative explanations that could have been tested had investigators properly controlled for covariates.

Kaufman *et al.*, 2004: A G × E or an Ethnicity × Environment Interaction?

With a mixed-ethnicity sample (32% African-American, 22% biracial, and 46% non–African-American; $n = 104$), Kaufman *et al.* (20) reported results showing that the depressogenic effect of a repeat polymorphism (short/long [s/l]) at the serotonin-transporter-linked polymorphic region (5-HTTLPR) depended on childhood maltreatment and on social support. I focus first on the two-way 5-HTTLPR × maltreatment interaction they describe. The investigators included ethnicity (the ancestral proportion score), age, and gender in the regression equation "given the relevance of these potential confounding variables in interpreting the study results" (20). The test of the 5-HTTLPR × maltreatment interaction was significant ($p = .007$), and this effect was primarily due to maltreated individuals with the s/s allele having significantly higher depression scores. However, as noted by the investigators, African Americans have a significantly higher frequency of the long repeat allele compared with non-African Americans. If, due to cultural norms, maltreated African Americans are less likely to report depression than maltreated non-African Americans, some or all of the detected G × E interaction might have been due to ethnicity moderating the effect of maltreatment. Somewhat less plausibly, it is also possible that the effect of 5-HTTLPR on depression depends on ethnicity. If African Americans in the sample had different rates of maltreatment, a 5-HTTLPR × ethnicity interaction might also have caused the apparent 5-HTTLPR × maltreatment interaction. Because the authors failed to include the environment × ethnicity and gene × ethnicity interaction terms, these alternative explanations for their findings cannot be ruled out.

Kaufman *et al.* (20) reported their 5-HTTLPR × maltreatment interaction in a model that also tested a two-way 5-HTTLPR × social support interaction (p = nonsignificant) and a three-way 5-HTTLPR × maltreatment × social support interaction ($p = .0001$). This raises two issues. First, in models testing three-way interactions, investigators must include not only all relevant two-way covariate × gene and covariate × environment interactions but also all relevant three-way interactions involving the covariate. With small sample sizes, this can eat up a relatively large number of available degrees of freedom, but it is necessary if investigators wish to eliminate these covariates as explanations for their interaction results.

Second, it is difficult and potentially misleading to interpret two-way interactions in the presence of three-way interactions. In such a model, the lower-order two-way interactions become "conditional" interactions, and the regression betas and p values are interpreted as the predicted two-way interactions when the other (omitted) variable is coded as zero (21). For example, the 5-HTTLRP × maltreatment interaction reported by Kaufman *et al.* (20) is the predicted effect of this interaction when social support is at zero. Whether "zero" is meaningful (e.g., the average level of social support) or not (e.g., outside the range of the data) is essential for interpreting the lower-order interactions (the exact same issue applies to "main" effects in the context of interactions). Because the authors do not mention their final coding scheme for social support, it is not possible to know whether the reported significant two-way interaction is meaningful, although in interpreting their aforementioned results, it was assumed that the authors centered social support so that the two-way 5-HTTLRP × maltreatment effect is the interaction predicted to occur among those at average levels of social support.

Caspi *et al.*, 2005: The Effect of Catechol-*O*-Methyltransferase on Psychosis Risk Depends on Adolescent Cannabis Use, But Is Cannabis the True Moderator?

