Chapter 8

Mutation Epidemiology

Contents

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INTRODUCTION

This report is about biochemical and genetic techniques for studying heritable mutations. These new techniques will ultimately be used to study people who are suspected of being at high risk for excess heritable mutations. The types of epidemiologic activities in which mutation detection techniques may eventually be used are: surveillance, monitoring, and ad hoc studies. Before any technique can be used for those purposes, a series of validation studies will be needed, calling for different populations that are appropriate for study at different stages of development of the technologies.

Surveillance is a routine activity whose aim, in the context of this report, would be to measure the "baseline" rate of mutations in a defined population over the course of time, and to facilitate rapid recognition of changes in these rates. Following the distinction made by Hook and Cross (44), the term monitoring is reserved for observations over time in populations thought to be at increased risk for heritable mutations because of a known or suspected exposure to a known or suspected mutagen, for the purpose of helping the specific population in whatever way possible. *Ad hoc studies* of a variety of designs are carried out to test *hypotheses* about suspected causes of mutations.

The different aims of surveillance, monitoring, and ad hoc studies require that different criteria be applied for deciding when and whether to carry out one or more of those activities. Surveillance and monitoring are not designed as hypothesis-testing activities, though they may be sources for hypothesis development.

The reasons a population is chosen for *surveillance* may be largely opportunistic. It is unlikely that an entirely new system of data collection would be put in place for mutation surveillance. It is more probable that mutation surveillance would be added to an existing program that is established for another purpose, for instance, birth defects surveillance. The population covered must be large enough to generate reliable rates for mutational events that may be relatively rare, but there is no fixed requirement for size. Of the three types of activities, surveillance generally would involve the smallest effort and resource expenditure per *individual,* but because large numbers of people would be routinely subject to the surveillance test, the total cost could be large. There is, therefore, a great need to consider the costs and benefits of such a program before embarking on one, and for choosing the detection technique accordingly. The threshold for initiating mutation surveillance would be relatively high. Information about trends sometimes can be obtained by means other than full-scale surveillance, and some such studies for that purpose have been done.

The main reason for instituting a mutation *monitoring* program is concern about the potential effects of a mutagenic exposure in a specific group of people. If there is enough concern, there undoubtedly will be a greater expenditure of resources and effort per person than is the case for surveillance, meaning that more extensive contact with the population and testing would be justified. Given today's knowledge, it is hard to conceive of a situation in which there would be a concern only about heritable mutations, so any mutation monitoring effort would most likely be part of a larger program. The obvious concurrent concerns for exposures thought to be mutagenic are cancer and birth defects. Strict criteria based on tests of the statistical power to detect certain levels of effects are not appropriate for monitored populations, since the concern is about that particular group of people. Any finding is of interest in that situation. The information maybe used incidentally to calculate upper limits of risk which could be generalized to other populations with similar exposures. The most important consideration in making a decision to monitor is that there is reasonable evidence suggesting that the population may be at a substantially increased risk.

This decision may well be influenced by political pressures to act, but ideally there should be a recognition that the best scientific judgment either does or does not support a monitoring effort.

The purpose of ad hoc *studies* is to test hypotheses about exposures and effects. This is the one place where it is imperative that studies be designed to achieve a high probability of detecting an effect if it is present. Such studies are valuable not only for the sake of the populations involved in the studies, but for their generalizability to other populations. Results of these studies can form the basis for public health actions, if levels of risk can be established.

VALIDATION OF MEASUREMENT TECHNIQUES

For the most part, the new technologies described in this report have not been "validated." They are new, and have not been applied to large numbers of people. Surveillance, monitoring, and ad hoc studies all require tools—in this case techniques for measuring mutations—with an acceptable degree of validity. That is, the tests must measure what they are designed to measure, within some definable limits. Generally, it is not possible to gather reliable information about a population and concurrently gather validating information about a technique used to measure outcome, unless another technique, with known validity, and known relationship to the new technique, is also applied in the study. Even though that is technically feasible, it is probably not an efficient way to gather validating data.

A first step in the validation process would be to use laboratory-prepared samples with known DNA sequences to confirm that the types of mutations that should theoretically be detected with a new technique actually are reliably detected. Beyond that stage, the need to move to clinical samples can be met using stored blood from individuals studied previously for other reasons. These stored samples need not be from parent/child triads initially, but triads will be needed at a later stage.

