



REVIEWS AND COMMENTARY

Nontraditional Epidemiologic Approaches in the Analysis of Gene-Environment Interaction: Case-Control Studies with No Controls!

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Although case-control studies are suitable for assessing gene-environment interactions, choosing appropriate control subjects is a valid concern in these studies. The authors review three nontraditional study designs that do not include a control group: 1) the case-only study, 2) the case-parental control study, and 3) the affected relative-pair method. In case-only studies, one can examine the association between an exposure and a genotype among case subjects only. Odds ratios are interpreted as a synergy index on a multiplicative scale, with independence assumed between the exposure and the genotype. In case-parental control studies, one can compare the genotypic distribution of case subjects with the expected distribution based on parental genotypes when there is no association between genotype and disease; the effect of a genotype can be stratified according to case subjects' exposure status. In affected relative-pair studies, the distribution of alleles identical by descent between pairs of affected relatives is compared with the expected distribution based on the absence of genetic linkage between the locus and the disease; the analysis can be stratified according to exposure status. Some or all of these methods have certain limitations, including linkage disequilibrium, confounding, assumptions of Mendelian transmission, an inability to measure exposure effects directly, and the use of a multiplicative scale to test for interaction. Nevertheless, they provide important tools to assess gene-environment interaction in disease etiology. *Am J Epidemiol* 1996;144:207-13.

case-control studies; environment; genes

As a result of advances in the Human Genome Project, the field of genetic epidemiology has assumed a prominent role in the investigation of gene-environment interaction for various diseases (1-9). Because of the relative ease of searching for a large number of DNA markers at several candidate gene

loci, the case-control method is increasingly used to search for gene-environment interaction (10-12). Using case-control studies, investigators can assess the associations of several genes and exposures with disease outcomes (12). This association approach complements the use of linkage analysis of family data to localize specific gene loci.

A crucial consideration in case-control studies is the choice of an appropriate control group. In these studies, the use of convenient control subjects to look for associations with disease status may lead to spurious findings as a result of confounding due to population stratification (e.g., if cases and controls come from different racial/ethnic backgrounds). To deal with potential confounding, some have suggested using the relatives of case subjects as controls (13, 14). Nevertheless, the use of relatives as control subjects may

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Abbreviations: COR, case-only odds ratio; OR, odds ratio; SIM, synergy index; TGF α , transforming growth factor alpha.

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lead to overmatching on a variety of genetic and environmental factors (14).

Over the last few years, several nontraditional approaches have emerged in the study of genetic factors in disease. These approaches essentially involve the use of an internal control group rather than an external one. In this commentary, we review three case-control study approaches without controls: 1) the case-only study, 2) the case-parental control study, and 3) the affected relative-pair study. The various approaches and their assumptions and limitations are summarized in table 1. These approaches will be used increasingly in the search for gene-environment interaction in disease etiology and pathogenesis.

GENE-ENVIRONMENT INTERACTION ANALYSIS IN THE CONTEXT OF A CASE-CONTROL STUDY

Let us first review the traditional analysis of gene-environment interaction in a regular case-control study, assuming appropriate control subjects have been chosen. For simplicity, we assume that an exposure is classified as being either present or absent, and that the underlying susceptibility genotype is also classified as present or absent. This genotype could reflect the presence of one or two alleles at one locus or a combination of alleles at multiple loci. To evaluate for gene-environment interaction, researchers could display data in a 2×4 table (table 2). Using unexposed subjects with no susceptibility genotype as the referent group, one can compute odds ratios for all other groups. Joint odds ratios (for the exposure and the genotype) (OR_{ge}) can be compared with odds ratios

TABLE 2. Gene-environment interaction analysis in the context of a case-control study

Exposure*	Susceptibility genotype	Cases	Controls	Odds ratio†
-	-	a	b	1.0
-	+	c	d	$OR_g = bc/ad$
+	-	e	f	$OR_e = be/af$
+	+	g	h	$OR_{ge} = bg/ah$

* -, absent; +, present.