In a sample of 803 Caucasian individuals, Caspi *et al.* (22) found that adolescent-onset cannabis use interacted with a single nucleotide polymorphism in the catechol-*O*-methyltransferase (*COMT*) gene to significantly predict several related adult psychotic symptoms. The investigators attempted to rule out the hypothesis that early cannabis use was a gateway to using amphetamines and hallucinogens, which in turn were the true moderators of the *COMT* polymorphism. They did this by including amphetamine/hallucinogen usage as a covariate in the model, which unsurprisingly (see Equation 3) had little effect on the *COMT* × cannabis use interaction. However, given that there is a relationship between early cannabis use and later usage of "harder" drugs (23), it is possible that the observed interaction had little to do with cannabis use but rather was driven by or was partially mediated by a *COMT* × hallucinogen/amphetamine interaction. Similarly, Caspi *et al.* (22) attempted to eliminate the counter-explanation that the *COMT* × cannabis interaction was driven by conduct disorder by including conduct disorder as a covariate, which again had little effect on the interaction result. However, given the relationship between cannabis use and conduct disorder reported by the investigators, it is also possible that the observed interaction was caused by *COMT* effects differing by level of conduct disorder. In other words, despite attempts to show the specificity of the interaction by controlling for covariates, their findings do not provide convincing evidence that adolescent cannabis use per se moderated the effect of *COMT*. Finally, if *COMT* itself is related either to hallucinogen/amphetamine usage or to conduct disorder (due to a passive or evocative gene–environment correlation or to subtle stratification effects), then it is possible that there is no G × E interaction here at all. Rather, the effect of conduct disorder (or hallucinogen/amphetamine usage, socioeconomic status, IQ, etc.) on psychosis might depend on cannabis usage, and the apparent G × E interaction might have actually been caused by a covariate × cannabis interaction.

Cicchetti *et al.*, 2007: A G × E or a Gender × Environment Interaction?

With a mixed-gender (54% male) sample of 267 individuals, Cicchetti *et al.* (24) found that a repeat polymorphism in the X-linked monoamine oxidase A (*MAOA*) gene interacted with childhood maltreatment to predict depression. The investigators coded "high activity" of the gene as having more than 3.5 repeats (63% allele frequency) and "low activity" as having fewer than 3.5 repeats. Because male subjects have only one copy of the gene, coding the genetic variable for male subjects was straightforward, but it was unclear how to code heterozygous (high/low) female subjects, who were therefore excluded. However, this coding strategy probably induced a relationship between *MAOA* activity and gender. The proportion of male subjects was approximately .63 for the high-activity allele and approximately .37 for the low-activity allele. However, for female subjects these proportions were approximately $.63^2 = .40$ for the high-activity and approximately $.37^2 = .14$ for the low-activity alleles (assuming Hardy-Weinberg equilibrium). Thus, female subjects were probably over-represented in the high-activity group: there were approximately 1.7 times more male subjects in the high- vs. low-activity groups but

approximately 2.9 times more female subjects in the high- vs. low-activity groups. Investigators controlled for the main effects of gender and ethnicity but not for their interactions with *MAOA* activity or childhood maltreatment. Therefore, a potential alternative to their findings is that the effects of maltreatment depend on gender, which presented itself as a *MAOA* × maltreatment interaction in their results. Last, this study also used a mixed-ethnicity sample of African Americans, European Americans, and Hispanics, and given large differences in *MAOA* allele frequencies between ethnicities (25), it is also possible that the observed interaction was driven by an ethnicity × maltreatment or an ethnicity × *COMT* interaction.

Literature Review

To understand the extent of improper usage of covariates in G × E studies, I selected all ($n = 47$) novel G × E studies that were identified in the Duncan and Keller (1) review of the first 10 years of candidate G × E studies in psychiatry. Novel studies (first reports of a given G × E finding) were selected, because replication attempts were likely to employ the same model used in the original report and therefore would provide redundant information about typical practices for controlling covariates. Studies were coded according to the following criteria: 1) whether they reported significant G × E findings or not; 2) whether the investigators properly controlled for covariates by including all relevant covariate × gene and covariate × environment interactions; and 3) whether the sample was ethnically heterogeneous or not. Of the 47 studies, 45 (96%) reported significant G × E results (Table 1). This high rate, when compared with the lower rate (27%) of positive results among replication attempts (not shown), is probably symptomatic of publication bias (1). As shown in Table 1, of the 41 studies that attempted to statistically control for potential confounders by including them as covariates in linear models, none used the properly specified model. Assuming that this sample of studies is representative of studies in the wider G × E literature, it is likely that almost all published G × E findings that have attempted to statistically control for covariates have done so improperly, and thus alternative explanations for these findings cannot be ruled out.