A number of research organizations are storing samples that would be appropriate for studies of mutations using new DNA techniques. The National Cancer Institute for example, is storing blood samples from cancer patients who have been treated with drugs and radiation, and the Radiation Effects Research Foundation has stored blood from Japanese citizens who were exposed to atomic bombs. In both of these cases, DNA is stored according to an established technique that uses Epstein-Barr virus to transform lymphocytes, thereby "immortalizing" the cells so they can be grown indefinitely. The transformed cells can be frozen for the long term in liquid nitrogen. Both sample preparation and long-term storage costs are substantial. With currently available technologies, it is unlikely that large numbers of samples will be stored. This limits the number and variety of samples available for validation studies, and also for later studies of people exposed to potential mutagens.

SURVEILLANCE AND DISEASE REGISTRIES

The methods and aims of disease surveillance have developed based on experience in infectious disease control. Reporting of vital statistics, in particular births and deaths for calculating birth and death rates, is also a form of surveillance. Surveillance of noninfectious diseases is a relatively recent development, with roots in the desire to track the incidence of cancer. Although the first national cancer surveillance system, which covers about 12 percent of the population, was put in place as recently as 1972, New York State instituted a reporting system for cancer cases in 1940, and Connecticut followed the next year. There are now dozens of cancer surveillance sys-

terns operating around the country and internationally, covering a range of populations from counties to whole countries (145).

Information about individual patients in cancer surveillance systems forms the basis for "cancer registries." The routine output of registries consists of cancer rates by sex, age, and race (where applicable) for each cancer site. Registries also are an important source for researchers investigating hypotheses about cancer causation. In this sense, cancer surveillance, with information about individuals recorded on registry forms, is similar to mortality statistics, with information about individuals recorded on death certificates.

It should come as no surprise that there are no "heritable mutation surveillance systems" now in place. There are, in various places around the world, registries of birth defects, which include records of at least some sentinel phenotypes. Beyond that, as this report shows, there are at present no techniques for detecting mutations that are suitable for use in a large-scale population surveillance program. Because developments have been so rapid, however, there maybe one or several good candidate techniques within 5 or 10 years.

Surveillance traditionally has involved reporting, to a central place, information *already collected* by some segment of the health care system for reasons directly related to the health of individuals. Even for infectious diseases, only cases that come to the attention of physicians are reported. Active "case-finding" in the population is not a usual feature of surveillance. For chronic disease, the same is true. Cancers diagnosed by physicians are entered into registries. Case-finding programs, such as breast cancer screening programs, are instituted on the basis of their effectiveness in identifying cases early in the course of disease, for the benefit of the individual with the disease.

Infants are examined for birth defects because of the potential impact on the children and their parents' lives, and not mainly for the purpose of computing the rates of birth defects in a population. Testing programs for newborns, including biochemical tests for metabolic diseases (not necessarily a result of a mutation), also have been instituted because of their importance to the health of the individual. Nearly all States now require testing newborns for phenylketonuria (PKU), and some require additional tests. For instance, New York requires testing for PKU, sickle-cell anemia, and congenital hypothyroidism, which are moderately rare, and also for very rare conditions including maple syrup urine disease, homocystinuria, histidinemia, galactosemia, and adenosine deaminase deficiency (102). The tests do not impose an added burden on the newborn, since all are carried out using the same blood sample.

Cytogenetic techniques have not been used for population-based surveillance of chromosome abnormalities, but some large hospital-born series of newborns have been tested (102). Most of the recorded cases of chromosome abnormalities found in this way might eventually have been detected later in life because of health and reproductive problems, but some others might otherwise have gone undetected.

There is no formula for deciding whether to institute a surveillance program, but there are characteristics of the disease, of the population, and of the particular test to be used that contribute to the decision: 1) the seriousness of the disease (if the measured endpoint is known to be associated with a disease); 2) the ability to alter its clinical course after diagnosis; 3) the prevalence of the disease in the population; 4) the reliability and validity of the test; 5) the acceptability of the test to the population; 6) the cost of the screening program; and 7) the cost of *not* screening (i.e., the cost of treatment and social support). It is worth considering these factors in thinking about screening and surveillance for heritable mutations.

