Open access • Journal Article • DOI:10.1056/NEJM200007133430213

Will genetics revolutionize medicine — Source link

Neil A. Holtzman, Theresa M. Marteau

Institutions: Johns Hopkins University

Published on: 13 Jul 2000 - The New England Journal of Medicine (Massachusetts Medical Society)

Topics: Genetic testing

Editorials

Cancer — Nature, Nurture, or Both

THE relative roles of genetic constitution and environmental exposure in the causation of cancer have been debated for decades.[1] Geographic differences, trends over time in the risk of cancer, and detailed studies of migrant populations overwhelmingly implicate environmental exposures as major causal factors and often identify the responsible carcinogens (e.g., tobacco, alcohol, radiation, occupational toxins, infections, diet, drugs). From this work has come the widely accepted estimate that 80 to 90 percent of human cancer is due to environmental factors.[2] Yet in the past 15 years, the explosion of molecular genetics has overshadowed environmental explanations by revealing genetic mechanisms underlying cancer. This is why the current confusion about environmental and genetic risk factors for cancer — on the part of patients, their families, clinicians, researchers, public policy makers, and the general public — is not surprising.

The gold standard for distinguishing genetic from environmental traits has been the study of twins. Comparing the incidence of disease in unrelated people, fraternal twins, and identical twins allows the heritable and environmental components of risk to be estimated. The study described by Lichtenstein and his colleagues in this issue of the *Journal*[3] has several advantages over previous studies of cancer in twins. It is population-based, the outcomes are derived from complete data on incidence, and the size of the population studied is four times as great as in any previous effort.

Although the current study has many strengths, its weaknesses illustrate the difficulties of using data on twins in studies of cancer. The study included more than 10,000 cancers in a total population of nearly 90,000 twins in Scandinavia, but the data effectively address cancer at only the four or five most common anatomical sites — and even for some of these, without much precision. The confidence intervals for the heritable proportion of susceptibility to stomach, colorectal, breast, and lung cancer all extend roughly from 5 percent to 50 percent, a fairly large range. The study lacks information on screening practices, which is quite possibly an issue in studies of twins. It also lacks data on specific types of exposure (e.g., tobacco use), so issues of interactions between genes and environment cannot be addressed. Indeed, the statistical model used specifically assumes no such interactions, ensuring that if any do exist, they will probably show up partly in the estimated environmental component of risk and partly in the heritable component. These practical limitations are inherent in studies of cancer in twins, and they indicate that delineation of the specific environmental and genetic components of the risk of cancer is likely to depend on the emerging new generation of large molecular epidemiologic studies — both population-based and family-based — rather than on studies of twins.

Despite its limitations, the study by Lichtenstein et al. provides new and valuable information for the nature-versus-nurture debate. In general, environmental factors were the dominant determinants of the site-specific risk of cancer. For cancer at four of the five common anatomical sites, estimates of the proportion of risk due to environmental effects were all 65 percent or greater. Though considerably less precise, estimates of the proportion of susceptibility that was due to environmental factors were generally even higher for cancer at the six next most frequent sites studied. These findings are consistent with the conclusions of studies of migrant groups. For example, rates of breast cancer among women who have recently immigrated to the United States from rural Asia are similar to those in their homelands and about 80 percent lower than the rates among third-generation Asian-American women, who have rates similar to or higher than those among white women in the United States.[4] This pattern is entirely consistent with the estimates by Lichtenstein et al. in this study that 73 percent of the causation of breast cancer is environmental and 27 percent heritable, particularly if a portion of the effect of heritable factors relates to genetic modification of environmental risk factors.

Although environmental effects may predominate, the findings with regard to heritability are noteworthy. Rates of concordance were generally higher in monozygotic pairs of twins than in dizygotic pairs, and the estimates of the proportion of susceptibility to cancer that was due to heritable effects ranged from 26 percent to 42 percent for cancer at the five common sites. These are substantial burdens of cancer risk, and substantially higher than estimates of risk based on a family history of a particular cancer. This degree of influence is also what would be expected if genetic effects are not limited to the rare, highly penetrant mutations that can result in familial cancer, but are also the result of polymorphisms that carry a much lower level of risk, do not result in an excess of cancer in families, and are much more prevalent than highly penetrant mutations in the general population. The most noteworthy effect of heritable factors is clearly that identified for prostate cancer (42 percent of risk). Like the other common cancers, prostate cancer shows marked international variation, and the risk among migrant groups tends to rise toward the level in the adopted country over several generations, indicating a substantial environmental component of the risk of this cancer.[5] Nonetheless,

a number of large-scale studies have searched for risk factors for prostate cancer and have found few. This lack of evidence stands in stark contrast to the situation with respect to breast, lung, and stomach cancer, for example, for which such studies have identified a variety of lifestyle-related, infectious, reproductive, and other environmental factors that are associated with moderate-to-high levels of risk. Perhaps prostate cancer does have a greater heritable component than cancer at these other sites. If some of the inherited factors are involved in modifying the risk associated with environmental factors, then success in identifying these two kinds of influences may depend on direct exploration of interactions between genes and environment.

The estimates of absolute concordance are telling. For cancer at the common sites in monozygotic twins, the rate of concordance is generally less than 15 percent. Thus, the fatalism of the general public about the inevitability of genetic effects should be easily dispelled. There is a low absolute probability that a cancer will develop in a person whose identical twin — a person with an identical genome and many similar exposures — has the same type of cancer. This should also be instructive to some scientists and others interested in individual risk assessment who believe that, with enough information, it will be possible to predict accurately who will contract a disease and who will not. With a few rare exceptions, any such deterministic approach to a disease as multifactorial as cancer seems doomed, and the data on twins seem to confirm that. This lack of absolute predictability, too, should not be surprising, given what we know about the risk of second primary cancers in paired organs.[6] For example, a woman's average annual risk of a contralateral breast cancer after the diagnosis of a first primary breast cancer is about 0.8 percent[7,8] — and this risk is for a person with, obviously, not only the identical genome, but also the identical complex of exposures.

Several things seem clear with respect to the importance of genetic and environmental factors in the causation and control of cancer. First, knowledge of one should expand our knowledge of the other. Information about types of environmental exposure that affect the risk of cancer should point to genes that might modify this risk, and the identification of genes associated with risk could help to indict previously unrecognized environmental risk factors.[9] Second, when genes and environment interact to produce a risk greater than the sum of their independent effects, this interactive component can be eliminated by removing either the genetic or the environmental factor. Finally, for cancer at many sites there are limited effective options for prevention. For this reason, unique opportunities to expand our knowledge of risk factors should be exploited regardless of their source. Perhaps it is time to drop the competition implied by talking about a debate over nature versus nurture in favor of efforts to exploit every opportunity to identify and manipulate both environmental and genetic risk factors to improve the control of cancer.