† Under an additive model: $OR_{ge} = OR_g + OR_e - 1$.

Under a multiplicative model: $OR_{ge} = OR_g \times OR_e$.

for the effects of exposure alone (OR_e) or of the genotype alone (OR_g). In a multiplicative model, the joint odds ratio is simply the product of individual odds ratios. To assess for interaction on a multiplicative scale, we can define a synergy index (SIM) as:

$$SIM = OR_{ge}/OR_g \times OR_e.$$

A SIM of more than one indicates more than multiplicative effects between the exposure and the genotype, while a SIM of less than one indicates less than multiplicative effects. An additive model of risk can also be derived (table 2). Adjustment for other potential confounding variables can be done by using stratified analyses or logistic models. Finally, for rare diseases, odds ratios approximate risk ratios derived from cohort studies.

A crucial point to keep in mind is that any association shown between a genotype and a disease in a case-control study may or may not reflect a true causal role of the allele. More often than not, the allele is in

TABLE 1. Characteristics of nontraditional case-only studies that can address gene-environment interaction

Feature	Case-only	Case-parental control	Affected relative-pair
Study subjects	Cases	Cases and their parents	Second case in family, proband, and parents
"Controls"	None	Expected genotype distribution based on parental genotypes	Expected distribution of alleles with Mendelian transmission
Assessment	Departure from multiplicative effects between exposure and genotype	Association between genotype and disease. Also departure from multiplicative effects	Linkage between locus and disease. Also departure from multiplicative effects
Assumptions	Independence between genotype and exposure.	Mendelian transmission	Mendelian transmission
Limitations	Although simple, cannot assess effects of exposure or genotype. Linkage disequilibrium	Requires one or both parents. Cannot assess exposure effects. Linkage disequilibrium	Need families with 2 or more cases. Cannot assess exposure. Cannot assess specific alleles

linkage disequilibrium with a disease susceptibility allele at a nearby locus. Occasionally, the association may be due to confounding as a result of population stratification (e.g., by race/ethnicity) (10).

For example, in a recent case-control study, Hwang et al. (11) assessed the effects of the interaction between maternal cigarette smoking and a transforming growth factor alpha (TGF α) polymorphism and the risk of oral clefts. They showed evidence of interaction between maternal smoking and the presence of *TaqI* polymorphism at this locus for cleft palate only. The results of crude analyses are shown in table 3. The authors had grouped persons with 1 or 2 *TaqI* polymorphisms as + genotype. As can be seen, the odds ratios for the exposure alone or for the genotype alone are close to unity, whereas the combined odds ratio for smoking and the genotype is 5.5 (95 percent confidence interval 2.1–14.6), indicating evidence of departure from multiplicative effects between the exposure and the genotype.

GENE-ENVIRONMENT INTERACTION ANALYSIS IN THE CONTEXT OF A CASE-ONLY STUDY

Increasingly, a case-series design has been promoted as an approach that can be used to evaluate gene-environment interaction in disease etiology (15, 16). In this method, investigators use case subjects only to assess the magnitude of the association between the exposure of interest and the susceptibility genotype. The basic setup for analysis is a new 2×2 table (table 4). Odds ratios and confidence intervals can be obtained by using standard crude analyses or logistic models after adjusting for other covariates (15, 16). How can odds ratios obtained with this methodology be interpreted? It has been shown (15, 16) that the odds ratio relating the exposure and the allele among case subjects only is a function of the odds ratios for the exposure alone, the genotype alone, and their joint effects in a standard case-control study,

$$\text{COR} = \text{OR}_{ge} / (\text{OR}_e \times \text{OR}_g) \times Z,$$

TABLE 4. Gene-environment interaction analysis in the context of a case-only study*

