Trials and Interventions in Molecular Epidemiology

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INTRODUCTION

Experimentation is an important resource for therapeutic and prevention research in the era of molecular epidemiology. It is understood that well-executed clinical trials can add greatly to our understanding of the impact of exposures on risk and of treatment on disease outcome. Trials add significantly to our understanding of genetic variability; this variability can predict or determine risk or treatment outcome; it can also be critical, interacting with exposure, predicting risk, and with treatment, predicting outcome.

The goal of molecular epidemiologic research is to understand the effects of different exposures on the risk of disease; genetic variability may alter these effects. For prevention, researchers seek to distinguish those exposures or experiences that can be modified or blocked from those that cannot. Within the therapeutic trial, the molecular epidemiologist assesses whether treatment alters the course of disease and whether genetic variation alters the impact of treatment. Within the prevention trial, the molecular epidemiologist considers both whether intervention alters the risk of disease and whether genetic variability alters the intervention effect. This chapter considers (1) the structure of and rationale for clinical trials, (2) critical distinctions between therapeutic and prevention trials, (3) the uses of biomarkers in clinical trials, and (4) pharmacogenetics within clinical trials.

STRUCTURE OF AND RATIONALE FOR CLINICAL TRIALS

The clinical trial is a relatively recent instrument of medical science; it did not become a mainstay of medical research until the late 1940s. To be sure, rudimentary clinical trials were known as early as the 18th century. Lind, for example, undertook a clinical trial of citrus fruit for 12 seamen stricken by scurvy (1). Two were assigned to receive a daily ration of citrus fruits; the others, in groups of two, received various other medicinal concoctions. Assignment to citrus fruit as opposed to the other compounds was not randomized; Lind reported, however, that all 12 men were about equally ill. The recovery
of the two men who received citrus fruits was, within two weeks, markedly better than that of the other 10. Application of the scientific method to medicine continued with contributions from Farr, from Louis, and from Guy early in the 19th century (2). Key statistical concepts and techniques, such as multiple regression, were introduced during the same general period (3). The clinical trial was proposed and promulgated by Austin Bradford Hill in the late 1940s to accomplish three important goals: (i) ensure uniformity in treatment or in nontreatment; (ii) ensure that subject and clinician expectations do not bias treatment assignment or outcome evaluation; and (iii) eliminate confounding, by ensuring that experimental and control subjects differ only by treatment (4).

The clinical trial is focused on intervention and is conducted prospectively. The subject is randomized to one among a series of distinct interventions; neither the subject nor the investigator picks the intervention the subject receives. Assignment to intervention is random, not haphazard; assignment is by preset, random allocation. When possible, the subject’s trial assignment is blinded. Neither the subject nor the investigator knows treatment assignment until after the trial is complete and the data analyzed. To the degree possible, the clinicians evaluating the patients are blinded to subject assignment. In some instances, such as with behavioral interventions, it is impossible to blind subjects to their experimental or control status; even so, the investigator attempts to blind the clinician evaluating the subject. In dietary intervention trials, subjects clearly know whether they have been assigned to the treatment or to the control condition: they are urged, however, not to discuss their intervention assignment with their clinician (5, 6). The statistical analyst knows only that some subjects received one intervention while others received one of the others. The goal of this blinding is to discourage the statistician from coaxing a finding from the data.

In the standard clinical trial, the intervention and the outcome are operationally defined. The investigator, on the basis of his or her understanding of the probable effect of the intervention, determines how many subjects need to be included in the trial, how long the treatment needs to extend, and for how long after treatment the subjects need to be observed. The subjects may be assigned to any one of two or more distinct interventions. Subjects are recruited to participate, are informed about the study, are then assigned by a random process to one of the treatments, and then observed for a fixed period of time.

The strength of the clinical trial resides in its superior ability to address one of the major threats to internal study validity: confounding. In observational research, a wide range of exposures may occur in concert; thus, variability in outcome can be attributed to the focal exposure, or to any of a number of the exposure’s correlates. The researcher uses the association of the outcome with the focal exposure to assess the exposure’s importance; the investigator will conclude that the focal exposure is a genuine risk factor if that association is substantively and statistically significant; the researcher’s confidence in this conclusion will be strengthened if the association is stronger than the association of this outcome with any other exposure. In testing this association, the investigator will also consider the degree to which varying levels of the exposure are associated with increased risk of the outcome (7).