ROBERT N. HOOVER, M.D.
National Cancer Institute
Rockville, MD 20892

REFERENCES

1. Fisher RA. Lung cancer and cigarettes? Nature 1958;182:108.
2. Higginson J, Muir CS. Détermination de l'importance des facteurs environnementaux dans le cancer humain: rôle de l'épidémiologie. Bull Cancer 1977;64:365-84.
3. Lichtenstein P, Holm HV, Verkasalo PK, et al. Environmental and heritable factors in the causation of cancer — analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 2000;343:78-85.
4. Ziegler RG, Hoover RN, Pike MC, et al. Migration patterns and breast cancer risk in Asian-American women. J Natl Cancer Inst 1993;85:1819-27.
5. Ross RK, Schottenfeld D. Prostate cancer. In: Schottenfeld D, Fraumeni JF Jr, eds. Cancer epidemiology and prevention. 2nd ed. New York: Oxford University Press, 1996:1180-206.
6. Doll R. The Pierre Denoix Memorial Lecture: nature and nurture in the control of cancer. Eur J Cancer 1999;35:16-23.
7. Harvey EB, Brinton LA. Second cancer following cancer of the breast in Connecticut, 1935-82. In: Boice JD, Storm HH, Curtis RE, et al., eds. Multiple primary cancers in Connecticut and Denmark. Bethesda, Md.: National Cancer Institute, 1985:99-112. (NIH publication no. 85-2714.)
8. Veronesi U, De Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early breast cancer. J Natl Cancer Inst 1999;91:1847-56.
9. Opportunity 1: genes and the environment. In: The nation's investment in cancer research. Bethesda, Md.: National Cancer Institute, 1999:38-44. (NIH publication no. 99-4323.)

BIOENGINEERED CORNEAS — THE PROMISE AND THE CHALLENGE

THE promise of bioengineered replacements for diseased or damaged tissues has become a reality, notably for skin and cartilage. The article by Tsai et al. in this issue of the *Journal*[1] demonstrates the promise of a nascent form of technology that may provide a new tool for reconstructing damaged ocular surfaces that previously would have been unrepairable.

Conditions such as the Stevens–Johnson syndrome, cicatricial pemphigoid, and chemical burns, among others, can severely compromise ocular surfaces and cause catastrophic visual loss in otherwise healthy eyes; such problems afflict thousands of patients in North America every year.[2] The global burden of blindness from these disorders is probably much greater because of the many cases of trachoma and the higher incidence of trauma outside the United States and Canada. A common pathogenic feature of this seemingly diverse group of disorders is the depletion of the stem-cell population responsible for repairing the damaged corneal epithelium.

Conventional corneal transplantation is simply not successful in patients with this type of chronic surface problem. The donor corneal epithelium is gradually replaced, and the remaining transplanted corneal stroma, which is immunologically nonreactive, must ultimately be resurfaced with epithelial cells derived from the recipient's corneal stem cells. An absence or deficiency of stem cells allows, or may even stimulate, conjunctival-cell ingrowth and its accompanying neovascularization and inflammation, resulting in failure of the corneal graft. Therapies aimed at replenishing the stem-cell population have evolved in tandem with increased knowledge of the biology of corneal stem cells.

As with other self-renewing epithelial tissues, the corneal epithelium is maintained by an ordered, hierarchical replication of stem cells, which reside in the basal layer of the limbus.[3] It is clear from both experimental studies and studies of human ocular disorders that depletion of the limbal stem-cell pool results in an abnormal corneal surface, which cannot be normalized without the introduction of a new source of stem cells. The most direct approach to correcting corneal-surface disease due to stem-cell deficiency has been transplantation of a segment of the stem-cell–laden limbus, either as a contralateral autograft or, more recently, as an allograft from either a cadaveric donor or a living relative.[2] However, this procedure requires the harvest of approximately half (or more) of the limbus, and the procedure therefore jeopardizes the donor eye, whether the transplant is autologous or allogeneic.[4] An ideal approach would use a bioengineered replacement tissue that replenished the pool of stem cells without endangering the corneal stem cells of the donor eye.

To engineer a successful replacement tissue, it is necessary to provide an ex vivo environment for stem cells that maintains both the replicative function of the tissue and its differentiated phenotype. However, it is also necessary to create a biologically compatible scaffold or stroma for the tissue. Tsai and colleagues[1] chose human amniotic membrane as the scaffold on which to grow their replacement corneal surface. That was a logical choice, given the already widespread use of human amniotic membrane for ocular-surface reconstruction.[5] Numerous studies (reviewed in the article by Tsai et al.) have demonstrated that amniotic membrane facilitates epithelialization without allowing fibrovascular growth, supports epithelial-cell differentiation, is gradually resorbed in vivo, is nonantigenic, and contains extracellular-matrix components resembling those of conjunctival basement membrane.

Indeed, the successful outcomes in the patients into whose eyes Tsai et al. transplanted limbal epithelial cells cultured on amniotic membrane, as well as the patients who underwent a similar procedure in our own study,[6] suggest the promise of the technique for patients with limbal stem-cell deficiency or disease. An advantage of the procedure is the reduction in the amount of donor limbal tissue required ($2\ mm^2$ in both studies), which minimizes the possibility of damage to the donor eye. Combining the expansion of cells ex vivo with cultivation on a modified amniotic membrane has the advantage of ensuring a compatible extracellular matrix for the graft, thus increasing its durability and manipulability. This method is an advance over earlier attempts at the use of engineered corneal surfaces, in which fragile sheets of epithelial cells, with no substantial underlying stromal support, were transplanted.[7,8]

Despite the promise of the procedure described by Tsai et al., challenges lie ahead. The difficulty remains of identifying stem cells and ensuring that enough of them are present in the bioengineered tissue to repopulate the ocular surface. Current techniques for identifying corneal stem cells rely on their ability to take up various substances or to serve as progenitor cells for large colonies in culture — characteristics difficult to identify once the cells have been incorporated into a tissue prepared for human transplantation. The identification of cell-surface markers, such as those identified for keratinocyte stem cells,[9] will help address this issue. A second challenge is to optimize the scaffold on which the replacement corneal surface is grown. Although amniotic membrane is currently the most widely used material for this purpose, a bioengineered laboratory equivalent for use as corneal stroma would minimize the possible risk, albeit remote, of the transmission of an as-yet-unidentified infectious disease through use of an allograft.