Because allele frequencies in the candidate genes typically investigated in G × E studies often differ across ethnicities, an ethnicity × environment interaction is a particularly plausible alternative explanation for G × E findings from ethnically heterogeneous samples. Of the 47 studies, 26 used an ethnically homogeneous sample, 10 used an ethnically heterogeneous sample, and 11 did not provide information about ethnicity. Most but not all of those studies that failed to provide information about the ethnic compositions of their samples were conducted in Europe and presumably used ethnically homogeneous samples. Thus, stratification is a possible alternative explanation for approximately one fifth of these G × E results.

Finally, although no study included all relevant covariate × environment or covariate × gene terms to control for the effects of the covariates on G × E interactions, it should be noted that several studies conducted follow-up analyses that went at least partway toward eliminating certain covariates as alternative explanations for the G × E findings. Dick *et al.* (26) tested gender × gene and age × gene interactions in separate models that did not include the G × E term and found they were not significant. This procedure does make it less likely that the two covariates investigated are responsible for the reported G × E interaction. However, it did not include the covariate × gene or covariate × environment interactions in the primary model, and as noted in the preceding section, even nonsignificant covariate interaction terms can substantively change conclusions about the interaction of interest. Caspi et al (27) stratified their sample by *MAOA* genotype and noted that the interaction held in each subsample. This is a highly

Table 1. G × E Studies

First Author	Year	Ref	Sig G × E?	Eth Het?	Proper Control?
Amstadter	2009	28	Y	Y	N
Aslund	2009	29	Y	Y	N/A
Bakermans-Kranenburg	2006	31	Y	U	N/A
Bau	2000	32	Y	Y	N
Bet	2009	33	Y	N	N
Binder	2008	34	Y	Y	N
Blomeyer	2008	35	Y	N	N
Bradley	2008	36	Y	Y	N
Caspi	2002	37	Y	N	N
Caspi	2003	27	Y	N	N
Caspi	2005	22	Y	N	N
Chotai	2003	38	Y	U	N
Covault	2007	39	Y	N	N
Dick	2006	26	Y	N	N
Fox	2005	40	Y	U	N
Gacek	2008	41	N	N	N
Gervai	2007	42	Y	Y	N
Grabe	2005	43	Y	N	N
Grabe	2009	44	Y	N	N
Haeffel	2008	45	Y	N	N/A
Henquet	2009	46	Y	U	N
Jokela	2007	47	Y	U	N
Jokela	2007	48	Y	U	N
Jokela	2007	49	Y	U	N
Jokela	2007	50	Y	U	N
Kahn	2003	51	Y	Y	N
Keltikangas-Järvinen	2004	52	Y	U	N
Koenen	2009	53	Y	Y	N
Lahti	2006	54	Y	U	N
Lasky-Su	2007	55	Y	U	N
Laucht	2007	56	Y	N	N
Nobile	2007	57	Y	N	N
Nobile	2009	58	Y	N	N
Ozkaragoz	2000	59	Y	N	N/A
Perroud	2008	60	Y	N	N
Racine	2009	61	N	N	N
Retz	2008	62	Y	N	N
Seeger	2004	63	Y	N	N/A
Stein	2008	64	Y	N	N
Stevens	2009	65	Y	N	N
Sun	2008	66	Y	N	N/A
Todd	2007	67	Y	Y	N
van Winkel	2008	68	Y	N	N
Vanyukov	2007	69	Y	N	N
Waldman	2007	30	Y	Y	N
Xu	2009	70	Y	N	N
Yen	2008	71	Y	N	N

Eth. Het? = whether the sample was ethnically heterogeneous [U = unknown]; Proper Control? = whether the investigators included all relevant covariate × gene and covariate × environment interactions in the model testing gene × environment (G × E) [N/A = not applicable because investigators did not attempt to control for any covariates]; Ref, reference number; Sig. G × E? = whether the primary G × E hypothesis was statistically significant. N, no; Y, yes.

conservative control for *MAOA* and essentially eliminates it as a potential confounder of their G × E finding, but the investigators did not control for their other covariate (gender) in the same way and did not control for any other potential confounders. Similarly, Amstadter *et al.* (28) and Aslund *et al.* (29) restricted follow-up analyses to ethnically homogeneous subsamples and found similar results to their original ones, eliminating stratification as a possible alternative explanation to their findings. However, the datasets were not similarly stratified on other covariates. Finally, the study by Waldman (30) was the only one of those investigated that included several covariate × gene (in this case) interaction terms in the primary model to eliminate several alternative explanations for the G × E finding, but the study failed to control for other covariates such as ethnicity in a similar way.