A critical test of the assertion that an exposure affects an outcome involves statistical control; the investigator uses any of a series of techniques to evaluate the association of this exposure and the outcome at fixed levels, or within categories, of other study variables. This process is also known as holding the other variables constant. Thus, for example, researchers may want to know whether a new drug, drug X, is more effective than drug Y. A straightforward way of understanding the relative effectiveness of drugs X and Y would be to compare cure or remission rates of patients who are treated with drug X as opposed to drug Y. But drug X may be more expensive than drug Y, or it may only be
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factors that might confound comparisons of drugs X and Y; it also requires that exposure
to these factors be measured. It is on this point that the randomized trial holds an
undeniable advantage: randomization almost inevitably, in studies of reasonably large
numbers of subjects, causes the intervention—the focal study variable—to be
uncorrelated with those other exposures. Thus, the clinical trial enables the researcher
to understand the importance of this one exposure, net of the impact of other suspected
exposures.

THERAPEUTIC AND PREVENTION TRIALS

There is little argument: the definitive test of an agent's therapeutic efficacy is a
randomized double-blinded clinical trial, with the agent compared to placebo or to the
accepted standard of care (8). Even with respect to prevention, the superiority of
experimentation is generally recognized. Nonetheless, therapeutic and prevention trials
are subject to substantially different challenges. Clinical trials are expensive in dollar
costs and time; this expense has blocked clinical trials from becoming a widely utilized
component of prevention and epidemiologic research. Subjects must, after treatment, be
monitored for the outcome of concern. Monitoring must be frequent; subjects must not be
lost to follow-up, and their experience of any outcome must be documented. Side effects
or complications of treatment or the experimental exposure must be accounted for; in
many settings, responding to these side effects will require modification of the exposure
protocol. These factors can add enormously to the expense of a prevention trial.

In general, it is more difficult to execute a prevention trial than a treatment trial. The
difference stems largely from the fact that cancer patients are dealing with a life-
threatening illness, with the conventional approach to administer for a short time a
frequently toxic agent that kills or disrupts both healthy and cancer cells: the agent's
effectiveness stems from its ability to kill a greater proportion of cancer than of healthy
cells.

Toxicity is widely understood to accompany chemotherapy, and patients and
clinicians in chemotherapeutic trials commonly expect it. In a prevention trial, on the
other hand, the patients are well; they may be, because of family history or a biomarker
such as a premalignant lesion, at elevated risk of subsequent cancer; nonetheless, they are
not sick, and they are much less willing than a cancer patient to tolerate an agent that
makes them feel sick. The design of the trial of finasteride for prevention of prostate
cancer included an allowance for substantial noncompliance due to modest sexual side effects (9). In colon cancer prevention trials of diet change and of dietary fiber supplements among adenomatous polyp patients, dropout or treatment noncompliance was substantial (5,10). Indeed, even if subjects are at increased risk, their actual risk—the probability that they will develop cancer—is relatively low.

Patients in a therapeutic trial will learn in relatively short order whether the intervention has been effective; the drug kills the tumor or lessens the tumor burden and the patient feels better, or it fails and the tumor remains or progresses. In a prevention trial, most patients, even those at elevated risk, will not experience the cancer the treatment is expected to prevent, and they will not know whether their chances have been improved until several years after the intervention begins. In the Polyp Prevention Trial, for example, some 2000 adenomatous polyp patients were randomized to diet change; by the end of the trial, only 40% of subjects had experienced an adenoma and fewer than 7% had experienced an advanced adenoma. Later, 15 years after the intervention began, fewer than 15—less than 1%—of the participants had developed colon cancer (11).

Third, as noted, in a therapeutic trial, treatment is of relatively short duration; in the trial of bevacizumab for treatment of metastatic colon cancer, median disease-free survival in the group that received bevacizumab along with a standard treatment cocktail was 10.6 months, compared with 6.2 months for those who received the standard. Median survival was 20.3 months for those whose treatment included bevacizumab, compared with 15.6 months for those who received the standard treatment. Clearly, toxicity experienced for 6 to 12 months is an issue most cancer patients would prefer to avoid, but it tends to be relatively short term (12). On the other hand, the Prostate Cancer Prevention Trial testing finasteride among average-risk men called for subject treatment by finasteride or placebo for up to seven years (9).