In addition, the persistence of transplanted donor stem cells, and their reintegration into the limbus of the recipient eye, have yet to be demonstrated in patients who have received bioengineered grafts. This issue is particularly important if the future of bioengineered corneal-surface tissue lies with allogeneic donor cells. Although the persistence of donor-specific DNA sequences has been detected as long as 30 months after conventional transplantation of allogeneic limbal tissue,[10] no such analyses have been performed in recipients of bioengineered grafts. Evaluation of bioengineered composite skin grafts has revealed that, despite the long-term persistence of the allogeneic epithelial cells, the donor cells are slowly replaced by host cells. This finding suggests that, in some cases, the cultured allogeneic graft acts as a biologic dressing, providing the cytokines and growth factors required for tissue repair in the appropriate temporal sequence. Finally, answering the question of what will happen to the tissue or organ decades after it is implanted will require years of careful observation and follow-up.

Bioengineered or cultured tissue products are currently being produced to replace other tissues, and the progress with corneal-surface replacement indicates that such products are likely to revolutionize

the treatment of many epithelial and even visceral diseases. Heartened as we should be by the rapid progress in this area, we must recognize that there is still much to be learned before bioengineered organs will be routinely available.

IVAN R. SCHWAB, M.D.
R. RIVKAH ISSEROFF, M.D.
University of California, Davis, Medical School
Sacramento, CA 95817

REFERENCES

1. Tsai RJ-F, Li L-M, Chen J-K. Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 2000; 343:86-93.
2. Holland EJ, Schwartz GS. Epithelial stem-cell transplantation for severe ocular-surface disease. N Engl J Med 1999;340:1752-3.
3. Cotsarelis G, Cheng SZ, Dong G, Sun TT, Lavker RM. Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. Cell 1989;57: 201-9.
4. Jenkins C, Tuft S, Liu C, Buckley R. Limbal transplantation in the management of chronic contact-lens-associated epitheliopathy. Eye 1993;7: 629-33.
5. Kim JL, Tseng SCG. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. Cornea 1995;14:473-84.
6. Schwab I, Reyes M, Isseroff R. Successful transplantation of bioengineered tissue replacements in patients with ocular surface disease. Cornea 2000;19:421-6.
7. Pellegrini G, Traverso CE, Franzi AT, Zingirian M, Cancedda R, De Luca M. Long-term restoration of damaged corneal surfaces with autologous cultivated corneal epithelium. Lancet 1997;349:990-3.
8. Torfi H, Schwab IR, Isseroff RR. Transplantation of cultural autologous limbal stem cells for ocular surface disease. In Vitro 1996;32:47A. abstract.
9. Slack JMW. Stem cells in epithelial tissues. Science 2000;287:1431-3.
10. Shimazaki J, Kaido M, Shinozaki N, et al. Evidence of long-term survival of donor-derived cells after limbal allograft transplantation. Invest Ophthalmol Vis Sci 1999;40:1664-8.

WHO IS AT LOW RISK AFTER HEAD OR NECK TRAUMA?

EMERGENCY physicians have traditionally relied heavily on diagnostic imaging in the evaluation of patients with head or neck trauma. This conservative approach is intended to reduce the risk of missing intracranial lesions or cervical-spine fractures to virtually zero, since the morbidity associated with these injuries can be great and the medicolegal consequences severe. Each year in the United States more than 2 million adults present on an emergency basis after trauma to the head. Of the 80 percent with apparently minor injury,[1] between 6 percent and 9 percent harbor unexpected, traumatic intracranial lesions, although fewer than 1 percent require neurosurgical intervention.[2,3] Approximately 800,000 radiographs of the spine are obtained annually because of potential cervical-spine trauma.[4] Spinal cord injuries occur in about 10,000 patients per year, and more than half of these injuries involve the cervical spine.[5] Optimally, diagnostic imaging of patients with head or neck trauma should be performed selectively, but with the ability to detect potentially devastating lesions.

Most recommendations for the use of computed tomography (CT) in patients with minor head injuries and plain radiography in those with potential cervical-spine injuries have been extremely cautious. However, these recommendations have been based mainly on findings in patients seen in consultation or admitted for surgical or neurosurgical care rather than on findings in the majority of patients, who are screened for injury and treated after the trauma by emergency physicians.

This issue of the *Journal* includes two studies with compelling data that should assist emergency physicians in identifying patients at low risk after minor head or neck trauma. Each study group sought to determine whether the number of unnecessary diagnostic imaging tests can be reduced through the adoption of a defined set of clinical criteria that has been validated for use in patients in the emergency department. Both sets of criteria were developed to address the clinically relevant question not of who requires imaging but, rather, of who does not.

Haydel et al.[6] ask whether the use of CT can be avoided in patients with apparently minor head injury who present within 24 hours after blunt trauma. In their population of patients at least three years old, minor head injury was defined by a loss of consciousness and, on arrival at the emergency department, a score on the Glasgow Coma Scale of 15 and normal findings on a brief neurologic examination. Only 57 of 909 patients who met the inclusion criteria (6.3 percent) had positive findings on CT. All 57 patients had at least one of seven clinical features: headache, vomiting, age greater than 60 years, drug or alcohol intoxication, deficits in short-term memory, trauma above the clavicles, and post-traumatic seizure. Most of the findings were broadly defined; that is, any history or evidence of headache, vomiting, craniofacial trauma, or seizure was deemed sufficient. Most important, all 212 patients with none of these seven features (23 percent) had normal CT scans.

Hoffman and colleagues[7] posed a similar question: Under what circumstances after blunt trauma is standard three-view cervical-spine radiography unnecessary? This enormous study at 21 centers included 34,069 patients. The five criteria for a low risk of injury were based on previous work[4]: no midline cervical tenderness, no focal neurologic deficit, normal alertness, absence of intoxication, and no painful, distracting injury. A total of 818 patients had cervical-spine injuries (2.4 percent of the study population). Of these 818 patients, only 8 fulfilled all the criteria for a low risk of injury (false negative rate, 1.0 per-

cent). In six of these patients, the injuries were clinically insignificant, since they required no specific management. Only the remaining two patients were categorized as having clinically significant injuries according to predefined criteria. However, one had a lesion that may have been an old injury, and the other was probably misclassified as not having a distracting, painful injury and thus did not in fact satisfy all the criteria for a low risk.