Conclusions

Because G × E research is inherently nonexperimental (even if the environmental variable is manipulated, the genetic variable cannot be), it is essential that investigators control for potential confounders to eliminate alternative explanations for G × E results. Unfortunately, it seems that virtually no G × E studies to date have appropriately controlled for covariates. This is not to say that previously published G × E findings are necessarily wrong; properly controlling for confounders would not have changed conclusions in some cases and might have even strengthened them in others. However, the point is that it is unknown how often G × E conclusions would have changed with properly specified models, and this is cause for concern.

There are at least two related potential objections to the recommendation to include all relevant covariate × gene and covariate × environment interactions to models estimating a G × E term. The first has to do with overfitting: with so many terms, it might be unrealistically hopeful to obtain precise estimates of all the covariate interaction terms, especially if sample sizes are small. However, the purpose of including covariate interaction terms is not to estimate their effects per se but rather to control for their effects on the G × E term of interest. The second potential objection is that, with a large number of interaction terms included in the model, multicolinearity might degrade evidence for the G × E term. However, this is entirely the point. To the degree interaction terms containing covariates are correlated with the G × E term, alternative explanations for the observed G × E interaction are possible. Moreover, inclusion of covariate interaction terms in a model tested on the full dataset is a much more statistically powerful approach for controlling potential confounders than splitting the data by covariates and testing the G × E term in each subset of the data. Finally, investigators should be assuaged by the fact that if covariate interaction terms have no true relationship with the G × E term, the G × E interaction estimate typically changes very little and is as likely to be strengthened as weakened by proper inclusion of covariate interaction terms.

The recommendations of this review extend to future genome-wide interaction studies as well. For such studies, it is not sufficient to control for stratification, site, platform, and plate effects as done in traditional (main effect) genome-wide studies. Rather, all relevant covariate × gene and covariate × environment interactions must also be included in the model to eliminate artifactual genome-wide signals that might otherwise swamp what are likely to be small true G × E signals.

In summary, G × E research has generated much excitement over the past decade. Findings from the field suggest an appealing possibility: genes are not destiny—their effects depend on environmental context. This might often be true, but to date, the field has not convincingly demonstrated that any particular G × E finding is robust. This is not only because investigators have failed to properly specify covariates in their models but also because sample sizes have typically been small, the appropriateness of multiple testing corrections has been difficult to verify, and the unpublished "file drawer" of negative findings might be large. These issues have led to an erosion of confidence in published G × E findings. This confidence will increase as investigators, reviewers, and editors acknowledge these issues and take steps to rectify them.