Thus, in a treatment trial, the success of the intervention is known within a reasonably short period; there is a partial or complete response, or there is none. A prevention trial, however, requires some understanding of the extended period during which a change in exposure or treatment will change outcome. In the Polyp Prevention Trial, participants were enrolled after a colonoscopy identified adenomatous polyps; patients were then randomized to a diet change program or to receive a copy of the National Cancer Institute dietary guidelines. Patients received a follow-up colonoscopy with polyp ablation after one year; they were scheduled to receive a final follow-up colonoscopy after being monitored for an additional three years (5). This trial design was based on the understanding that diet change would change polyp recurrence within three years. One explanation of its failure to do so has been that it was of too short duration. The recent follow-up, nearly 15 years after the intervention began, confirmed that the intervention had not altered polyp recurrence risk (11).

Fourth, because of the reluctance of healthy patients to accept even minor toxicity, of the fact that most prevention trial participants will not ever know whether their participation decreased their risk of disease, and because treatment in a prevention trial can well last for several years, noncompliance can be substantial. The design of the just mentioned Prostate Cancer Prevention Trial of finasteride included an assumption that compliance with the medication would be a good deal less than 100% (9,13).

Prevention clinical trials are particularly burdensome in expense and time requirements. Several colon cancer prevention trials have been focused on a risk biomarker—adenomatous polyp recurrence—rather than on colon cancer. Adenomatous polyps, a necessary precursor to colon cancer, occur at a much higher rate than colon cancer does; in addition, the occurrence of adenomatous polyps in people who have already had one or more polyps diagnosed has been understood to be much higher than it
is among those who have never had them diagnosed. Although not all adenomatous polyps eventuate in cancer, virtually all colon cancers began as adenomatous polyps. Thus, a trial of an agent’s ability to prevent adenomatous polyp recurrence can be smaller and involve a shorter period of follow-up than a trial of the same agent’s ability to prevent colon cancer.

Whether the advantages of clinical trials are great enough to legitimize the expense they engender has been widely argued. Some have argued that it is enough simply to rely on observation, taking advantage, through cohort and case-control studies of the natural experiments that lead to variance in exposure (14). Competing hypotheses are tested by means of statistical control. Others have argued that many of the exposures that are of interest for prevention cannot be induced except by an experimental intervention (15,16).

Control for confounding remains an enormous challenge. Unless exposure to confounders can be measured with great accuracy, under circumstances involving minimal measurement error, effective statistical control is impossible.

It has been understood in epidemiology for over 50 years that random error in the measurement of an exposure tends to cause attenuation of the exposure’s association with the study outcome (17). Errors in measurement of exposure to suspected confounders will lead to underestimation of their importance. Thus, if several exposures are equally predictive of an outcome, but they are measured with varying degrees of error, the association of the exposures with the outcome will be inversely associated with the degree of error in their measurement (18). Thus, the variables that are measured well will be strongly associated with risk, those measured poorly will be more weakly associated with risk. Or, they will be uncorrelated with risk. It has also been well documented (19) that statistical control is inhibited by error in the measurement of these exposures: the need for statistical control is obscured, and the effectiveness of this control is lessened (18).

A final distinction between observational and trial-based prevention research concerns change in exposure. What one generally learns from a cohort or case-control study is the impact of a given level of exposure; whether those who more frequently consume red meat, for example, may be at elevated risk of colon cancer (20). Researchers may seek to evaluate the impact of change in exposure, but it is extremely difficult to measure this change in an observational setting; there is little evidence that it can readily be gauged. In the face of an association of risk with exposure, it may be tempting to propose that the risk of those at a given level of exposure would tend to merge with the risk of those with a level of exposure, if those at the given level assumed the higher exposure. As attractive as this proposition might be, it is based on little to no evidence and is generally without foundation. For example, those with elevated levels of red meat intake may experience elevated risks of colon cancer; whether those people would soon—or ever—decrease their risk by decreasing their red meat intake is not addressed by the data: how long it would take and how much change would be required to effect any substantial change are not known. Whether the risk of those consuming red meat at a given level can be changed after a given age is similarly not known.

