**Table of Contents**

**This Week in the Journal**

**Article Summaries**

**Perspective**

Without Conscience
E. Wiesel

Global Health: A Nutrition Paradox — Underweight and Obesity in Developing Countries
B. Caballero

Marathon Maladies
B. D. Levine and P. D. Thompson

**Original Articles**

Daily versus As-Needed Corticosteroids for Mild Persistent Asthma
H. A. Boushey and Others

DNA Topoisomerase II in Therapy-Related Acute Promyelocytic Leukemia
A. R. Mistry and Others

The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure
J. G.F. Cleland and Others

Hyponatremia among Runners in the Boston Marathon
C. S.D. Almond and Others

Brief Report: Modification of Human Hearing Loss by Plasma-Membrane Calcium Pump PMCA2
J. M. Schultz and Others

**Images in Clinical Medicine**

An Unusual Case of Pulmonary Embolism
T. Routledge and D. Jenkins

Airway Dilatation after Inhalation of a Beta-Agonist
G. Tamura and Y. Suda

**Case Records of the Massachusetts General Hospital**

Case 11-2005 — A 32-Year-Old Pregnant Woman with an Abnormal Fetal Karyotype
L. B. Holmes, A. R. Gargiulo, A. S. Nadel, and C. Racowsky

**Editorials**

Does Mild Persistent Asthma Require Regular Treatment?
L. M. Fabbri

Insights into Leukemogenesis from Therapy-Related Leukemia
J. Pedersen-Bjergaard

Resynchronizing Ventricular Contraction in Heart Failure
J. A. Jarcho

Listening to Genetic Background Noise
J. H. Nadeau
REVIEW ARTICLES
Drug Therapy: Effectiveness of Antimalarial Drugs
J. K. Baird

CLINICAL IMPLICATIONS OF BASIC RESEARCH
Arrest in the Liver — A Genetically Defined Malaria Vaccine?
U. Frevert and E. Nardin

CORRESPONDENCE
C-Reactive Protein Levels and Outcomes after Statin Therapy
Molecular Prediction of Recurrence of Breast Cancer
Attention Deficit–Hyperactivity Disorder
Prophylaxis against Rabies
Case 38-2004: A Large Tumor of the Skull
Clinical Trial Registration
Distribution of C-Reactive Protein Values in the United States
Altered Mental Status after a Marathon

BOOK REVIEWS
The Great Betrayal: Fraud in Science
Hope or Hype: The Obsession with Medical Advances and the High Cost of False Promises
Double Standards in Medical Research in Developing Countries
Twentieth Century Ethics of Human Subjects Research: Historical Perspectives on Values, Practices, and Regulations
Daily treatment with a controller medication is currently recommended for patients with mild persistent asthma. These investigators compared lung function and the number of episodes of asthma in the presence and absence of daily treatment with either an inhaled corticosteroid or a leukotriene-receptor antagonist; all patients were instructed to initiate inhaled corticosteroid treatment should asthma symptoms arise. There were no significant differences among the groups in morning peak flow or the time to the first exacerbation of asthma.

Although not designed to show equivalence, these data lay the groundwork for a large trial to evaluate whether patients with mild persistent asthma can safely be treated only when they have symptoms.

SEE P. 1519; EDITORIAL, P. 1589; CME, P. 1621
**ORIGINAL ARTICLE**

**DNA Topoisomerase II in Acute Promyelocytic Leukemia**

Formation of the chromosomal translocation t(15;17) was studied in cases of acute promyelocytic leukemia (APL) that developed after treatment of cancer with mitoxantrone, a topoisomerase II poison. In the presence of the drug, topoisomerase II damaged DNA in ways that caused breakpoint “hot spots” capable of forming t(15;17).

Drugs commonly used in cancer chemotherapy increase the rate of DNA cleavage by topoisomerase II or decrease rejoining of the two strands of DNA by the enzyme. Such drugs also increase susceptibility to APL. This article shows how a chemical attack on topoisomerase II causes the genetic changes of APL.

