Am. J. Hum. Genet. 42:089–095, 1988

An Epidemiologic Approach to Ecogenetics

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Summary

Although "ecogenetics" seeks to examine genetically mediated differences in susceptibility to environmental agents, researchers often examine the relation between genetic markers and disease without regard to environmental determinants. By using epidemiologic definitions of genotype-environment interaction, it can be shown that the relative risk of disease for the genetic marker is a function of the frequency of exposure to the environmental agent, the strength of interaction between the genotype and the agent, and the specificity of the environmental effect vis-à-vis the genotype. Using examples from the literature, we illustrate under six patterns of genotype-environment interaction that the relative risk associated with the marker can fluctuate markedly. However, with infrequent exposures, the relative risk is close to unity (implying no genetic effect) even in the face of strong genotype-environment interaction. Alternatively, elevated relative risks imply a frequent environmental exposure or a strong pattern of interaction. We suggest that genetic marker-disease associations be evaluated within the context of an epidemiologic study design that considers specific environmental determinants of risk.

Introduction

Over the past decade, ecogenetics has been established as a distinct branch of human genetics that seeks to study genetically determined differences among individuals in their susceptibility to the actions of physical, chemical, and biological agents in the environment (Calabrese 1984; Mulvihill 1984; Goedde 1978; Motulsky 1978; Omenn and Motulsky 1978). Ecogenetics has grown as an offspring of pharmacogenetics, which refers to the study of the genetic basis for differential drug response and reactions (Kalow 1962; Vesell 1973); for example, several enzyme systems have been found to be associated with several diseases (e.g., acetylator phenotype system with bladder cancer [Cartwright et al. 1982] and a variety of other disorders [Evans 1984] and debrisoquine oxidation phenotype with lung cancer [Ayesh et al. 1984]). Although the concept of genetic-environmental interaction is central to ecogenetics and has long been recognized by geneticists (Haldane 1946), studies in this area have primarily examined the relationship between genetically determined enzyme systems and disease, without considering environmental determinants (see, e.g., Ayesh et al. 1984; Barbeau et al. 1985).

In this commentary, we provide an epidemiologic framework for evaluating genetic-environmental interactions in the context of ecogenetic studies. We provide definitions for disease risk and relative risks in terms of probabilities observed in epidemiologic studies. We illustrate that, in the presence of six patterns of interaction, the failure to consider environmental components of the disease concomitantly with measurement of the genetic marker may lead to erroneous inferences concerning the role of the genetic marker in disease etiology.

Formulation of Genotype-Environment Interaction Model

Under a simple genotype-environment interaction scheme, we consider that there is a susceptibility

Received March 13, 1987; revision received July 20, 1987.

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0002-9297/88/4201-0013$02.00

Table 1

Formulation and Notations for a Genotype-Environment Interaction Model in Cohort and Case-Control Studies

Genotype, Environment	Cohort Study		Case-Control Study		
	Disease Risk	Relative Risk	No. of Cases	No. of Controls	Odds Ratio
−, −	I	1	A_1	B_1	1
−, + ...	IR_e	R_e	A_2	B_2	A_2B_1/A_1B_2
+, −	IR_g	R_g	A_3	B_3	A_3B_1/A_1B_3
+, + ...	IR_{ge}	R_{ge}	A_4	B_4	A_4B_1/A_1B_4

Note.—A plus sign (+) denotes presence; and a minus sign (−) denotes absence.

genotype and an environmental exposure, each of which is dichotomous (either present or absent) as shown in table 1. It is to be noted that genotype is presented here in terms of a single gene locus. However, the method can be extended to multiple genetic loci as well. In studies of genetic marker–disease associations in which environmental effects are not considered, the quantity usually estimated is the relative risk of disease for the marker, which can be defined as the ratio of the probability of disease among individuals with the susceptibility genotype ($P[D|G+]$) to the probability of disease among individuals without the genotype ($P[D|G-]$). To simplify, we assume that the probability of exposure to the environmental agent f is independent of the genotype, an assumption that can be written as

$$P(E|G+) = P(E|G-) = P(E) = f \ . \quad (1)$$

The probability of disease for a person with a given genotype is the sum of the joint conditional probability of disease and exposure and the joint conditional probability of disease and no exposure.