On the other hand, a trial imposes and tests a change. In a therapeutic trial, the patient is treated by an agent—chemotherapeutic—to which he or she has not been previously exposed. In prevention trials many of the participants are already exposed to the agent or intervention being tested. The object of the intervention is to substantially increase or decrease the exposure. In the trial of wheat bran fiber supplementation for adenomatous polyp patients, the intervention was an increase in fiber; virtually all of the participants were already consuming some dietary fiber, and all had developed their index adenoma while consuming this preintervention diet. On average, they more than doubled their fiber intake (5). In the trial of change to a plant-based diet among women who had
undergone definitive first-line treatment for breast cancer, all the participants were already consuming a diet that contained some plant-based foods. Many, in fact, consumed a diet that was intensely focused on plant-based foods (6).

**BIOMARKERS IN CLINICAL TRIALS**

Biomarkers in trials and interventions perform a range of functions. They reflect exposure to possible confounders, effect-modifying genetic factors, the extent to which treatment has reached the target tissue, and biologic response. As in all prospective studies, it is valuable to measure exposure to disease risk factors as well as possible. In a clinical trial, exposure to most risk factors will be by study design uncorrelated with the intervention: as there will be no correlation between the risk factor exposure and the intervention, there will, in all likelihood, be no confounding. Nonetheless, properly interpreted biomarkers of exposure to possible confounders offer the opportunity to substantially lessen the attenuation of associations that would result from more error-laden verbal reports.

A salient issue for the clinical trial is the impact of baseline risk factor exposure as a modifier of any intervention effect. For example, in a widely cited trial of selenium treatment of nonmelanoma skin cancer patients, the baseline selenium blood level was associated with substantial alteration of the impact of treatment (21). In the Women’s Health Initiative dietary intervention study, the intervention was not successful in decreasing breast cancer risk; it did, however, decrease risk among women with baseline fat intake in the highest quartile (22). Genetically governed modification of intervention effects is a very real possibility that has only recently begun to attract widespread attention.

Poor measurement of baseline exposure status could, however, well obscure the evidence that that baseline status alters the impact of the intervention. This is especially important for prevention trials that evaluate lifestyle interventions; recent studies have considered nutritional supplements and diet change. The subjects in these studies have in many cases had some exposure to these supplements or to a given dietary pattern prior to their enrollment in the intervention. In evaluating supplementation, or dietary or lifestyle change, prior status will be critical to interpreting the findings. To the degree that biomarkers increase the precision of baseline exposure intake, they will be valuable for evaluating baseline status’s modification of the impact of intervention. Biomarkers of genetic status will be critical to understanding baseline modification of the impact of intervention. An individual’s baseline exposure status may modify the impact especially of preventive interventions: nutritional status with respect to vitamin or trace element repletion; baseline stores of toxic chemicals; any status likely to result from a constellation of exposures and genetic predispositions. Biomarkers of such facets of baseline vulnerability play and will continue to play critical roles.

A critical issue in any biologic experiment is that the intervention be documented to have affected the target organ or organ system. In drug treatment trials, this will have been established prior to the intervention. It is not always as clear in prevention trials. Thus, in an important study of antioxidant administration to prevent the recurrence of adenomatous polyps, Greenberg (23) presented data documenting that the blood antioxidant levels of subjects were substantially increased by the intervention. In the Women’s Healthy Eating and Living study, Pierce and colleagues documented that dietary biomarkers of a plant-based diet—blood carotenoids—were increased by the intervention (6). In some instances, what reaches the target organ may be a metabolite of the agent administered; in prostate cancer prevention trials presently underway at Roswell Park,
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In prevention, biomarkers are used both as risk indicators and as interventional targets. By identifying populations at higher risk, they enable intervention to focus on individuals who can be expected to experience the outcome of interest. In addition, risk biomarkers are more likely to be seen than the endpoint of cancer. For example, the use of a biomarker endpoint may enable a study to be conducted and completed in far less time than would be necessary if cancer were the study endpoint. The adenomatous polyp is considered, as a premalignant lesion, to denote populations at elevated colon cancer risk. Many adenomas will not ever progress to colon cancer; nonetheless, colon cancers virtually always emerge from an adenoma. Thus, the adenoma is not a sufficient, but a necessary premalignant lesion, for colon cancer (25). The focus of several large interventional trials has been the recurrence of adenomatous polyps among individuals who, having had adenomas identified and ablated, are at elevated risk, not just of having new ones detected, but also of colon cancer (5,10,26). In each instance, the adenomatous polyp is used as a biomarker to denote a population risk; the recurrence of adenomatous polyps is then used as a biomarker of interventional efficacy.