SEE P. 1529; EDITORIAL, P. 1591

**ORIGINAL ARTICLE**

**Effect of Cardiac Resynchronization on Heart Failure**

Cardiac resynchronization improves left ventricular function and functional status in patients who have left ventricular systolic dysfunction and interventricular dyssynchrony due to a conduction delay. In a randomized trial comparing medical therapy alone with medical therapy plus cardiac resynchronization, combined therapy was associated with a significant reduction in the risk of death from any cause.

SEE P. 1539; EDITORIAL, P. 1594

**ORIGINAL ARTICLE**

**Hyponatremia among Runners in the Boston Marathon**

The development of hyponatremia during a marathon may have grave consequences. In this study of 488 runners in the 2002 Boston Marathon, 13 percent had hyponatremia, and 0.6 percent had critical hyponatremia (serum sodium concentration, <120 mmol per liter). Weight gain during the race, longer racing time, and body-mass-index extremes were associated with hyponatremia. Better efforts to monitor and regulate fluid balance may reduce the frequency of this largely preventable condition.

SEE P. 1550; PERSPECTIVE, P. 1516; CME, P. 1622

**BRIEF REPORT**

**Genetic Modifier of Human Hearing Loss**

This study implicates a variant of a gene encoding PMCA2, a plasma membrane calcium pump, in the degree of severity of hearing loss caused by the mutation of another gene. The findings suggest that the mutant PMCA2 allele, which is carried by approximately 3 to 5 percent of persons of European descent, is a risk factor for presbycusis and noise-induced hearing loss. Studies to investigate this possibility are warranted.

SEE P. 1557; EDITORIAL, P. 1598

**DRUG THERAPY**

**Effectiveness of Antimalarial Drugs**

A global resurgence of malaria has taken place as a result of a lapse in preventive efforts and the emergence of resistance to standard antimalarial drugs. New therapies are available, but because of social, economic, and clinical factors, the use of older drugs persists. This review considers current approaches to the prevention and treatment of malaria.

SEE P. 1565; CME, P. 1623

**CLINICAL IMPLICATIONS OF BASIC RESEARCH**

**A Malaria Vaccine in the Mouse**

Deleting a gene critical to the development of *Plasmodium berghei* in the liver transforms the sporozoite — the infectious stage of the pathogen — into an organism that acts as a vaccine.

SEE P. 1600

**CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL**

**Pregnant Woman with an Abnormal-Karyotype Fetus**

A 32-year-old pregnant woman sought genetic counseling after her fetus had been found to have an abnormal karyotype. Ultrasonography for the evaluation of an ovarian cyst at 14 weeks of gestation showed increased fetal nuchal translucency; amniocentesis showed extra material on the short arm of fetal chromosome 18. The mother recalled a family history of birth defects. Diagnostic testing was performed, and the role of preimplantation genetic testing for future pregnancies was discussed.

SEE P. 1579
Without Conscience

Elie Wiesel

This is one of those stories that invite fear.

Now we know. During the period of the past century that I call Night, medicine was practiced in certain places not to heal but to harm, not to fight off death but to serve it.

In the conflict between Good and Evil during the Second World War, the infamous Nazi doctors played a crucial role. They preceded the torturers and assassins in the science of organized cruelty that we call the Holocaust. There is a Talmudic adage, quite disturbing, that applies to them: Tov she-barofim le-gehinom — “The best doctors are destined for hell.” The Nazi doctors made hell.

Inspired by Nazi ideology and implemented by its apostles, eugenics and euthanasia in the late 1930s and early 1940s served no social necessity and had no scientific justification. Like a poison, they ultimately contaminated all intellectual activity in Germany. But the doctors were the precursors. How can we explain their betrayal? What made them forget or eclipse the Hippocratic Oath? What gagged their conscience? What happened to their humanity?

In all truth, the medical field was not the only one to subscribe to Hitler’s plan. There was the judicial profession. And in some ways, the church. Only the literary world retained its sense of honor: the great writers, for the most part, were exiled. Not only Jews — Thomas Mann and Bertolt Brecht were not Jewish, but they were unable to breathe in the stifling air of the Third Reich. Doctors, on the other hand, mostly stayed — not the Jewish ones, but the others.