The idea of surveillance for heritable mutations represents a departure from the traditional applications of surveillance. It appears to be the case that most heritable mutations are not related to disease over the course of an individual's lifetime, and no predictions useful to the individual can currently be made about the effect of a heritable mutation in the absence of recognizable disease, beyond those that are associated with sentinel phenotypes and major chromosome abnormalities. As mutation detection techniques become more and more sensitive, in fact, a greater per-

centage of the mutations detected may not be related to a known effect on health.

Heritable mutation surveillance beyond reporting sentinel phenotypes will require more than just a reporting of events already detected. It will require imposing a test burden on a population for the sole purpose of collecting information about mutations that may never affect an individual's life. This argues against instituting surveillance. A reason in favor of surveillance is that it is clear that increased mutation rates will be looked for in special populations, those being monitored because of worries that they have been exposed to a mutagen. Surveillance systems can provide a range of estimates of "baseline" or "background" rates, even though they may be from different populations. In a more general sense, one of the original aims of surveillance is relevant: to substantiate long-term trends and patterns in health events and to detect changes that may be addressed by public health action.

MONITORING AND EXPOSURE REGISTRIES

Monitoring is the "long-range observation of individuals who are at presumptive high risk for adverse outcomes because of specific life events, " (44) in particular, exposures to suspected mutagens. The event may be catastrophic, such as exposure at the time of detonation of an atomic bomb, or a chemical plant explosion. Or the "event" may be long term, such as an occupational or an environmental exposure. There are about two dozen populations around the world currently monitored for long-term health effects, and some of those programs include various studies of heritable mutations.

The most extensive population monitoring, including monitoring for mutations, is of the Japanese residents of Hiroshima and Nagasaki, many of whom were exposed to substantial amounts of radiation during World War II when atomic bombs were detonated in those cities. The population around a chemical plant that exploded near Seveso, Italy in 1976, releasing several pounds of dioxin, is the subject of health monitoring activities, including monitoring for birth defects. The people exposed to methyl isocyanate in Bhopal, India, will undoubtedly be followed for years to come. Because these groups were exposed, and because it is conceivable that something could be done to alleviate health problems if they are detected early, or if warning signals are picked up, they are being monitored; the scientific knowledge gained as a result is a secondary benefit.

The most prominent examples of chronic exposures are from occupational activities and toxic chemicals in the environment. The populations exposed to hazards often are not geographically determined, but may be a collection of workers from around the country. Workers exposed to radiation in the nuclear power industry are an example of this. There are several "exposure registries" in existence worldwide, though none specifically because of a perceived increased risk of mutations. One such registry has the names of all workers who were exposed to dioxin during the manufacture of various chemicals in this country. There also is an international dioxin registry, with names of workers from all around the world. The registry does not, however, have information about the health status of those workers. A similar registry for workers exposed to beryllium exists in this country. A report prepared for the Nuclear Regulatory Commission in 1980 recommended that a registry be started for workers exposed to low-level ionizing radiation in certain types of workplaces, because of a possible increased cancer risk (29). These registries could be used for monitoring and as a potential population to include in ad hoc studies.

AD HOC EPIDEMIOLOGIC STUDIES

Surveillance, monitoring, medical case reports, and laboratory research can all lead to hypotheses about possible causes of heritable mutations. An investigator wishing to test a hypothesis must find suitable subjects to study, in contrast to a monitoring activity, where the existence of the exposed population is the reason for acting. A study should be undertaken only if there is a good chance of answering the question of interest. Disease and exposure registries are common sources of individuals to study, depending on the question.

A cohort design will probably prove the most useful approach for studies of heritable mutations, though case-control studies of sentinel phenotypes may also prove valuable. A cohort study involves identifying a group of individuals, some exposed to the suspected mutagen and some not exposed. The health outcomes, i.e., the presence or absence of mutations in offspring, of the two sub-cohorts are compared. A higher rate of mutations in the exposed group would signify an "association" between the exposure and heritable mutations. Statistical tests are applied to the results to estimate the likelihood of the result occurring if in fact there was no real difference in mutation rates between the two groups.