Breast cancer biomarkers have been considered in several intervention trials. These biomarkers include mammographic density or pattern, and indicators of cellular proliferation and apoptosis. Atypical ductal hyperplasia, recognized as predictive of substantially increased breast cancer risk, has been used as a risk biomarker (27,28).

High grade prostate intraepithelial neoplasia (HGPIN), in the opinion of many a premalignant lesion, has been used to identify individuals at increased risk of prostate cancer (29). The rationale for linking HGPIN to prostate cancer stems from the fact that prostate cancer patients often have extensive fields of HGPIN, HGPIN occasionally appears to have cancer emanating from it, and populations at elevated risk of prostate cancer have elevated HGPIN prevalence. Nonetheless, the predictive value of HGPIN has become the subject of increasing debate. Early studies (30-34) that found foci of HGPIN without cancer often, on rebiopsy, found cancer; whether these were because HGPIN leads to cancer or because these early studies were limited by inadequate prostate sampling is not clear. On the other hand, some recent studies based on very complete prostatic sampling have found HGPIN to be highly predictive of subsequent prostate cancer (35). Three studies to date have focused chemoprevention interventions on men with HGPIN: Steiner et al, in a study of several doses of an estrogen modulator, toremifene (36); Alberts et al, in a study of an antiandrogen, flutamide (37); and Marshall et al, in a study of selenomethionine (29).

PHARMACOGENETICS

This chapter has already noted that the impact of individual variability in the myriad processes that affect carcinogenesis, the effects of chemotherapeutic or chemopreventive agents is in all likelihood profound. A part of the experience of any living organism is exposure to a range of chemicals and compounds; irritants and toxins are particularly important. A range of metabolic systems have evolved to detoxify these compounds and to protect the organism from them. These systems can be crudely characterized as phase I and phase II systems. The phase I systems transform possibly harmful substances for excretion or for additional modification by a second set of systems. The products of these
phase I systems can be more toxic than the initial agents they modify. At that point, phase II systems can proceed to further degradation or to excretion. Genetically governed variance in the speed and the efficiency with which the organism deals with these compounds can be substantial.

Oxidative stress, recognized as a likely source of genetic damage, stems from cellular exposure to both endogenously generated and exogenous compounds. Genes damaged by oxidative stress may function aberrantly. Living organisms are equipped with extensive and highly redundant systems to regulate oxidative stress; Individual variability in genes that govern these systems is highly possible. Thus, variability in systems that protect against oxidative stress could alter the efficiency of the body in coping with an excess of oxidative stress (38). In an important recent paper, Ahn et al. (39) used a functional assay to show that intake of fruits and vegetables, likely sources of antioxidants, interacted with allelic variation of a gene, catalase, that regulates intracellular oxidative stress.

Detoxification and protection against oxidative stress directed toward cellular damage are likely governed by pharmacogenetic processes. After cells are damaged, however, a series of steps involved in carcinogenesis governs the formation and advance of neoplasia to invasion. According to Hanahan and Weinberg (40), at least six distinct processes play roles in carcinogenesis: enhanced replicative potential, angiogenesis, apoptosis evasion, growth signal self-sufficiency, insensitivity to antigrowth signaling, tissue invasion, and metastasis. All of these in carcinogenesis represent aberrations of normal, genetically governed cellular processes; potentially, there is great variability in the genes that normally govern each of these processes. This naturally occurring variability could affect the degree to which neoplastic growth advances and becomes invasive. Clearly, the clinical trial, in which an intervention of great interest is administered and evaluated such that confounding is extremely unlikely, provides a distinctly advantageous setting for evaluating pharmacogenetics for both therapeutic and for preventive intervention.

Gene systems could readily alter one another’s effects: thus, to take the example from phase I and phase II enzyme systems, one phase I enzyme could lead subjects to very efficiently metabolize a foreign substance to an intermediate stage; the stage could be highly reactive and toxic to the cell. A phase II enzyme could then prepare it for excretion. An active form of the phase I enzyme coupled with an inactive form of the phase II enzyme could lead to excessive cellular damage, and to increased risk of carcinogenesis. If both enzymes were active, or inactive, or if the phase I enzyme were inactive and the phase II enzyme active, exposure might be less likely to have an effect. Clearly, as has been mentioned, it is possible that environmental exposures could interact with critical gene systems to govern the extent to which the organism is subject to environmentally induced damage.