We know the facts. The motives as well. One day, Hitler and Himmler’s health minister made it known to leaders in the medical field that, according to a secret decision made at the highest level, it was necessary to get rid of “useless mouths” — the insane, the terminally ill, children, and elderly people who were condemned to misfortune by nature and to suffering and fear by God. Few in the German medical profession believed it worthy or good to refuse.

Thus, instead of doing their job, instead of bringing assistance and comfort to the sick people who needed them most, instead of helping the mutilated and the handicapped to live, eat, and hope one more day, one more hour, doctors became their executioners.

In October 1939, several weeks after the beginning of hostilities, Hitler gave the first order concerning the Gnadentod, or “charitable death.” On the 15th of that month, gas was used for the first time to kill “patients” in Poznán, Poland. But similar centers had already been created in Germany three years earlier. Now, psychiatrists and other doctors collaborated in a professional atmosphere exemplary for its camaraderie and efficiency. In less than two years, 70,000 sick people disappeared into the gas chambers. The Gnadentod program was going so well that the head of the Wehrmacht Hospital psychiatric ward, Professor Wurth, worried, “With all the mentally ill being eliminated, who will want to pursue studies in the burgeoning field of psychiatry?” The program was interrupted only when the bishop of Münster, Clemens August Graf von Galen, had the courage to denounce it from his cathedral’s pulpit; protest, in other words, came not from the medical profession, but from the church. Finally, public opinion was moved: too many German families were directly affected.

Like the fanatical German theorists, Nazi doctors did their work without any crisis of conscience.
They were convinced that by helping Hitler to realize his racial ambitions, they were contributing to the salvation of humanity. The eminent Nazi doctor responsible for “ethical” questions, Rudolf Ramm, did not hesitate to declare that “only an honest and moral person may become a good doctor.”

Thus, the doctors who tortured, tormented, and killed men and women in the concentration camps for “medical” reasons had no scruples. Human guinea pigs, prisoners both young and not so young, weakened or still in good health, were subjected to unspeakable suffering and agony in laboratories managed by doctors from the best German families and the most prestigious German universities. As a consequence, after the war, there were survivors of occupied Germany who refused to receive care from German doctors. They were scared. They remembered other doctors — or the same ones — from elsewhere.

In Ravensbrück, Dachau, Buchenwald, and Auschwitz, German scientists operated on their victims without anesthesia in an effort to discover cures for obscure diseases. The researchers let them die of hunger, thirst, cold; they drowned them, amputated their limbs, suffocated them, dissected their still-living bodies to study their behavior and measure their stamina.

At the first trial of doctors before the international court at Nuremberg in 1946, 23 of the accused were charged with having initiated, directed, and organized criminal activities against prisoners. Acting under their authority, a number of well-respected doctors caved in to their orders. How did they turn into assassins?

I personally met only one: Josef Mengele, who was known best not as a doctor but as a criminal and a murderer. Like so many other deportees, I saw him the night of my arrival in Birkenau. I remember the thought that crossed my mind: he looked elegant. I remember his calm voice as he asked me my occupation and age (warned by an inmate, I made myself older). And I recall his fateful gesture that separated the living from the soon-to-be dead. I learned his name only later. Morbid rumors went around about him. Wherever he sprang up, Death spread its shadow. It was known that he was always on the lookout for little twins and children with spinal problems. In the camp for Gypsies, he came across as likeable, warm, and tender toward one particular boy. He had the boy dressed in nice clothes, gave him the best food. This was his favorite prisoner. And on the night the Gypsies were liquidated, the doctor himself led this boy to the gas chamber.

Did I meet other doctors? In my barracks at Buna, some of them supervised the division of those permitted to live from those who were to die. I have described elsewhere the silence that preceded this event: it filled our being. We were afraid to look at one another. As on Yom Kippur evening, I had the feeling that the dead were mixed with the living. As for the doctors, I knew not who they were and have forgotten their faces.