In a case-control design, a group of "cases," individuals with conditions of interest, e.g., sentinel phenotypes, is compared to a group of individuals who do not have the condition of interest, but who are otherwise similar demographically. The cases and controls are compared according to their past histories of exposures or other characteristics that might be associated with the mutation and an assessment made as to whether their histories differ in important ways.

The important question for all studies is not just whether the exposure is "associated with" mutations, but whether it *causes* them. That is a difficult if nearly impossible judgment to make in most instances, but there are some generally accepted guidelines for evaluating the likelihood of an association being causal based on epidemiologic evidence. These are:

1. Consistency: The association is observed in studies by different investigators, at different times and in different populations, and in studies of different designs.
2. **Strength:** The size of the effect of an exposure is the measure of strength of association. This is usually measured as an estimate of relative risk (a ratio of the rate of mutations in an exposed group to the rate in an unexposed group). The presence of a dose-response relationship, that is, the size of the effect changes in a logical way with the level of exposure and in at least some cases, with the dose rate.
3. Specificity: Specificity refers to the degree to which the exposure is associated exclusively with the outcome of interest, in this case a mutation, and the degree to which a mutation is associated exclusively with the exposure. The concept of specificity derives from study of infectious disease and is relevant to the study of mutations (and chronic diseases generally) only in special cases, for example, a specific mutation that almost never occurs in the general population but appears to be exclusively related to a particular exposure. While a highly specific relationship can provide positive evidence for a causal relationship, a lesser degree of specificity does not necessarily argue strongly against causality.
4. Temporal Relationship: The exposure must occur before the effect. In the case of heritable mutations, the picture is more complicated. See chapter 6 for a discussion of the timing of exposure for males and females for a plausible effect on germ cells.
5. **Coherence:** All available information from medical and biological science, and from epidemiologic observations and studies, fits together in a way that supports the hypothesis. The greater the variety of information, and types of study designs, the stronger the finding of coherence.

These criteria are quite stringent, and even in the best of cases, often cannot be met, but they are useful as standards.

POPULATIONS TO STUDY

There are elements in the environment that damage human health under certain conditions of exposure. Biologic, chemical, and physical agents cause acute and chronic diseases in humans. At present, there are no exposures unequivocally known to cause heritable mutations in human beings. A combination of factors, including the rather insensitive methods for detecting heritable mutations that have been available, and the possibility that human germ cells may not be very susceptible to some mutagens, probably contribute to this situation. As a consequence, investigators looking for the effects of mutagens must do so in people who have been highly exposed to agents that are very likely to be mutagenic in germ cells. There are not very many large groups of people fitting that description, a fact that many might find surprising.

Radiation= Exposed Groups

Radiation causes heritable mutations in laboratory mice and is the most likely potential germ-cell mutagen to which large numbers of human beings have been exposed, either intentionally or accidentally. The largest population with a known high radiation exposure, the Japanese atomic bomb survivors, continue to be followed for effects on cancer incidence, birth outcomes, and heritable mutations. Heritable mutations have been studied by clinical observations, cytogenetic techniques, one-dimensional electrophoresis of blood proteins, and more recently with the most sensitive technique of two-dimensional gel electrophoresis of blood proteins (see ch. 3).

A report was prepared in 1980, under contract to the Nuclear Regulatory Commission (NRC), that evaluated opportunities for studying the health effects of low-level ionizing radiation (29). The report is focused on cancer, but the evaluation methods apply equally to studying mutations. The authors initially identified 100 candidate populations. About 30 remained after two broad criteria were applied: 1) that there be data identifying exposed individuals, and 2) that there were at least 10,000 people in a single population group or one comprising several similar groups. Those 30 populations were evaluated further, and recommendations made that if additional studies were to be undertaken, three occupational groups and one group with environmental exposure held out the greatest promise of yielding a reliable result. Even the best of these, however, has a relatively low power: less than a 50 percent chance of finding an excess of cancer if it exists. In general, this level of power would be unacceptable in an epidemiologic study. Although political considerations might influence a decision to go ahead with a study, they do nothing to increase the power of the method.