Two critical statistical issues are raised by pharmacogenetics. The first is the significance of interactions in the face of null overall genetic or environmental effects. The second concerns statistical hypotheses testing and exploration.

A profound implication of the specter of substantial gene-environment interactions is that a common means of sifting data—evaluating bivariate associations between each exposure and each variant gene under study—is not adequate. A polymorphic variant of the same gene could reverse its effect. Not taking the polymorphic variants of this gene into account, the investigator would understand treatment to have no effect; he or she would only see its effects by categorizing subjects by their status on the gene. Similarly, the effects of the variant forms of the gene would not be seen unless the subjects were categorized by their treatment or nontreatment.
The second implication issues from the sheer numbers of genetic variants that can be examined. It is well known that statistical significance testing refers to the probability that an association of a given magnitude or larger would be seen, given the absence of a real association. A common approach is to denote as statistically significant as a result that would have only a 5% probability of being observed, given the truth of the null hypothesis. In other words, a test of a null variable has about a 95% probability of not indicating that the null variable has an effect.

If two genes are evaluated, the possible outcomes are that the first gene is statistically significant and the other is not, or that the first gene is not statistically significant and the other one is, that neither is statistically significant, or that both are. The probability that both are not statistically significant, even with neither representing a genuine association, is smaller than the probability that, if only one gene is tested, it is statistically nonsignificant. If three null genes are tested, the probability that none of the three appears statistically significant is smaller. If 10 null genes are tested, the probability that none appears statistically significant is smaller yet. In general, the probability that none in a series of null genes will be found to be statistically significant is inversely proportional to the number of genes tested.

If the investigator is testing a large number of null genes, say 30, the probability that none of the 30 will be found to be statistically significant is only about 20%; in other words, the probability that one or more of these null genes will be found to be statistically significant is approximately 80%. If several hundred even null genes are tested, then the probability that none of them will be found to be statistically significant is essentially zero. Several have suggested adjustments or corrections for the testing of multiple hypothesis; essentially, these require increasing the strength of the association required for the association to be recognized as statistically significant.

In a study in which a number of exposures and a number of genes are equally of interest, the problem of multiple hypothesis testing reaches astronomical proportions; the number of combinations is equal to the product of the number of gene constellations and the number of exposures; in a study in which 35 exposures and 30 gene patterns are of interest, the number of two-way interactions alone is 1050. The number of gene-exposure interactions studied could readily be several times the number of subjects in the study. In a clinical trial, this problem is lessened because only one intervention is focal. The number of genetic factors interacting with treatment may well be large, but the number of interactions is restricted by there being only one treatment. Clearly, of course, using baseline exposures as predictive of risk increases the number of genetic interactions that can be considered.

At present, a number of options have been proposed for handling the vast amounts of data generated by pharmacogenetic analysis. Adjusting for multiple hypothesis testing offers one approach; another is to simply regard statistical significance tests as convenient fictions, using these more as means of sifting and comparing the data on associations than as strict hypothesis testing exercises.

**SUMMARY**

This chapter has considered the clinical trial as a resource for molecular epidemiology. Although the clinical trial is in general more expensive than the purely observational study, it is in many ways superior: it provides for standardization of treatment, for eliminating bias in treatment assignment, and for control of confounding. There is no question but that the clinical trial is the criterion standard of therapeutic research. While the clinical trial holds great potential to strengthen inference for preventive options, it is to an order of magnitude more difficult than the therapeutic trial to execute.
Biomarkers in observational epidemiology are of particular value for control of confounding; their value in the clinical trial, especially for molecular epidemiology, resides primarily in their ability to predict differences in response to treatment. Biomarkers as linked to pharmacogenomics may prove critical to progress in molecular epidemiology, but their use raises two critical issues: discovery of gene-environment interactions may not follow from the standard epidemiologic approach of beginning with the evaluation of first-order associations of exposure and risk. Finally, the number of interactions to be potentially evaluated is so large as to render meaningless the common use of statistical significance criteria.

REFERENCES