These two clinical decision rules share a number of noteworthy features. Both deal with common and extremely expensive clinical problems. The percentage of negative diagnostic studies is tremendous, as indicated by the 95.7 percent and 97.6 percent rates of negative studies in the cohorts screened for cervical-spine injury and minor head injury, respectively. These investigations were methodologically sound. Each was prospective and used a standardized data-collection instrument. The two clinical decision rules are simple, brief, and pragmatic, with only five and seven clinical features to evaluate. They can be learned with minimal instruction and can be applied consistently, as shown by the acceptably low rates of interobserver variability. Each rule relies on mostly qualitative assessments that can be made rapidly at the bedside and are therefore practical in the emergency setting. In addition, these studies were designed to ensure patients' safety; if there was any pathologic finding, the CT scan of the head was considered positive, whether or not it led to any change in treatment. Moreover, clinical significance was conservatively defined.

Both rules could save far more than money. Unneeded diagnostic examinations are inconvenient, uncomfortable, and potentially unsafe for patients. They waste time, space, and the effort of skilled personnel. As a direct result, time spent in busy emergency departments increases, and patients' satisfaction declines. Finally, failure to diagnose clinically significant head or neck lesions could have consequences so grave that only an exceptionally sensitive rule would be acceptable to clinicians. In fact, the negative predictive values of the criteria used to rule out minor, closed head injury and cervical-spine injury in these studies were 100 percent and 99.8 percent, respectively, with impressive confidence intervals as a result of the large numbers of patients evaluated, particularly in the study of cervical-spine injury.

These studies, while remarkable, are not without precedent. Other investigations in emergency departments have led to decreased use of expensive or limited resources in the evaluation of patients with trauma who have a low risk of injury. For instance, clinical decision rules formulated by Stiell et al. and Plint et al. and developed and tested in emergency departments have succeeded in greatly reducing the number of radiographs obtained in the emergency evaluation of adults and children with knee injuries[8] or ankle injuries.[9,10] Although emergency departments can be chaotic, it is now routine for well-controlled, prospective, randomized trials to be conducted in them. Indeed, for rigorous testing of the initial management of limb-threatening and life-threatening conditions, the emergency department should be considered the essential laboratory for clinical research.

What, then, are the next steps? The study by Hoffman et al. was large and was undertaken at multiple centers. The decision rule that it examined for ruling out cervical-spine injury can be adopted with great confidence in most clinical settings. This study reinforces the empirical sense of many emergency physicians and should reassure clinicians who, on the basis of previous work, have already cautiously adopted similar guidelines. The data in the study by Haydel et al. come from a smaller cohort at a single site and cannot be considered quite as convincing. Corroborative investigations may be needed before conservative clinicians will implement the decision rule for minor, closed head injury. For both these problems, prospective validation studies with follow-up are needed but will require a great expenditure of time and enormous resources. Further research may refine the rules and extend their use to practice by nurses and paramedics.

These two valuable clinical decision rules can diminish the imperative for health care providers to seek diagnostic perfection. The beauty of these instruments is their ability to identify, on the basis of clinical evaluation alone, patients who have a low risk of serious injury. Their application should provide benefit to patients, providers, and insurers. Certainly, isolated exceptions to these rules are bound to occur. However, the high-quality data from which these rules are derived should be reassuring to patients and physicians and persuasive to the health care industry and the medicolegal system.

JOHN A. MARX, M.D.
Carolinas Medical Center
Charlotte, NC 28232-2861

MICHELLE H. BIROS, M.D.
Hennepin County Medical Center
Minneapolis, MN 55415-1829

REFERENCES

1. Borczuk P. Mild head trauma. Emerg Med Clin North Am 1997;15:563-79.
2. Miller EC, Derlet RW, Kinser D. Minor head trauma: is computed tomography always necessary? Ann Emerg Med 1996;27:290-4.
3. Jeret JS, Mandell M, Anziska B, et al. Clinical predictors of abnormality disclosed by computed tomography after mild head trauma. Neurosurgery 1993;32:9-15.
4. Hoffman JR, Wolfson AB, Todd KH, Mower WR. Selective cervical spine radiography in blunt trauma: methodology of the National Emergency X-Radiography Utilization Study. Ann Emerg Med 1998;32:461-9.
5. Tator CH, Duncan EG, Edmonds VE, Lapczak LI, Andrews DF. Changes in epidemiology of acute spinal cord injury from 1947 to 1981. Surg Neurol 1993;40:207-15.
6. Haydel MJ, Preston CA, Mills TJ, Luber S, Blaudeau E, DeBlieux PMC. Indications for computed tomography in patients with minor head injury. N Engl J Med 2000;343:100-5.
7. Hoffman JR, Mower WR, Wolfson AB, Todd KH, Zucker MI. Validity

of a set of clinical criteria to rule out injury to the cervical spine in patients with blunt trauma. N Engl J Med 2000;343:94-9.
8. Stiell IG, Greenberg GH, Wells GH, et al. Prospective validation of a decision rule for the use of radiography in acute knee injuries. JAMA 1996; 275:611-5.
9. Stiell IG, McKnight RD, Greenberg GH, et al. Implementation of the Ottawa ankle rules. JAMA 1994;271:827-32.
10. Plint AC, Bulloch B, Osmond MH, et al. Validation of the Ottawa Ankle Rules in children with ankle injuries. Acad Emerg Med 1999;6: 1005-9.

Sounding Board

Will Genetics Revolutionize Medicine?

ON both sides of the Atlantic, revolutionary claims have been made about the ultimate impact of genetics on clinical medicine. John Bell at Oxford has asserted that "within the next decade genetic testing will be used widely for predictive testing in healthy people and for diagnosis and management of patients. . . . The excitement in the field has shifted to the elucidation of the genetic basis of the common diseases."[1] And in the United States the director of the National Human Genome Research Institute, Francis Collins, has stated that the good that would come from mapping the human genetic terrain "would include a new understanding of genetic contributions to human disease and the development of rational strategies for minimizing or preventing disease phenotypes altogether."[2]

Statements like these clothe medicine in a genetic mantle. The result of efforts to identify genes that have a role in common diseases suggests a different picture: the genetic mantle may prove to be like the emperor's new clothes. In this article we argue that the new genetics will not revolutionize the way in which common diseases are identified or prevented. Mapping and sequencing the human genome will lead to the identification of more genes causing mendelian disorders and to the development of diagnostic and predictive tests for them. The development of safe and effective treatments, however, will usually lag behind,[3] although occasionally a treatment does precede the discovery of the disease-causing allele, as was the case for hemochromatosis.[4] Furthermore, only a small proportion of the population has mendelian disorders, and this will limit the ultimate impact of the Human Genome Project.