Exposure	Susceptibility genotype	
	-	+
-	a	b
+	c	d

* COR, case-only odds ratio = ad/bc . Under assumption of independence between exposure and genotype among controls: $\text{COR} = \text{OR}_{ge} / \text{OR}_e \times \text{OR}_g = \text{SIM}$, where SIM is the synergy index.

where COR is the case-only odds ratio and Z refers to the odds ratio among control subjects relating the exposure and the susceptibility genotype (similar to table 4). If the genotype and the exposures are independent, this factor becomes unity and the odds ratio obtained from a case-only study becomes simply the synergy index on a multiplicative scale derived from a regular case-control study as shown earlier. Under the null hypothesis of no multiplicative effects, the SIM and COR are expected to be unity; if there is more than multiplicative effects, the odds ratio will be more than one. In this commentary, we do not deal directly with protective factors.

This approach provides a simple tool with which to screen for gene-environment interaction in disease etiology. It can be used in the context of crude analysis of a 2×2 table or in the context of logistic models when other covariates need to be adjusted for. As shown by Piegorsch et al. (15), this approach can result in greater precision in estimating interactions (i.e., smaller standard errors) than can be obtained by traditional case-control analyses. Furthermore, Begg and Zhang (16) observed that the odds ratio (SIM) could indicate the presence of underlying etiologic heterogeneity in the disease. Investigators can also adjust for other potential confounding factors using logistic regression analysis.

For illustration, we apply the case-only analysis to the data of Hwang et al. (11) on the association among oral clefts, maternal smoking, and TGF α polymorphisms (table 3). The case-only analysis shows a

TABLE 3. Case-control analysis of the interaction between maternal cigarette smoking, transforming growth factor alpha (*TaqI*) polymorphism, and the risk of cleft palate. Adapted from Hwang et al. (11)

Smoking	<i>TaqI</i> polymorphism	No. of cases	No. of controls	Odds ratio*,†	95% confidence interval
-	-	36	167	1.0	Referent
-	+	7	34	1.0	0.3–2.4
+	-	13	69	0.9	0.4–1.8
+	+	13	11	5.5	2.1–14.6

* Crude odds ratios are presented.

† Odds ratio based on a case-only study is 5.1 (95% confidence interval 1.5–18.5) $((13 \times 36)/(13 \times 7))$.

marked departure from multiplicative effects of the genotype and the exposure. The COR obtained from this analysis is 5.1, comparable with the SIM of 6.1 obtained from the regular case-control analysis. Also, the assumption of independence between exposure and genotype among controls is reasonable.

What are some of the methodological issues involved in applying this approach? First, the choice of cases is still subject to the usual rules of case selection for any case-control study. For example, population-based incident cases allow researchers to maximize the generalizability of their findings.

Second, researchers must assume independence between exposure and genotype in order to apply this method. This assumption may seem reasonable for a wide variety of genes and exposures. There are some genes, however, that may lead to a higher or lower likelihood of the exposure on the basis of some biologic mechanisms. For example, genetic variation in alcohol and aldehyde dehydrogenases, the main enzymes involved in alcohol metabolism, are suspected risk factors for alcoholism and alcohol-related liver damage. However, individuals with delayed alcohol metabolism as a result of this genetic variation may have an increased flushing response after alcohol ingestion and thus be less likely to seek alcohol, possibly leading to a negative correlation between alcohol exposure and alcohol dehydrogenase polymorphisms in different populations (17, 18). On the other hand, the assumption of independence between genes and exposures is more likely to be valid than the assumption of independence between nongenetic risk factors (e.g., nutritional factors, smoking, and occupational exposures). This may be an important reason why a case-only study is less valuable when interactions between different non-genetic risk factors are examined.

Third, the case-only approach does not allow the investigators to evaluate the independent effects of the exposure alone or the genotype alone, merely their interaction.

Fourth, as with a regular case-control study, associations may be due to linkage disequilibrium between the genetic marker and the true susceptibility allele(s) at a neighboring locus.