Over the succeeding years, as I studied documents and archives about the Final Solution, I became familiar with the dominant role played by Nazi medicine and science. They were integral to the concentration-camp system and were as guilty as the various branches of Hitler’s armed services and police force of the monstrous crimes committed in occupied Europe out of hatred for the Jews and other so-called inferior races and groups. Yet after Germany’s defeat, with rare exceptions, criminal doctors calmly returned home to resume normal practices and ordinary life. No one bothered them at home, nothing threatened them. Only on the occasion of the trial of Adolf Eichmann in Jerusalem did German justice suddenly remember their crimes. The police found their addresses in telephone books.

But if an Eichmann shocks us, a Mengele revolts us. Eichmann was a rather ordinary low-life, without education or culture, whereas Mengele spent a number of years at a university. The existence of an Eichmann casts doubt on the nature and mentality of the German people, but the possibility of a Mengele throws into question the very basis of German education and culture. If the former represents Evil at a bureaucratic level, the latter embodies Evil at an intellectual level. Eichmann denied having been anti-Semitic and pleaded not guilty: he was
only following orders. But the Nazi doctors? None among them acted under duress — neither those who presided over the nocturnal division of new arrivals, nor those who killed the prisoners in their laboratories. They could have slipped away; they could have said no. Until the end, they considered themselves public servants loyal to German politics and science. In other words, patriots, devoted researchers. Without too great a stretch, maybe even societal benefactors. Martyrs.

Must one conclude that, since a humane science exists, there was also a science that wasn’t humane? I won’t even consider racist theorists who tried to treat racism as an exact science. Their vulgar stupidity deserves nothing but disdain. But there were excellent physicians, well-informed chemists, and great surgeons — all racist. How could they seek truth and happiness for human beings at the same time that they hated some of them solely because they belonged to human communities other than their own?

One of the brutal shocks of my adult life came the day I discovered that many of the officers of the Einsatzgruppen — the death commandos in Eastern Europe — had received degrees from Germany’s best universities. Some held doctorates in literature, others in philosophy, theology, or history. They had spent many years studying, learning the lessons of past generations, yet nothing kept them from killing Jewish children at Babi Yar, in Minsk, Ponár. Their education provided them with no shield, no shelter from the temptation and seduction of cruelty that people may carry within. Why? This question still haunts me.

It is impossible to study the history of German medicine during the Nazi period in isolation from German education in general. Who or what is to blame for the creation of the assassins in white coats? Was the culprit the anti-Semitic heritage that German theologians and philosophers were dredging up? The harmful effects of propaganda? Perhaps higher education placed too much emphasis on abstract ideas and too little on humanity. I no longer remember which psychiatrist wrote a dissertation demonstrating that the assassins hadn’t lost their moral bearings: they knew how to discern Good and Evil; it was the sense of reality that was missing.

In their eyes, the victims did not belong to humankind; they were abstractions. The Nazi doctors were able to manipulate their bodies, play with their brains, mutilate their future without remorse; they tortured them in a thousand ways before putting an end to their lives.

Yet inside the concentration camps, among the prisoners, medicine remained a noble profession. More or less everywhere, doctors without instruments or medications tried desperately to relieve the suffering and misfortune of their fellow prisoners, sometimes at the price of their own health or their own lives. I knew several such doctors. For them, each human being represented not an abstract idea but a universe with its secrets, its treasures, its sources of anguish, and its poor possibilities for victory, however fleeting, over Death and its disciples. In an inhumane universe, they had remained humane.

When I think about the Nazi doctors, the medical executioners, I lose hope. To find it again, I think about the others, the victim-doctors; I see again their burning gazes, their ashen faces.

Why did some know how to bring honor to humankind, while others renounced humankind with hatred? It is a question of choice. A choice that even now belongs to us — to uniformed soldiers, but even more so to doctors. The killers could have decided not to kill.

Yet these horrors of medical perversion continued beyond Auschwitz. Traces may be found, for example, in the hellish Stalin and post-Stalin eras. Communist doctors betrayed their brethren. Psychiatrists collaborated with the secret police to torture prisoners.