Our doubts stem from the incomplete penetrance of genotypes for common diseases, the limited ability to tailor treatment to genotypes, and the low magnitude of risks conferred by various genotypes for the population at large. Consequently, most people will have little interest in learning their genotypes. In the following sections, we use the term "genotype" to denote the alleles that a person possesses at a single gene locus on homologous chromosomes.

PENETRANCE

In contrast to genotypes for mendelian disorders such as Huntington's disease, which is due to a single, highly penetrant autosomal dominant gene, most genotypes for common, complex diseases are incompletely penetrant, and correlations between the genotype and the phenotype are therefore weak. Associations between a disease and a genetic marker can occur by chance,[5,6] and some have proved to be spurious.[7-9] Although many disease-related genes have been mapped to regions of specific chromosomes, highly penetrant susceptibility-conferring genotypes at loci related to asthma, hypertension, schizophrenia, bipolar disorder, and other disorders have not been found despite intensive efforts.

Searches for susceptibility-conferring genotypes for breast cancer,[10] colon cancer,[11] rare, early-onset forms of type 2 diabetes,[12] and Alzheimer's disease[13] have been successful, but in each case these genotypes account for less than 3 percent of all cases. One explanation is that the risk of disease conferred by alleles at one locus depends not only on alleles at other, independently segregating loci, which by themselves do not increase the risk,[14,15] but also on environmental factors.[16] The problem of identifying susceptibility-conferring genotypes is compounded when different combinations of gene loci are implicated in a disease, for it means that finding enough patients to serve as research subjects in a study will be extremely difficult.[9,17]

Frequently occurring genotypes, or polymorphisms (frequency of 1 percent or more), are unlikely to have a high penetrance for diseases that reduce reproductive fitness; such genotypes would be selected against except when the presence of a gene on only one chromosome (a single gene dose) confers a selective advantage that counterbalances the disadvantage of its presence on both chromosomes (a double, or homozygous, gene dose). Polymorphisms may confer higher risks for diseases that usually begin after the reproductive years end (e.g., Alzheimer's disease) or diseases for which selection pressures have not had a chance to reduce their frequency because the environment or lifestyles have changed only in recent generations. In populations that have been relatively isolated, genotypes that confer susceptibility to diseases with a recent increase in incidence may have frequencies in the polymorphic range.[18]

TAILORING TREATMENTS TO GENOTYPES

A recent article in the *New York Times* echoed the assertions of proponents of the genetic revolution: "Health care will shift from a focus on detection and treatment to a process of prediction and prevention."[19] One researcher was quoted as saying, "You can imagine having an infant tested at birth . . . and a result that says you are susceptible to diseases A, B, and C." Physicians will, the argument goes, be able to tailor drugs to a patient's genetic profile.

Finding drugs to thwart a disease will depend on the complexity of the genetic contribution to the disease. If genotypes at only one locus markedly increase the risk of disease, drugs to compensate for the malfunction could be devised. Yet, over 40 years have

passed since the molecular basis of sickle cell anemia was discovered,[20] and no definitive treatment has emerged. If genotypes at more than one locus must be present simultaneously in order to increase the risk of disease, finding the loci will be difficult. Once they are found, a drug that blocks the effect of only one allele might interrupt the pathogenic process, but this remains to be proved.

Inherited differences in sensitivity to drugs may be more amenable to pharmacologic tailoring than differences in susceptibility to disease. Determining patients' genotypes before they are given certain drugs may lead physicians to avoid administering drugs that could be harmful or to lower the dosages in sensitive patients, but the overall risk of adverse reactions may not be very high because of the low penetrance or low frequency of the genotypes. Alternatively, patients could begin taking a drug, be carefully monitored, and undergo genotyping only after an adverse reaction has occurred. This approach has been recommended for women in whom deep-vein thrombosis develops while they are taking oral contraceptives and who may have a susceptibility-conferring genotype at the prothrombin gene locus.[21]

THE MAGNITUDE OF ABSOLUTE, RELATIVE, AND ATTRIBUTABLE RISKS

The lifetime risk of breast cancer is 12.6 percent for women, the lifetime risk of prostate cancer is 15.9 percent for men, and the lifetime risk of colon cancer is 5.6 percent for men and women combined.[22] The prevalence of asthma at all ages is 5.5 percent; at the age of 45 to 64 years, the prevalence of ischemic heart disease is 5.2 percent and that of diabetes is 5.8 percent.[23] The lifetime risk of a major depressive episode is 17.1 percent, and the lifetime risk of nonaffective psychosis is 0.7 percent.[24] We can use these data together with genotype frequencies and penetrance to calculate the relative risks of genotypes that confer susceptibility. Thus, susceptibility-conferring genotypes at the *BRCA1* and *BRCA2* gene loci confer a relative risk of breast cancer of about 5.[22,25] Susceptibility-conferring genotypes at DNA-mismatch–repair gene loci confer a relative risk of colon cancer of about 9.3.[22,26] Susceptibility-conferring genotypes with polymorphic frequencies would be expected to confer relative risks that are not much more than 2 for various diseases.[27] Given the high prevalences of these disorders, even a relative risk of 2 could make the absolute risk conferred by susceptibility-conferring genotypes appreciable, and people might therefore flock to be tested for these genotypes.

Several other factors must be considered in the decision whether or not to be tested. First, the probability that the disease will develop in a person with a positive test result (the positive predictive value) is approximately equal to the penetrance of the disease and is usually low. As illustrated in Table 1, the positive predictive value is a function of the frequency of a susceptibility-conferring genotype, the relative risk of the disease, and the risk of disease in a given population.[28] Only if the frequency of the susceptibility-conferring genotype is 1 percent or less and if the relative risk approaches 20 will the positive predictive value exceed 50 percent when the risk of disease in a given population is 5 percent. When the risk in a given population is lower, the positive predictive value will also be lower.

Table 1. Positive Predictive Value of Tests for Susceptibility-Conferring Genotypes for a Disease with a Lifetime Risk in a Given Population of 5 Percent.*

Frequency of Susceptibility-Conferring Genotype	Relative Risk				
	1.5	2.0	5.0	10.0	20.0
%	positive predictive value (%)				
0.1	7.5	10.0	24.9	49.6	98.1
0.5	7.5	10.0	24.5	47.8	91.3
1.0	7.5	9.9	24.0	45.9	84.0
10.0	7.1	9.1	17.9	26.3	34.5
30.0	6.5	7.7	11.4	13.5	14.9

*The positive predictive value can be calculated with use of the following formula: $[R(D)\times 100]\div[G(R-1)+1]$, where R is the relative risk, D is the incidence of a disease (in this case, 0.05), and G is the frequency of a susceptibility-conferring genotype.