$$P(D|G) = P(DE|G) + P(D\bar{E}|G) \ . \quad (2)$$

Equation (2) can be written as

$$P(D|G) = P(D|EG)P((E|G) + P(D|\bar{E}G)P(\bar{E}|G) \ . \quad (3)$$

Equation (3) can be written as

$$P(D|G) = P(D|EG)f + P(D|\bar{E}G)(1 - f) \ . \quad (4)$$

To obtain the conditional probabilities of disease in equation (4), one must determine disease risk for each of the four possible genotype-environment combinations. As shown in table 1, we assume that among unexposed individuals without the susceptible genotype there exists a certain background risk of disease, I, which reflects etiologic heterogeneity. Exposed individuals without the genotype have a disease risk IR_e (where R_e refers to the relative risk of disease for the exposure in the absence of the genotype). R_e reflects the specificity of environment effect vis-à-vis the genotype. If $R_e = 1$, then the exposure is not a risk factor in the absence of the genotype, whereas if $R_e > 1$, then the exposure exerts an effect even among individuals without the genotype (i.e., it is not specific to the genotype). Also, unexposed individuals with the genotype have a disease risk IR_g (where R_g refers to the relative risk of the genotype in the absence of the exposure). If $R_g = 1$, then the genotype requires an environmental trigger to increase disease risk. If $R_g > 1$, then the genotype alone can produce excess disease risk through a mechanism other than the environmental exposure. If $R_g < 1$, then in the absence of the specific environment exposure the genotype is protective against the disease. Individuals with both the genotype and exposure have a disease risk IR_{ge} (where R_{ge} is the ratio of disease risk in exposed individuals with the genotype to disease risk in unexposed people without the genotype and reflects the strength of interaction). R_g, R_e, and R_{ge} are relative risks estimated in case-control studies by the corresponding odds ratios as shown in table 1. If we replace the appropriate parameters in equation (4), the marginal probability of disease among individuals with the genotype is written as follows:

$$P(D|G+) = IR_{ge} \times f + IR_g \times (1 - f) \ . \quad (5)$$

Similarly, among individuals without the genotype the marginal probability of disease can be written as

follows:

$$P(D|G-) = IR_e \times f + I \times (1 - f) \quad . \quad (6)$$

Thus, the relative risk associated with the genotype when the exposure is neglected is the ratio of two marginal probabilities:

$$R_* = [(1 - f)R_g + fR_{ge}]/[1 - f + fR_e] \quad . \quad (7)$$

The relative risk of disease for the susceptibility genotype can also be estimated using estimates of R_g, R_e, and R_{ge} obtained from the case-control design (table 1). The relationship among R_{ge}, R_g, and R_e depends on the pattern of interaction that exists between the genotype and the exposure. In epidemiologic studies, two commonly considered statistical models are the additive model where $R_{ge} = R_g + R_e - 1$ and the multiplicative model where $R_{ge} = R_g \times R_e$ (Kahn 1983; Rothman 1986). However, no assumptions will be made here about the underlying statistical model of interaction.

Patterns of Genotype-Environment Interaction

We illustrate the effects of six biologically plausible patterns of genotype-environment interaction on the relative risk of the marker R_*. These patterns are summarized in table 2 and are illustrated graphically in figure 1. In each situation, it is assumed that the combination of the genotype and the exposure is deleterious (i.e., $R_{ge} > 1$), that the exposure is a risk factor (i.e., $R_e > 1$), but that the genotype may be associated with either increased or decreased risk (i.e., $R_g < 1$ or $R_g > 1$). In the first pattern, the genotype alone and the exposure alone do not cause excess disease risk (i.e., $R_g = 1$ and $R_e = 1$). Two