Finally, the measure obtained from this analysis can only be interpreted as a departure from multiplicative effects. There continues to be discussion in the epidemiology literature on appropriate definitions and interpretation of interaction (19–21). It is sufficient to state here that the SIM index has the limitation of not being able to assess departures from joint additive effects of the exposure and the genotype. To the extent that departure from additivity may represent the true underlying model of joint effects, this approach could

miss such interaction. On the other hand, many biologically plausible modes of gene-environment interaction do involve rather extreme departures from multiplicative effects (22).

GENE-ENVIRONMENT INTERACTION ANALYSIS IN THE CONTEXT OF THE CASE-PARENTAL CONTROL STUDY

Over the last few years, a number of authors (23–33) have published on similar analytic strategies in which the parents of case subjects are used as a sort of control group to look for genetic markers that could be associated with increased disease risk or be in linkage disequilibrium with alleles at a neighboring locus. These approaches can be collectively referred to as the case-parental control method (28). This approach is also called the haplotype relative risk (23), the genotype relative risk (26), and the transmission disequilibrium test (27).

The case-parental control method requires the availability of genotypic information on the parents of case subjects, although the method has been extended to situations in which only one parent is available (29). In its simplest form, the genotype of each case can be compared with the genotype of a fictitious control formed by the non-transmitted alleles from each parent. Because this is a matched analysis, one can construct a 2×2 table comparing case and control subjects with respect to the presence or absence of the allele (or genotype), as shown in table 5. Odds ratios can be simply obtained, and the statistical testing involves matched-pair analysis. This method can also be used to stratify case subjects according to the presence or absence of the pertinent interacting exposure, and odds ratios can be derived with or without the exposure (table 5). In spite of this method's simplicity, its

TABLE 5. Gene-environment interaction analysis in the context of a case-parental control study: analysis of non-transmitted alleles

	Susceptibility genotype in cases	
	–	+
A. Exposure: absent		
Parental non-transmitted alleles		
–	a	b
+	c	d
Relative risk (among non-exposed)	1	b/c
B. Exposure: present		
Parental non-transmitted alleles		
–	e	f
+	g	h
Relative risk (among exposed)	1	f/g

main limitation is that the "control" group may or may not be representative of the unaffected underlying population, especially given that certain parental genotypes may be associated with disease status that may interfere with reproduction.

Recently, we have proposed a method of analysis for this type of study (29). The method is noniterative and leads to a closed estimate of the risk ratio comparing risk among persons with a specific genotype with the risk among persons with a comparison genotype. Essentially, for each combination of parental genotypes, the observed distribution of the offspring (case) genotype is compared with the distribution expected on the basis of Mendelian transmission probabilities. This approach is reminiscent of a case-base study design, because the odds that case subjects have a specific genotype are compared with the odds that the entire risk set (which includes case subjects) has the genotype.

To illustrate the parental non-transmitted alleles method (29) with a very simple example, we will assume that 100 case subjects have a rare fully penetrant autosomal recessive disease, with no etiologic heterogeneity. Both parents are obligate heterozygotes (say NS, N for normal allele, and S for susceptibility allele). All the case subjects will have an SS genotype, and the analysis can proceed as shown in table 6. In this situation, the odds ratio comparing the risk for the disease among SS individuals compared with that among NN individuals is infinity, and the population attributable fraction is 100 percent. This approach also allows researchers to assess gene-environment interaction in stratifying the analysis according to case-exposure status. Departure from multiplicative effects between the gene and the exposure can be inferred if these odds ratios differ. The approach is particularly advantageous for perinatal conditions such as birth defects, because parental information is usually available. Nevertheless, one needs to assume that parents' survival and reproduction should not be affected by carrying specific alleles. The method may also be applicable to adult-onset conditions that do not affect reproduction and are not quickly fatal. As with the

case-only design, this approach does not allow researchers to assess the independent effect of the exposure, merely whether the effect of the genotype is different for persons with an exposure than for persons without the exposure. This effect variation is also measured on a multiplicative scale similar to the case-only design. Nevertheless, this approach is superior to the case-only design in that it permits researchers to assess the effect of the genotype (with and without the exposure), whereas the case-only approach does not. Finally, any association found may be the result of linkage disequilibrium between the allele and the underlying susceptibility genotype.