Second, the proportion of cases of a common disease that can be attributed to susceptibility-conferring genotypes is small under the most likely circumstances (Table 2).[29] Other factors, such as the environment, can have a substantial role. Consequently, healthy people will gain little reassurance that a negative test result means they will remain free of a particular disease. For instance, only about 0.25 percent of women carry *BRCA1* or *BRCA2* susceptibility-conferring genotypes,[30] and only about 0.1 percent of people have susceptibility-conferring genotypes at the DNA-mismatch–repair loci.[31] Given the relative risks associated with these susceptibility-conferring genotypes, people who have them will account for fewer than 5 percent of all patients with breast or colon cancer. Only in the case of polymorphisms that have frequencies in the range of 10 to 30 percent and that increase susceptibility to disease is the attributable risk appreciable. However, the risk of disease conferred by polymorphic genotypes is usually low, as we have already discussed (Table 1).

The I1307K allele at the adenomatous polyposis coli (*APC*) gene locus, which is found in about 6 percent of Ashkenazi Jews,[32] and the apolipoprotein E

TABLE 2. PROPORTION OF CASES OF A DISEASE THAT CAN BE ATTRIBUTED TO A SUSCEPTIBILITY-CONFERRING GENOTYPE (THE ATTRIBUTABLE RISK).*

FREQUENCY OF SUSCEPTIBILITY-CONFERRING GENOTYPE	RELATIVE RISK				
	1.5	2.0	5.0	10.0	20.0
%	attributable risk (%)				
0.1	0.05	0.1	0.4	0.9	1.9
0.5	0.25	0.5	2.0	4.3	8.7
1.0	0.5	1.0	3.9	8.3	16.0
10.0	4.8	9.0	28.6	47.4	65.5
30.0	13.0	23.1	54.6	73.0	85.1

*The attributable risk can be calculated with use of the following formula: $[G(R-1)\times 100] \div [G(R-1)+1]$, where G is the frequency of a susceptibility-conferring genotype and R is the relative risk. The general formula has been described previously.[29]

ϵ4 allele, which is found in about 20 percent of a predominantly white population,[33] confer relative risks of approximately 2 for colon cancer and Alzheimer's disease, respectively. According to the formula given in Table 2, these alleles will account for 5.7 percent of cases of colon cancer and 16.7 percent of cases of Alzheimer's disease, respectively, in the general population. When susceptibility-conferring genotypes at two or more independent gene loci must be present simultaneously for a disease to occur, the attributable risk will be much smaller.

Third, only in the case of very few diseases are interventions available that could prevent the disease in healthy people with positive test results or that could improve their survival or quality of life if the disease eventually developed. No interventions based on the identification of disease-related genes have yet proved safe and effective. Approaches already in use, such as prophylactic surgery and monitoring for incipient disease, may prolong life in people with an inherited susceptibility to breast and colon cancer, but the extent of improvement has not been established and the effects on the quality of life have not been studied.

THE DEGREE OF PUBLIC INTEREST IN LEARNING ABOUT DISEASE RISKS

Given the uncertainties surrounding test results and the questionable effectiveness of interventions in persons with positive results, how much interest will people have in being tested or in making lifestyle changes or undergoing medical or surgical interventions that might reduce their risk of future disease? With respect to predictive genetic testing, people want to know the probability of their getting a disease if the test result is positive (i.e., the positive predictive value) or negative (i.e., the false negative rate, calculated as 1 − sensitivity).[34] When the test result is positive, they also want to know what can be done to prevent the disease or improve its outcome. Many people will decide not to be tested if the positive predictive value and sensitivity of a test are low[35] and when no treatment is available.[36] Even when the positive predictive value of a test is high and interventions are available, as is the case for hereditary nonpolyposis colorectal cancer, interest in testing has been lower than anticipated.[37] Interest is also influenced by how convenient it is to be tested,[35,38] raising questions about the extent to which enthusiastic suppliers can manipulate demand.

Some evidence suggests that when risks have been determined by genetic testing, persons perceive the risks as less amenable to change,[39] suggesting that the likelihood that genetically based risk assessment will result in behavioral changes is even lower than the likelihood of this outcome after traditional assessments of health risks. When medical or surgical interventions are available, healthy people might not want to undergo them. Most women with a family history of breast cancer say they would not undergo prophylactic mastectomy if they were found to have a susceptibility-conferring genotype.[40] When treatment is not available for a specific disease, a few people may want the information on risk just for the sake of knowing and, possibly, planning. Finding out that a test for highly penetrant genotypes is negative may also reduce a person's anxiety and the need for other tests. For incompletely penetrant susceptibility-conferring genotypes, negative results may provide those tested with a false sense of security.

CONCLUSIONS

We do not want to downplay the importance of highly penetrant susceptibility-conferring genotypes or inherited drug sensitivity. Nonetheless, neither category represents a large enough proportion of the population to warrant widespread screening.[41] Testing in families with a history of the disease would be a more efficient approach but does not a revolution make.

It would be revolutionary if we could determine the genotypes of the majority of people who will get common diseases. The complexity of the genetics of common diseases casts doubt on whether accurate prediction will ever be possible. Alleles at many different gene loci will increase the risk of certain diseases only when they are inherited with alleles at other loci, and only in the presence of specific environmental or behavioral factors. Moreover, many combinations of predisposing alleles, environmental factors, and behavior could all lead to the same pathogenic effect.

In our rush to fit medicine with the genetic mantle, we are losing sight of other possibilities for improving the public health. Differences in social struc-

ture, lifestyle, and environment account for much larger proportions of disease[42,43] than genetic differences. Although we do not contend that the genetic mantle is as imperceptible as the emperor's new clothes were, it is not made of the silks and ermines that some claim it to be. Those who make medical and science policies in the next decade would do well to see beyond the hype.

NEIL A. HOLTZMAN, M.D., M.P.H.
Johns Hopkins Medical Institutions
Baltimore, MD 21205-2004

THERESA M. MARTEAU, PH.D.
Guy's, King's and St. Thomas' Medical School
London SE1 9RT, United Kingdom

Supported by funds from the Wellcome Trust (to Dr. Marteau).