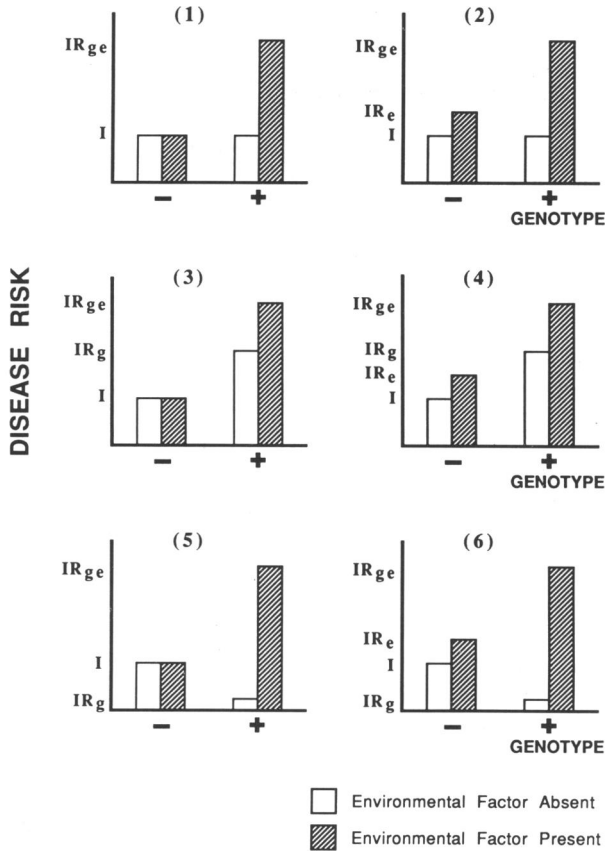

Figure 1 Patterns of genotype-environment interaction depicted in table 2.

examples can be given. The first example is that of a rare environmental exposure, succinyl choline administration during anesthesia, and its interaction with pseudocholinesterase deficiency in producing postoperative apnea (Evans 1983). The second is an example of a universal environmental exposure, phenylalanine in the diet and its interaction with the

Table 2

Patterns of Genotype-Environment Interactions Observed in Ecogenetic Studies

Pattern	Effect of Genotype in Absence of Environment	Specificity of Environment Effect vis-à-vis Genotype	Notations
1	Innocuous	Specific	$R_g = 1, R_e = 1$
2	Innocuous	Nonspecific	$R_g = 1, R_e > 1$
3	Risk factor	Specific	$R_g > 1, R_e = 1$
4	Risk factor	Nonspecific	$R_g > 1, R_e > 1$
5	Protective	Specific	$R_g < 1, R_e = 1$
6	Protective	Nonspecific	$R_g < 1, R_e > 1$

phenylketonuria genotype in producing mental retardation. In both examples, neither exposure nor genotype alone produces excess disease risk, but they do increase risk when present in combination.

A second pattern of interaction is that of an innocuous genotype in the absence of the specific exposure (i.e., $R_g = 1$) but an environmental exposure effect in individuals without the genotype (i.e., $R_e > 1$). An example of this type of interaction is xeroderma pigmentosa, i.e., exposure to sunlight and the production of skin cancer (Mulvihill 1984). In this case, $R_g = 1$ because the genotype requires an environmental trigger (UV light) but $R_e > 1$ because sunlight is a risk factor for skin cancer regardless of the presence of xeroderma pigmentosa (Frank and Slesis 1986).

In the third pattern of interaction, the genotype alone is associated with excess disease risk whereas the exposure alone is not (i.e., $R_g > 1$ and $R_e = 1$). An example of this type of interaction may be G6PD deficiency and fava beans. In this case, eating fava beans alone does not produce hemolytic anemia, whereas G6PD deficiency can do so if there is exposure to certain antimalarial drugs (Evans 1983).

In the fourth pattern of interaction, both the genotype and the environment alone are associated with excess risk of disease (i.e., $R_g > 1$ and $R_e > 1$). An example here is alpha-1 antitrypsin deficiency and cigarette smoking in pulmonary emphysema. Individuals with the PiZ phenotype have a very high risk of chronic obstructive pulmonary disease even if they do not smoke (i.e., $R_g > 1$), and smokers have a high risk of chronic obstructive pulmonary disease even if they do not have the deficiency gene (i.e., $R_e > 1$)(Tockman et al. 1985).