GENE-ENVIRONMENT INTERACTION ANALYSIS IN THE CONTEXT OF THE AFFECTED RELATIVE-PAIR STUDY

The third type of case-only design is well known in the genetics literature as the affected sib-pair or affected relative-pair method (34–38). Essentially, this method allows investigators to look at the genotypic distribution of pairs of affected relatives. Most commonly applied to siblings, this method allows researchers to examine the number of alleles at any particular locus that are identical by descent between these affected relatives. The setup for the analysis for siblings is shown in table 7. Under the assumption of no genetic linkage, the expected distribution of alleles shared by descent between two siblings is 25 percent for 0 alleles, 50 percent for one allele, and 25 percent for two alleles. Departure from this distribution suggests linkage between the disease and the marker locus. For example, this method has been used recently in the search for gene loci for Alzheimer's disease (39), non-insulin-dependent diabetes mellitus (40), and rheumatoid arthritis (41).

To look for gene-environment interaction using this method, researchers can stratify the affected individuals by their exposure status. Such an analysis is complicated by the need to consider exposure status of each of the affected relatives, leading to concordance or discordance of the relatives in terms of specific exposures. The affected relative-pair study is a preliminary test for linkage that can be used effectively as a fishing expedition for candidate gene loci that are associated with increased disease susceptibility. However, because it requires the presence of two affected members in the family, its use may reduce the number of case subjects available for the study. It often requires testing of the parents or other intervening relatives to infer whether or not the alleles shared among case subjects are identical by descent. The affected relative-pair study does not assess the effects of specific alleles on disease susceptibility; rather, it assesses

TABLE 6. Illustration of the case-parental control analysis for a rare autosomal recessive disorder*

Parental controls	Case genotype		Total
	Not SS	SS	
Not SS	0	100	100
SS	0	0	0
Total	0	100	100

* All cases have an SS genotype. Parents are obligate heterozygotes NS × NS. Therefore, the untransmitted genotype is always NN. The odds ratio is 100/0 or infinity.

TABLE 7. Gene-environment interaction analysis in the context of an affected sib-pair study

No. of alleles identical by descent with proband	Unexposed case	Exposed case	Expected	Odds ratio (unexposed)	Odds ratio (exposed)
0	<i>a</i>	<i>b</i>	0.25	1.0	1.0
1	<i>c</i>	<i>d</i>	0.50	<i>c/2a</i>	<i>d/2b</i>
2	<i>e</i>	<i>f</i>	0.25	<i>e/a</i>	<i>f/b</i>

linkage at the locus. It also cannot assess independent effects of exposures. Furthermore, because the affected relative-pair study assumes Mendelian transmissions for expected distributions, any departure from independent segregation and random assortment could affect the results of this approach (38). Finally, selection factors, including survival, chronicity, and method of case ascertainment, may heavily affect the types of case subjects that could be available for this analysis (38).

CONCLUSION

In this commentary, we have reviewed three non-traditional methods for assessing gene-environment interaction in epidemiologic studies that involve no external control group. All three methods can test for any departure from multiplicative effects on disease risk between genotype and exposure. Because all these methods have assumptions and limitations, they can never substitute for well-conducted case-control studies. Finally, we do not discuss here the likelihood of genetic heterogeneity or interactions between several genes and several non-genetic factors. These phenomena can further dilute the magnitude of any gene-environment interaction detected in these study designs and the regular case-control method. Nevertheless, these alternative methods provide important additional tools in the epidemiologic armamentarium with which to screen for gene-environment interaction in disease etiology. We encourage the epidemiologic community to become familiar with these approaches and to apply them under appropriate conditions.

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