And how can the recent, shameful torture to which Muslim prisoners were subjected by American soldiers be justified? Shouldn’t the prison conditions in Iraq have been condemned by legal professionals and military doctors alike?

Am I naive in believing that medicine is still a noble profession, upholding the highest ethical principles? For the ill, doctors still stand for life. And for us all, hope.

This article has been modified by the author from an essay in his collection D’où viens-tu? (Editions du Seuil, 2001) and was translated from the French by Jamie Moore.
A few years ago, I was visiting a primary care clinic in the slums of São Paulo. The waiting room was full of mothers with thin, stunted young children, exhibiting the typical signs of chronic undernutrition. Their appearance, sadly, would surprise few who visit poor urban areas in the developing world. What might come as a surprise is that many of the mothers holding those undernourished infants were themselves overweight.

The combination of underweight in children and overweight in adults, frequently coexisting in the same family, is a relatively new phenomenon in developing countries undergoing the nutrition transition — the changes in diet, food availability, and lifestyle that occur in countries experiencing a socioeconomic and demographic transition. In such countries, as many as 60 percent of households with an underweight family member also have an overweight one, a situation that has been dubbed the “dual burden household” (see graph). Among countries at an intermediate level of development (middle-income countries, with a per capita gross national product [GNP] of about $3,000 per year), overweight ranks fifth among the top 10 causes of disease burden — right below underweight. This is the same position held by overweight as a cause of disease burden in the developed world.

Traditionally, obesity has been linked with abundance, and it was anticipated that as developing countries improved their economic status and their GNP, undernutrition would decrease and obesity would begin to appear among members of the upper socioeconomic classes. But the relationship between GNP and overweight is complex. Although being poor in the poorest countries (those with a per capita GNP of less than $800 per year) indeed “protects against” obesity, being poor in a middle-income country is actually associated with a higher risk of obesity than being richer in the same country.

The reasons are not completely clear, but it is obvious that in poor countries, the dietary energy intake of the poorest people may be limited by the scarcity of food, and the high energy demands of manual labor and daily-survival activities make it difficult for people to achieve a net positive energy balance and therefore to gain weight. In more urbanized developing countries with a higher GNP, food scarcity may no longer be the driving factor behind energy intake. Instead, the availability of cheap, energy-dense foods (including those from street vendors and fast-food restaurants) may facilitate the consumption of more calories. Widespread access to television would favor an indoor, sedentary lifestyle, further reducing the average daily energy expenditure. In the wealthier segments of a given population, these influences may be counterbalanced by access to better education about health and nutrition, sufficient income to purchase healthier foods (which are usually more expensive), greater quantities of leisure time for physical activity, and better access to health care that would help to address problems of excess weight. The contribution of the urban environment to the underweight–overweight para-
dox will probably continue to increase, since it is predicted that most of the population growth in the next 30 years will occur in urban areas, and almost all these new urban areas will be located in developing countries.

These factors explain the development of obesity among people who are marginally poor. But what about the persistence of underweight? It seems evident that the improvement in per capita GNP in countries undergoing a socioeconomic transition does not benefit all citizens equally. Data from the World Bank show that the rates of poverty and underweight have actually increased among children younger than five years of age in urban areas of countries in socioeconomic transition. People who move from rural to urban areas usually lose the ability to grow their own food and become dependent for their calories on a cash market. It is also more likely that women who move to the city will join the labor force and therefore become less available to prepare food at home, relying more heavily on commercially prepared foods for themselves and their families. For people with sufficient money, such a reliance may improve food choices and permit a more stable, if not better-quality, supply of dietary energy. But for those with an inadequate income, the urban environment may not offer the safety net of extended family and subsistence agriculture that is common in rural areas. These factors may explain why in Brazil women with incomes in the lowest quartile of the income distribution have a higher prevalence of underweight as well as a higher prevalence of overweight than do women with incomes in the top quartile. Because food costs consume a much larger proportion of family income in developing countries than in developed countries — more than 50 percent, in many cases — prices have a strong effect on the selection of particular foods. The globalization of food markets has resulted in the introduction of mass-produced, low-cost foods to the domestic food supply of many developing countries. This change, along with advertising campaigns, may have a powerful effect on the food choices and dietary patterns of low-income families. For example, the introduction of low-cost vegetable oils from industrialized countries greatly increased the proportion of fat calories in the average diet in countries undergoing the nutrition transition. Although many of these low-cost commercial foods are energy-dense, they may be nutrient-poor. And nutrient density is particularly important for growing children. For example, on a per-calorie basis, a five-year-old boy needs five times as much iron in his diet as a man. Cheap, energy-dense, nutrient-poor foods may adversely affect the growth of the child but may provide sufficient calories for the adult to gain excess weight.