The fifth and sixth patterns of interaction occur when there is a reversal of the genotype's effect depending on the presence or absence of the environment. In this case, the genotype is protective in the absence of the environment (i.e., $R_g < 1$) but deleterious in the presence of the environment (i.e., $R_{ge} > 1$). Although no clear-cut examples in human genetics can be cited, a somewhat related example is that of the sickle cell trait and its advantage in the face of malaria (Luzatto et al. 1970) but its possible disadvantage in the absence of malaria (Calabrese 1984). These types of interaction are included to illustrate that, under reversal of the genotypic effect in different environments, the failure to consider such environmental components can lead to even more serious errors in assessing the role of the genotype in disease etiology.

Table 3

Relative Risks Associated with Genotype That Are Seen in Type I Interaction, by Frequency of Exposure and R_{ge}

Frequency of Exposure	R_{ge}		
	5	10	100
.001	1.004	1.009	1.099
.01	1.04	1.09	1.99
.10	1.40	1.90	10.9
.50	3.0	5.5	50.5
1.0	5.0	10.0	100.0

Note.—In this type 1 interaction the genotype alone is innocuous and environmental factor specific (i.e., $R_g = 1$ and $R_e = 1$).

Illustrations

In the illustrations given here, the observed theoretical relative risks ($R*$) for genetic markers are shown for various types of interaction, exposure frequencies (i.e., f), and R_{ge}.

Type I Interaction

As shown in table 3, with R_{ge} and frequency of exposure to the environmental component, the relative risk associated with the genetic marker $R*$ as measured in the population tends to increase. However, with infrequent exposures (<1% of the population), the relative risk associated with the marker is close to unity, implying no measured genotype effect. Even in the face of strong interaction (i.e., $R_{ge} = 100$), the relative risk is <2. Thus, a low relative risk for the genetic marker does not negate the importance of the genetic marker in the etiology of the disease if there is interaction with an environmental trigger and if the exposure frequency is low. Alternatively, high relative risks imply either a frequent environmental exposure, strong genotype-environment interaction, or both. Under this scheme, it will be easy to detect phenylketonuria as a risk factor for mental retardation in the general population because of the universality of the environmental trigger (i.e., a normal diet).

Type 2 Interaction

As shown in table 4, under type 2 interaction, the $R*$ associated with the marker tends to decrease with increasing R_e at a given exposure frequency. The im-

Table 4

Relative Risks Associated with Genotype That Are Seen in Type 2 Interaction, by Frequency of Exposure and R_e

Frequency of Exposure	R_e		
	2	5	10
.001	1.098	1.095	1.089
.01	1.97	1.91	1.83
.10	9.91	7.79	5.74
.50	33.7	16.8	9.1
1.0	50.0	20.0	10.0

NOTE.—R_{ge} is assumed = 100. In this type 2 interaction the genotype alone is innocuous and the environmental effect is present in the absence of genotype (i.e., $R_g = 1$ and $R_e > 1$).

pact of R_e is larger at higher exposure frequencies. This effect is intuitively understood by considering that the probability of disease among individuals without the genotype will be inflated by an excess risk due to the exposure to the environmental factor in a fraction of individuals. Thus, the nonspecificity of the environmental effect vis-à-vis the genotype will tend to dilute the measured effect of the genotype in the general population.

Type 3 Interaction

As shown in table 5, when the genotype confers excess disease risk in the absence of the environmental component (i.e., $R_g > 1$), the net effect will be to increase the measured effect of the genotype in the population $R*$ at any given R_{ge}, R_e, and f. Thus, if the genotype confers excess risk of disease regardless of the environmental exposure, a genotypic effect is easier to detect in the general population, especially

Table 5

Relative Risks Associated with Genotype That Are Seen in Type 3 Interaction, by Frequency of Exposure and R_g

Frequency of Exposure	R_g		
	2	5	10
.001	2.098	5.095	10.09
.01	2.98	5.95	10.9
.10	11.8	14.5	19.0
.50	51.0	52.5	55.0
1.0	100	100	100

NOTE.—R_{ge} is assumed = 100. In this type 3 interaction the genotype alone is a risk factor and the environmental effect is specific to genotype (i.e., $R_g > 1$ and $R_e = 1$).