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1. Duncan LE, Keller MC (2011): A critical review of the first ten years of candidate gene-by-environment interaction research in psychiatry. *Am J Psychiatry* 168:1041–1049.
2. Eaves LJ (2006): Genotype x Environment interaction in psychopathology: Fact or artifact? *Twin Res Hum Genet* 9:1–8.
3. Munafo MR, Durrant C, Lewis G, Flint J (2009): Gene X environment interactions at the serotonin transporter locus. *Biol Psychiatry* 65: 211–219.
4. Munafo MR, Flint J (2009): Replication and heterogeneity in gene x environment interaction studies. *Int J Neuropsychopharmacol* 12: 727–729.
5. Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J, *et al.* (2009): Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression: A meta-analysis. *JAMA* 301: 2462–2471.
6. Flint J, Munafo MR (2008): Forum: Interactions between gene and environment. *Curr Opin Psychiatry* 21:315–317.
7. McClelland GH, Judd CM (1993): Statistical difficulties of detecting interactions and moderator effects. *Psychol Bull* 114:376–390.
8. Ioannidis JP (2005): Why most published research findings are false. *PLoS Med* 2:e124.
9. Bosker FJ, Hartman CA, Nolte IM, Prins BP, Terpstra P, Posthuma D, *et al.* (2011): Poor replication of candidate genes for major depressive disorder using genome-wide association data. *Mol Psychiatry* 16:516–532.
10. Collins AL, Kim Y, Sklar P, O'Donovan MC, Sullivan PF (2012): Hypothesis-driven candidate genes for schizophrenia compared to genome-wide association results. *Psychol Med* 42:607–616.
11. Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, *et al.* (2009): A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet* 5:e1000373.
12. Sanders AR, Duan J, Levinson DF, Shi J, He D, Hou C, *et al.* (2008): No significant association of 14 candidate genes with schizophrenia in a large European ancestry sample: Implications for psychiatric genetics. *Am J Psychiatry* 165:497–506.
13. Sullivan PF, Lin D, Tzeng JY, van den Oord E, Perkins D, Stroup TS, *et al.* (2008): Genomewide association for schizophrenia in the CATIE study: Results of stage 1. *Mol Psychiatry* 13:570–584.
14. Murcray CE, Lewinger JP, Gauderman WJ (2009): Gene-environment interaction in genome-wide association studies. *Am J Epidemiol* 169: 219–226.
15. Hewitt JK (2012): Editorial policy on candidate gene association and candidate gene-by-environment interaction studies of complex traits. *Behav Genet* 42:1–2.
16. Johnston C, Lahey BB, Matthys W (2013): Editorial policy for candidate gene studies. *J Abnorm Child Psychol* 41:511–514.
17. Hull JG, Tedlie JC, Lehn DA (1992): Moderator variables in personality research: The problem of controlling for plausible alternatives. *Pers Soc Psychol B* 18:115–117.