REFERENCES

1. Bell J. The new genetics in clinical practice. BMJ 1998;316:618-20.
2. Collins FS. Shattuck Lecture — medical and societal consequences of the Human Genome Project. N Engl J Med 1999;341:28-37.
3. Treacy E, Childs B, Scriver CR. Response to treatment in hereditary metabolic disease: 1993 survey and 10-year comparison. Am J Hum Genet 1995;56:359-67.
4. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. Nat Genet 1996; 13:399-408.
5. Kidd KK. Associations of disease with genetic markers: deja vu all over again. Am J Med Genet 1993;48:71-3.
6. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241-7.
7. Schwab SG, Albus M, Hallmayer J, et al. Evaluation of a susceptibility gene for schizophrenia on chromosome 6p by multipoint affected sib-pair linkage analysis. Nat Genet 1995;11:325-7.
8. Risch N, Botstein D. A manic depressive history. Nat Genet 1996;12: 351-3.
9. Owen MJ, Cardno AG. Psychiatric genetics: progress, problems, and potential. Lancet 1999;354:Suppl 1:SI11-SI14.
10. Welcsh PL, Owens KN, King MC. Insights into the functions of BRCA1 and BRCA2. Trends Genet 2000;16:69-74.
11. Potter JD. Colorectal cancer: molecules and populations. J Natl Cancer Inst 1999;91:916-32.
12. Mandrup-Poulsen T. Diabetes. BMJ 1998;316:1221-5.
13. Lendon CL, Ashall F, Goate AM. Exploring the etiology of Alzheimer disease using molecular genetics. JAMA 1997;277:825-31.
14. Dragani TA, Manenti G. Mom1 leads the pack. Nat Genet 1997;17: 7-8.
15. Kajiwara K, Berson EL, Dryja TP. Digenic retinitis pigmentosa due to mutations at the unlinked peripherin/RDS and ROM1 loci. Science 1994; 264:1604-8.
16. Balaban E. Eugenics and individual phenotypic variation: to what extent is biology a predictive science? Sci Context 1998;11:331-56.
17. Friddle C, Koskela R, Ranade K, et al. Full-genome scan for linkage in 50 families segregating the bipolar affective disease phenotype. Am J Hum Genet 2000;66:205-15.
18. Fodor FH, Weston A, Bleiweiss IJ, et al. Frequency and carrier risk associated with common BRCA1 and BRCA2 mutations in Ashkenazi Jewish breast cancer patients. Am J Hum Genet 1998;63:45-51.
19. Fisher LM. Biology meets high technology: biochips signal a critical shift for research and medicine. New York Times. December 21, 1999:C1.
20. Ingram VM. Abnormal human haemoglobins. III. The chemical difference between normal and sickle cell haemoglobins. Biochim Biophys Acta 1959;36:402-11.
21. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. N Engl J Med 1998;338:1793-7.
22. Ries LAG, Kosary CL, Hankey BF, Clegg L, Edwards BK, eds. SEER cancer statistics review: 1973-1996. Bethesda Md.: National Cancer Institute, 1999.
23. National Center for Health Statistics, Adams PF, Hendershot GE, Marano MA. Current estimates from the National Health Interview Survey, 1996. Vital and health statistics. Series 10. No. 200. Washington, D.C.: Government Printing Office, 1999.
24. Kessler RC, McGonagle KA, Zhao S, et al. Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States: results from the National Comorbidity Survey. Arch Gen Psychiatry 1994;51:8-19.
25. Struewing JP, Hartge P, Wacholder S, et al. The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. N Engl J Med 1997;336:1401-8.
26. Dunlop MG, Farrington SM, Carothers AD, et al. Cancer risk associated with germline DNA mismatch repair gene mutations. Hum Mol Genet 1997;6:105-10.
27. Todd JA. Interpretation of results from genetic studies of multifactorial diseases. Lancet 1999;354:Suppl 1:SI15-SI16.
28. Khoury MJ, Newill CA, Chase GA. Epidemiologic evaluation of screening for risk factors: application to genetic screening. Am J Public Health 1985;75:1204-8.
29. Lilienfeld AM, Lilienfeld DE. Foundations of epidemiology. 2nd ed. New York: Oxford University Press, 1980.
30. Peto J, Collins N, Barfoot R, et al. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst 1999;91:943-9.
31. Aaltonen LA, Salovaara R, Kristo P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338:1481-7.
32. Laken SJ, Petersen GM, Gruber SB, et al. Familial colorectal cancer in Ashkenazim due to a hypermutable tract in APC. Nat Genet 1997;17:79-83.
33. Evans DA, Beckett LA, Field TS, et al. Apolipoprotein E epsilon4 and incidence of Alzheimer disease in a community population of older persons. JAMA 1997;277:822-4.
34. Holtzman NA, Bernhardt BA, Doksum T, Helzlsouer KA, Geller G. Education about BRCA1 testing decreases women's interest in being tested. Am J Hum Genet 1996;59:Suppl:A56. abstract.
35. Tambor ES, Bernhardt BA, Chase GA, et al. Offering cystic fibrosis carrier screening to an HMO population: factors associated with utilization. Am J Hum Genet 1994;55:626-37.
36. Marteau TM, Croyle RT. The new genetics: psychological responses to genetic testing. BMJ 1998;316:693-6.
37. Lerman C, Hughes C, Trock BJ, et al. Genetic testing in families with hereditary nonpolyposis colon cancer. JAMA 1999;281:1618-22.
38. Bekker H, Modell M, Denniss G, et al. Uptake of cystic fibrosis testing in primary care: supply push or demand pull? BMJ 1993;306:1584-6.
39. Senior V, Marteau T, Weinman J. Impact of genetic testing on causal models of heart disease and arthritis: analogue studies. Psychol Health 2000;14:1077-88.
40. Eisinger F, Geller G, Burke W, Holtzman NA. Cultural basis for differences between US and French clinical recommendations for women at increased risk of breast and ovarian cancer. Lancet 1999;353:919-20.
41. Wald NJ, Hackshaw AK, Frost CD. When can a risk factor be used as a worthwhile screening test? BMJ 1999;319:1562-5.
42. Rose G. Sick individuals and sick populations. Int J Epidemiol 1985; 14:32-8.
43. McKinlay JB, Marceau LD. A tale of 3 tails. Am J Public Health 1999; 89:295-8.

INFORMATION FOR AUTHORS

These guidelines are in accordance with the "Uniform Requirements for Manuscripts Submitted to Biomedical Journals." (The complete document appears in N Engl J Med 1997;336:309-15.)