Table 6

Relative Risks Associated with Genotype That Are Seen in Type 5 Interaction, by Frequency of Exposure and R_g

Frequency of Exposure	R_g		
	½	⅕	1/10
.001	0.60	0.30	0.20
.01	1.50	1.20	1.10
.10	10.4	10.2	10.1
.50	50.2	50.1	50.0
1.0	100	100	100

NOTE.—R_{ge} is assumed = 100. In this type 5 interaction the genotype alone is protective and the environmental effect is specific to genotype ($R_g < 1$ and $R_e = 1$).

with increasing levels of R_g. Type 4 interaction is a combination of type 2 and type 3 interaction.

Type 5 and Type 6 Interactions

When the genotypic effect varies depending on the presence or absence of the environmental component, unpredictable effects on the observed $R*$ can be obtained. As shown in table 6, measured values of $R*$ can vary from less than unity (protective effect) to more than unity (risk-factor effect) depending on values of f and R_g. At a given exposure frequency, the more protective the R_g, the lower the measured $R*$ in the population. On the other hand, at a given R_g effect, the higher the exposure frequency, the higher the measured $R*$ in the population. In this situation, if the environmental component is neglected, some studies might find a protective effect of the genotype, whereas others will find a risk-factor effect; and controversy thus will ensue.

Comments

The concept of genetic-environmental interaction is not new. In 1946, Haldane wrote a classic paper on the issue, in which he illustrated six forms of interaction (which are equivalent to three types in the present paper). In Haldane's exposition, the genotype was defined in terms of genetic strains (thus implying genetic effects at multiple loci) and the phenotype was measured in terms of means and variance of a continuous trait in the population (instead of in terms of disease risk, relative risk, or odds ratio, as we have done here). The effects of genetic-environmental interactions on the measured phenotype are further

complicated by the number of genetic loci involved, the nonadditivity of the genetic effects (Ward 1985), and the presence of etiologic heterogeneity (Sing et al. 1985). Nevertheless, numerous studies have examined the relationship between genetic traits and disease entities without considering environmental factors. Examples range from HLA studies (Bias 1981) to studies of specific enzyme systems (Ayesh et al. 1984; Evans 1984). In the face of accumulating evidence on genetic-environmental interactions (Calabrese 1984), this one-sided approach may not be ideal in unraveling disease etiology. As shown in these illustrations, depending on the pattern of interaction, on the frequency of exposure to the environmental component, which can vary among different populations, and on the strength of interaction, the importance of the genotype in disease etiology may not be appreciated. Likewise, a one-sided approach by epidemiologists in the study of the role of environmental factors in disease etiology—an approach that does not consider differential genetic susceptibility—can also lead to incorrect interpretation of the effects of the environmental factors (Khoury et al. 1987).

In this commentary, we advocate an epidemiologic approach to evaluating genetic marker–disease associations and their interaction with specific environmental risk factors. However, prior to conducting such studies, a major challenge is to determine which environmental factors might be relevant to which genetic markers in the etiology of a specific disease. This initial step depends on prior epidemiologic data and on biologic and molecular knowledge of disease mechanisms and is beyond the scope of this discussion. Once a genetic marker(s) is identified, the case-control design is frequently the preferred approach because it allows for the evaluation of multiple environmental risk factors that might interact with multiple genotypes of interest. Moreover, the case-control design is often more feasible and less expensive than other approaches, particularly for rare diseases. After appropriate selection of cases and controls, genetic marker(s) of interest are measured and information is collected systematically from the two groups concerning documented and suspected risk factors for the disease under study and for environmental exposures that can interact with the genotype of interest. The stratified analysis for disease risks (odds ratio that is used to estimate values of R_e, R_g, and R_{ge} (as shown in table 1) could potentially determine whether an interaction exists between a genotype and an environmental factor and the type of interaction. This simple analysis can also point to the statistical pattern of interaction (i.e., additive or multiplicative) between the two variables. Adjustment for potential confounding variables can be accomplished via either study design (such as matching) or appropriate statistical procedures (such as the Mantel-Haenzel procedure or multivariate analysis [Kahn 1983]). In addition, the case-control method allows for evaluation of both duration and multiple levels of the environmental exposure in its interaction (dose response) with the genotype.

In summary, the failure to consider environmental determinants of risk can lead to erroneous inferences concerning the role of genetic markers in disease causation. The application of epidemiologic principles and techniques to ecogenetics can potentially lead to a better understanding of disease mechanisms in terms of genetic-environmental interactions.

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