18. Yzerbyt VY, Muller D, Judd CM (2004): Adjusting researchers' approach to adjustment: On the use of covariates when testing interactions. *J Exp Soc Psychol* 40:424–431.
19. Muller D, Judd CM, Yzerbyt VY (2005): When moderation is mediated and mediation is moderated. *J Pers Soc Psychol* 89:852–863.
20. Kaufman J, Yang BZ, Douglas-Palumberi H, Houshyar S, Lipschitz D, Krystal JH, *et al*. (2004): Social supports and serotonin transporter gene moderate depression in maltreated children. *Proc Natl Acad Sci U S A* 101:17316–17321.
21. Cohen J, Cohen P, West SG, Aiken LS (2003): *Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences*. Mahwah, NJ: Lawrence Erlbaum.
22. Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, *et al*. (2005): Moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catechol-*O*-methyltransferase gene: Longitudinal evidence of a gene X environment interaction. *Biol Psychiatry* 57:1117–1127.
23. Lynskey MT, Heath AC, Bucholz KK, Slutske WS, Madden PA, Nelson EC, *et al*. (2003): Escalation of drug use in early-onset cannabis users vs co-twin controls. *JAMA* 289:427–433.
24. Cicchetti D, Rogosch FA, Sturge-Apple ML (2007): Interactions of child maltreatment and serotonin transporter and monoamine oxidase A polymorphisms: Depressive symptomatology among adolescents from low socioeconomic status backgrounds. *Dev Psychopathol* 19: 1161–1180.
25. Sabol SZ, Hu S, Hamer D (1998): A functional polymorphism in the monoamine oxidase A gene promoter. *Hum Genet* 103:273–279.
26. Dick DM, Agrawal A, Schuckit MA, Bierut L, Hinrichs A, Fox L, *et al*. (2006): Marital status, alcohol dependence, and GABRA2: Evidence for gene-environment correlation and interaction. *J Stud Alcohol* 67: 185–194.
27. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, *et al*. (2003): Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene. *Science* 301:386–389.
28. Amstadter AB, Koenen KC, Ruggiero KJ, Acierno R, Galea S, Kilpatrick DG, *et al*. (2009): Variant in RGS2 moderates posttraumatic stress symptoms following potentially traumatic event exposure. *J Anxiety Disord* 23:369–373.
29. Aslund C, Leppert J, Comasco E, Nordquist N, Oreland L, Nilsson KW (2009): Impact of the interaction between the 5HTTLPR polymorphism and maltreatment on adolescent depression. A population-based study. *Behav Genet* 39:524–531.
30. Waldman ID (2007): Gene-environment interactions reexamined: Does mother's marital stability interact with the dopamine receptor D2 gene in the etiology of childhood attention-deficit/hyperactivity disorder? *Dev Psychopathol* 19:1117–1128.
31. Bakermans-Kranenburg MJ, van Ijzendoorn MH (2006): Gene-environment interaction of the dopamine D4 receptor (DRD4) and observed maternal insensitivity predicting externalizing behavior in preschoolers. *Dev Psychobiol* 48:406–409.
32. Bau CH, Almeida S, Hutz MH (2000): The TaqI A1 allele of the dopamine D2 receptor gene and alcoholism in Brazil: Association and interaction with stress and harm avoidance on severity prediction. *Am J Med Genet* 96:302–306.
33. Bet PM, Penninx BW, Bochdanovits Z, Uitterlinden AG, Beekman AT, van Schoor NM, *et al*. (2009): Glucocorticoid receptor gene polymorphisms and childhood adversity are associated with depression: New evidence for a gene-environment interaction. *Am J Med Genet B Neuropsychiatr Genet* 150B:660–669.
34. Binder EB, Bradley RG, Liu W, Epstein MP, Deveau TC, Mercer KB, *et al*. (2008): Association of FKBP5 polymorphisms and childhood abuse with risk of posttraumatic stress disorder symptoms in adults. *JAMA* 299:1291–1305.
35. Blomeyer D, Treutlein J, Esser G, Schmidt MH, Schumann G, Laucht M (2008): Interaction between CRHR1 gene and stressful life events predicts adolescent heavy alcohol use. *Biol Psychiatry* 63:146–151.
36. Bradley RG, Binder EB, Epstein MP, Tang Y, Nair HP, Liu W, *et al*. (2008): Influence of child abuse on adult depression: Moderation by the corticotropin-releasing hormone receptor gene. *Arch Gen Psychiatry* 65:190–200.
37. Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, *et al*. (2002): Role of genotype in the cycle of violence in maltreated children. *Science* 297:851–854.