The power figures for these studies refer to cancer detection, and the probability of detecting heritable mutations is undoubtedly far lower, making it unlikely at best that anything could be learned about radiation and heritable mutations by studying any of these groups with currently-available methods.

The report to the NRC contained one other recommendation, that **a** registry for radiation workers be initiated. The registry would maintain information about radiation doses and some information about other exposures. This recommendation has not been acted on. There are examples of radiation-exposure registries, but these are mainly for people acutely exposed accidentally, and not for the more usual long-term chronic exposures of workers.

Cancer Patients

Treatment for many cancers includes chemotherapy with cytotoxic drugs, some of which are carcinogenic in laboratory animals and mutagenic in vitro, and treatment with high doses of radiation. There is a growing body of evidence that cancer patients are at a severalfold increased risk of developing second cancers, and some of these second cancers may be attributable to treatment of the first cancer with drugs and radiation (see, e.g., ref. 149). Cancer is mostly a disease of old age, but certain cancers have their peak incidence in younger people. Hodgkin's disease, for instance, occurs with greatest frequency in young

men. Childhood leukemias, some brain cancers, and tumors with strong genetic components, e.g. Wilms' tumor, retinoblastoma, and neuroblastoma, occur in the first few years of life. As treatment for these early cancers has improved over the last two decades, larger numbers of people are surviving, and it is these survivors who are at an increased risk of a second cancer, and possibly of heritable mutations.

Results from four studies of the offspring of childhood cancer survivors, and nine studies of offspring of adult cancer patients have been published as of mid-1985. Several other studies are in progress (82).

The combined published studies represent more than 700 cancer patients (both male and female) and more than 1,500 pregnancies, about 1,200 of which resulted in live births. Four percent of the liveborn babies had major birth defects, which is similar to the incidence in the general population. Two of the liveborn children had cancer. One had a hereditary bilateral retinoblastoma, as his father had. The other, the daughter of a brain cancer survivor, had acute myelocytic leukemia. One child had a condition that could have been the result of a new mutation, the Marfan syndrome, which fits the definition of a sentinel phenotype. Several other children had defects that might have had genetic components, but none of these represented sentinel phenotypes.

The largest study of offspring of childhood cancer survivors, including about 2,300 individuals from five population-based cancer registries, is nearing completion. Preliminary results indicate no increased risk of cancer in offspring compared with a control group, but the analysis is not yet final (82). Another long-term followup study, with more than 3,3oo **cases** enrolled to date, is under way in the United Kingdom. No results are yet available from that study (82).

The findings of a large international cooperative study of second tumors in children treated for cancers are provocative (142). Overall, 12 percent of children who survive at least 2 years after a first cancer develop a second cancer sometime during the 25 years following the first cancer. Most of the patients in the study were treated with high-dose radiation therapy. The risk of second cancers was highest among children with cancers known to be strongly genetically influenced. In that group, there may well be a genetic defect that predisposes to mutations, e.g., a faulty repair mechanism, which could also be related to a higher risk of heritable mutations in that group.

Cancer registries are the most numerous registries of any type, and cohorts of treated patients and patients with second tumors are relatively easy to identify, compared with identifying other populations potentially exposed to mutagens. These groups should be considered when studies of heritable mutations using the new technologies become feasible.

Other Populations

A study of birth outcomes in people who had attempted suicide by self-poisoning in Hungary is an example of opportunistic *use* of available information (23), A cohort of about 1,300 individuals who took large doses of drugs in suicide attempts has been studied since 1976. Early on, the investigators looked for short-term effects on somatic cells, using cytogenetic and biochemical testing. Long-term followup of birth outcomes examined spontaneous abortions, ectopic pregnancies, stillbirths, low birthweight, and congenital anomalies. The study suffered a large loss of followup of study subjects, but in those evaluated, no important excesses in any of these endpoints were discovered.

CONCLUSIONS

A very important question is answered by simply observing birth outcomes in people thought to be at high risk, namely whether those individuals are at risk of having children with serious diseases and disabilities. The new mutation detection technologies discussed in this report may greatly increase the power to identify mutations in studies such as those described above, adding another dimension to knowledge about the relationship between exposure to mutagens, the presence of detectable mutations in DNA, and the existence of observable health effects.