MANUSCRIPTS Manuscripts containing original material are accepted for consideration if neither the article nor any part of its essential substance, tables, or figures has been or will be published or submitted elsewhere before appearing in the *Journal*. This restriction does not apply to abstracts or press reports published in connection with scientific meetings. Authors should submit to the Editor copies of any published papers or other manuscripts in preparation or submitted elsewhere that are related to the manuscript to be considered by the *Journal*. The *Journal* discourages the submission of more than one article dealing with related aspects of the same study.

Submit an original manuscript with one set of original figures and two copies of the complete manuscript. Manuscripts must be no longer than 3000 words. Please supply a word count (not including abstract or references). Use standard-sized paper, and triple-space throughout. Address all submissions to the Editor, New England Journal of Medicine, 10 Shattuck St., Boston, MA 02115-6094. A covering letter *signed by all authors* should identify the person (with the address and telephone number) responsible for negotiations concerning the manuscript; the letter should make it clear that the final manuscript has been seen and approved by all authors and that they have taken due care to ensure the integrity of the work. At least one person's name must accompany a group name — e.g., Thelma J. Smith, for the Boston Porphyria Group. As stated in the Uniform Requirements (see above), credit for authorship requires substantial contributions to: (a) conception and design, or analysis and interpretation of data; and (b) the drafting of the article or critical revision for important intellectual content. If more than 12 authors are listed for a multicenter trial, or more than 8 for a study from a single institution, each author must sign a statement attesting that he or she fulfills the authorship criteria of the Uniform Requirements. No more than 12 names will be listed under the title; other names will appear in a footnote. Acknowledgments will be limited to a column of *Journal* space, and those acknowledged will be listed only once. (See editorial, Nov. 21, 1991, issue.)

LETTERS TO THE EDITOR See the *Journal* correspondence section.

CONFLICT OF INTEREST Authors of research articles should disclose at the time of submission any financial arrangement they may have with a company whose product figures prominently in the submitted manuscript or with a company making a competing product. Such information will be held in confidence while the paper is under review and will not influence the editorial decision, but if the article is accepted for publication, the editors will usually discuss with the authors the manner in which such information is to be communicated to the reader.

Because the essence of reviews and editorials is selection and interpretation of the literature, the *Journal* expects that authors of such articles will not have any financial interest in a company (or its competitor) that makes a product discussed in the article.

COPYRIGHT Authors agree to execute copyright transfer forms as requested. Copyright in any contribution is owned by the Massachusetts Medical Society. The Society and its licensees have the right to use, reproduce, transmit, derivate, publish, and distribute the contribution, in the *Journal* or otherwise, in any form or medium. Authors will not use or authorize the use of the contribution without the Society's written consent, except as may be allowed by U.S. fair use law.

UNITS OF MEASUREMENT Authors should express all measurements in conventional units, with Système International (SI) units given in parentheses throughout the text. Figures and tables should use conventional units, with conversion factors given in legends or footnotes. In accordance with the Uniform Requirements, however, manuscripts containing only SI units will not be returned for that reason.

TITLES AND AUTHORS' NAMES With the manuscript, provide a page giving the title of the paper; titles should be concise and descriptive (not declarative). Also include a running head of fewer than 40 letter spaces; the name(s) of the author(s), including the first name(s) and no more than two graduate degrees; the name of the department and institution in which the work was done; the institutional affiliation of each author; and the name and address of the author to whom reprint requests should be addressed. Any grant support that requires acknowledgment should be mentioned on this page.

ABSTRACTS Provide on a separate page an abstract of not more than 250 words. This abstract should consist of four paragraphs, labeled Background, Methods, Results, and Conclusions. They should briefly describe, respectively, the problem being addressed in the study, how the study was performed, the salient results, and what the authors conclude from the results.

KEY WORDS Three to 10 key words or short phrases should be added to the bottom of the abstract page; these will help us index the article and may be published with the Abstract. Use terms from the Medical Subject Headings from *Index Medicus.*

REFERENCES References must be triple-spaced and numbered consecutively as they are cited. References first cited in tables or figure legends must be numbered so that they will be in sequence with references cited in the text. The style of references is that of *Index Medicus.* List all authors when there are six or fewer; when there are seven or more, list the first three, then "et al." The following is a sample reference:

1. Lahita R, Kluger J, Drayer DE, Koffler D, Reidenberg MM. Antibodies to nuclear antigens in patients treated with procainamide or acetylprocainamide. N Engl J Med 1979;301:1382-5.

Numbered references to personal communications, unpublished data, and manuscripts either "in preparation" or "submitted for publication" are unacceptable (see "Permissions"). If essential, such material may be incorporated in the appropriate place in the text.

TABLES Double-space tables and provide a title for each. If an article is accepted, the *Journal* will arrange to deposit extensive tables of important data with the National Auxiliary Publications Service (NAPS); we will pay for the deposit and add an appropriate footnote to the text. This service makes available microfiche or photocopies of tables at moderate charges to those who request them.

ILLUSTRATIONS Figures should be professionally designed. Symbols, lettering, and numbering should be clear and large enough to remain legible after the figure has been reduced to fit the width of a single column.

The back of each figure should include the sequence number, the name of the author, and the proper orientation (e.g., "top"). Do not mount the figure on cardboard. Photomicrographs should be cropped to a width of 8 cm, and electron photomicrographs should have internal scale markers.

If photographs of patients are used, either the subjects should not be identifiable or their pictures must be accompanied by written permission to use the figure. Permission forms are available from the Editor.

Legends for illustrations should be triple-spaced on a separate sheet and should not appear on the illustrations.

Color illustrations are encouraged. Send both transparencies and prints for this purpose.

ABBREVIATIONS Except for units of measurement, abbreviations are discouraged. Consult *Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers* (Sixth edition. New York: Cambridge University Press, 1994) for lists of standard abbreviations. The first time an abbreviation appears it should be preceded by the words for which it stands.

DRUG NAMES Generic names should generally be used. When proprietary brands are used in research, include the brand name in parentheses in the Methods section.

PERMISSIONS Materials taken from other sources must be accompanied by a written statement from both author and publisher giving permission to the *Journal* for reproduction.

Obtain permission in writing from at least one author of papers still in press, unpublished data, and personal communications.

REVIEW AND ACTION Manuscripts are examined by the editorial staff and are usually sent to outside reviewers. We encourage authors to suggest the names of possible reviewers, but we reserve the right of final selection. Only one copy of rejected manuscripts will be returned, usually within six weeks. Decisions about potentially acceptable manuscripts may take longer.