38. Chotai J, Serretti A, Lattuada E, Lorenzi C, Lilli R (2003): Gene-environment interaction in psychiatric disorders as indicated by season of birth variation in tryptophan hydroxylase (TPH), serotonin transporter (5-HTTLPR) and dopamine receptor (DRD4) gene polymorphisms. *Psychiatry Res* 119:99–111.
39. Covault J, Tennen H, Armeli S, Conner TS, Herman AI, Cillessen AH, *et al*. (2007): Interactive effects of the serotonin transporter 5-HTTLPR polymorphism and stressful life events on college student drinking and drug use. *Biol Psychiatry* 61:609–616.
40. Fox NA, Nichols KE, Henderson HA, Rubin K, Schmidt L, Hamer D, *et al*. (2005): Evidence for a gene-environment interaction in predicting behavioral inhibition in middle childhood. *Psychol Sci* 16:921–926.
41. Gacek P, Conner TS, Tennen H, Kranzler HR, Covault J (2008): Tryptophan hydroxylase 2 gene and alcohol use among college students. *Addict Biol* 13:440–448.
42. Gervai J, Novak A, Lakatos K, Toth I, Danis I, Ronai Z, *et al*. (2007): Infant genotype may moderate sensitivity to maternal affective communications: Attachment disorganization, quality of care, and the DRD4 polymorphism. *Soc Neurosci* 2:307–319.
43. Grabe HJ, Lange M, Wolff B, Volzke H, Lucht M, Freyberger HJ, *et al*. (2005): Mental and physical distress is modulated by a polymorphism in the 5-HT transporter gene interacting with social stressors and chronic disease burden. *Mol Psychiatry* 10:220–224.
44. Grabe HJ, Spitzer C, Schwahn C, Marcinek A, Frahnow A, Barnow S, *et al*. (2009): Serotonin transporter gene (SLC6A4) promoter polymorphisms and the susceptibility to posttraumatic stress disorder in the general population. *Am J Psychiatry* 166:926–933.
45. Haeffel GJ, Getchell M, Koposov RA, Yrigollen CM, Deyoung CG, Klinteberg BA, *et al*. (2008): Association between polymorphisms in the dopamine transporter gene and depression: Evidence for a gene-environment interaction in a sample of juvenile detainees. *Psychol Sci* 19:62–69.
46. Henquet C, Rosa A, Delespaul P, Papiol S, Fananas L, van Os J, *et al*. (2009): COMT ValMet moderation of cannabis-induced psychosis: A momentary assessment study of 'switching on' hallucinations in the flow of daily life. *Acta Psychiatr Scand* 119:156–160.
47. Jokela M, Lehtimaki T, Keltikangas-Jarvinen L (2007): The influence of urban/rural residency on depressive symptoms is moderated by the serotonin receptor 2A gene. *Am J Med Genet B Neuropsychiatr Genet* 144B:918–922.
48. Jokela M, Lehtimaki T, Keltikangas-Jarvinen L (2007): The serotonin receptor 2A gene moderates the influence of parental socioeconomic status on adulthood harm avoidance. *Behav Genet* 37:567–574.
49. Jokela M, Keltikangas-Jarvinen L, Kivimaki M, Puttonen S, Elovainio M, Rontu R, *et al*. (2007): Serotonin receptor 2A gene and the influence of childhood maternal nurturance on adulthood depressive symptoms. *Arch Gen Psychiatry* 64:356–360.
50. Jokela M, Raikkonen K, Lehtimaki T, Rontu R, Keltikangas-Jarvinen L (2007): Tryptophan hydroxylase 1 gene (TPH1) moderates the influence of social support on depressive symptoms in adults. *J Affect Disord* 100:191–197.
51. Kahn RS, Khoury J, Nichols WC, Lanphear BP (2003): Role of dopamine transporter genotype and maternal prenatal smoking in childhood hyperactive-impulsive, inattentive, and oppositional behaviors. *J Pediatr* 143:104–110.
52. Keltikangas-Jarvinen L, Raikkonen K, Ekelund J, Peltonen L (2004): Nature and nurture in novelty seeking. *Mol Psychiatry* 9:308–311.
53. Koenen KC, Aiello AE, Bakshis E, Amstadter AB, Ruggiero KJ, Acierno R, *et al*. (2009): Modification of the association between serotonin transporter genotype and risk of posttraumatic stress disorder in adults by county-level social environment. *Am J Epidemiol* 169: 704–711.
54. Lahti J, Raikkonen K, Ekelund J, Peltonen L, Raitakari OT, Kelitkangas-Jarvinen (2006): Socio-demographic characteristics moderate the association between DRD4 and Novelty seeking. *Pers Indiv Differ* 40: 533–543.
55. Lasky-Su J, Faraone SV, Lange C, Tsuang MT, Doyle AE, Smoller JW, *et al*. (2007): A study of how socioeconomic status moderates the relationship between SNPs encompassing BDNF and ADHD symptom counts in ADHD families. *Behav Genet* 37:487–497.
56. Laucht M, Skowronek MH, Becker K, Schmidt MH, Esser G, Schulze TG, *et al*. (2007): Interacting effects of the dopamine transporter gene and psychosocial adversity on attention-deficit/hyperactivity disorder

symptoms among 15-year-olds from a high-risk community sample. *Arch Gen Psychiatry* 64:585–590.
57. Nobile M, Giorda R, Marino C, Carlet O, Pastore V, Vanzin L, *et al*. (2007): Socioeconomic status mediates the genetic contribution of the dopamine receptor D4 and serotonin transporter linked promoter region repeat polymorphisms to externalization in preadolescence. *Dev Psychopathol* 19:1147–1160.
58. Nobile M, Rusconi M, Bellina M, Marino C, Giorda R, Carlet O, *et al*. (2009): The influence of family structure, the TPH2 G-703T and the 5-HTTLPR serotonergic genes upon affective problems in children aged 10–14 years. *J Child Psychol Psychiatry* 50:317–325.
59. Ozkaragoz T, Noble EP (2000): Extraversion. Interaction between D2 dopamine receptor polymorphisms and parental alcoholism. *Alcohol* 22:139–146.
60. Perroud N, Courtet P, Vincze I, Jaussent I, Jollant F, Bellivier F, *et al*. (2008): Interaction between BDNF Val66Met and childhood trauma on adult's violent suicide attempt. *Genes Brain Behav* 7:314–322.
61. Racine SE, Culbert KM, Larson CL, Klump KL (2009): The possible influence of impulsivity and dietary restraint on associations between serotonin genes and binge eating. *J Psychiatr Res* 43:1278–1286.
62. Retz W, Freitag CM, Retz-Junginger P, Wenzler D, Schneider M, Kissling C, *et al*. (2008): A functional serotonin transporter promoter gene polymorphism increases ADHD symptoms in delinquents: Interaction with adverse childhood environment. *Psychiatry Res* 158:123–131.
63. Seeger G, Schloss P, Schmidt MH, Ruter-Jungfleisch A, Henn FA (2004): Gene-environment interaction in hyperkinetic conduct disorder (HD + CD) as indicated by season of birth variations in dopamine receptor (DRD4) gene polymorphism. *Neurosci Lett* 366:282–286.
64. Stein MB, Schork NJ, Gelernter J (2008): Gene-by-environment (serotonin transporter and childhood maltreatment) interaction for anxiety sensitivity, an intermediate phenotype for anxiety disorders. *Neuropsychopharmacology* 33:312–319.
65. Stevens SE, Kumsta R, Kreppner JM, Brookes KJ, Rutter M, Sonuga-Barke EJ (2009): Dopamine transporter gene polymorphism moderates the effects of severe deprivation on ADHD symptoms: Developmental continuities in gene-environment interplay. *Am J Med Genet B Neuropsychiatr Genet* 150B:753–761.
66. Sun N, Xu Y, Wang Y, Duan H, Wang S, Ren Y, *et al*. (2008): The combined effect of norepinephrine transporter gene and negative life events in major depression of Chinese Han population. *J Neural Transm* 115:1681–1686.
67. Todd RD, Neuman RJ (2007): Gene-environment interactions in the development of combined type ADHD: Evidence for a synapse-based model. *Am J Med Genet B Neuropsychiatr Genet* 144B:971–975.
68. van Winkel R, Henquet C, Rosa A, Papiol S, Fananas L, De Hert M, *et al*. (2008): Evidence that the COMT(Val158Met) polymorphism moderates sensitivity to stress in psychosis: An experience-sampling study. *Am J Med Genet B Neuropsychiatr Genet* 147B:10–17.
69. Vanyukov MM, Maher BS, Devlin B, Kirillova GP, Kirisci L, Yu LM, *et al*. (2007): The MAOA promoter polymorphism, disruptive behavior disorders, and early onset substance use disorder: Gene-environment interaction. *Psychiatr Genet* 17:323–332.
70. Xu Y, Li F, Huang X, Sun N, Zhang F, Liu P, *et al*. (2009): The norepinephrine transporter gene modulates the relationship between urban/rural residency and major depressive disorder in a Chinese population. *Psychiatry Res* 168:213–217.
71. Yen YC, Rebok GW, Yang MJ, Lung FW (2008): A multilevel analysis of the influence of Apolipoprotein E genotypes on depressive symptoms in late-life moderated by the environment. *Prog Neuropsychopharmacol Biol Psychiatry* 32:479–486.