Factors other than diet and lifestyle also link early undernutrition with overweight in adulthood. The hypothesis of "fetal origins of disease," which is supported by a number of observational epidemiologic studies, postulates that early (intrauterine or early postnatal) undernutrition causes an irreversible differentiation of metabolic systems, which may, in turn, increase the risks of certain chronic diseases in adulthood. For example, a fetus of an undernourished mother will respond to a reduced energy supply by switching on genes that optimize energy conservation. This survival strategy causes a permanent differentiation of regulatory systems that result in an excess accumulation of energy (and consequently of body fat) when the adult is exposed to an unrestricted dietary energy supply. Because intrauterine growth retardation and low birth weight are common in developing countries, this mechanism may result in the establishment of a population in which many adults are particularly susceptible to becoming obese.

The coexistence of underweight and overweight poses a challenge to public health programs, since the aims of programs to reduce undernutrition are obviously in conflict with those for obesity preven-
tion. As pointed out by Doak et al., these programs will have to identify and consider the magnitude and demographic composition of dual-burden households at the local and regional levels and then develop more targeted interventions. It will be essential to educate health care workers about the underweight–overweight phenomenon. Fortunately, some important interventions for reducing the rate of undernutrition may also be beneficial in terms of reducing the burden of obesity: promoting breast-feeding, improving the nutritional status of women of reproductive age, and reducing the rates of fetal growth retardation and low birth weight.

Improving the “obesogenic” environment in urban areas of the developing world may be more challenging. Governments and nongovernmental organizations must play an active role in promoting and protecting an environment that supports the growth and development of infants and children, monitoring the food market, and facilitating community-based initiatives that aim to promote healthy eating and physical activity. The World Health Organization’s Global Strategy on Diet, Physical Activity, and Health, endorsed by all member countries in May 2004, outlines a program and process for achieving these goals. But the other major challenge for countries in transition is to reduce socioeconomic and health disparities in urban areas. Until we close these gaps, we will continue to find malnourished children in the arms of overweight mothers.


Patriots’ Day, a Massachusetts holiday commemorating the Revolutionary War Battles of Lexington and Concord, is also the date of the annual Boston Marathon, a 42-km footrace. It was first run in 1897, one year after members of the Boston Athletic Association returned from the reincarnation of the Olympic Games in Greece.

As traditional as the marathon itself is the use of the event for research and of its runners as research subjects. In the second year of its existence, two physicians, Harold Williams and Horace D. Arnold, examined urine specimens from some of the runners and noted urinary casts and proteinuria — findings that would later be known as “athletic pseudonephritis.” Clarence DeMar, a legendary Boston runner, won the marathon an incredible seven times. His total would probably have been higher had he not been advised against competing by a physician who detected what was undoubtedly an innocent flow murmur produced by DeMar’s augmented cardiac stroke volume. DeMar was also a subject in studies performed by the noted Boston cardiologist Paul Dudley White, who had a lifelong interest in the marathon and had studied the heart rate of Boston participants in the 1915 and 1916 races. When DeMar died of colon cancer in 1958, White arranged for an autopsy on the already embalmed body. A report in 1961 presented results from both White’s earlier studies of DeMar and the autopsy, which showed that the diameter of DeMar’s coronary arteries was approximately two to three times that in normal adults. White, a great advocate of exercise who often rode his bicycle to

Marathon Maladies
Benjamin D. Levine, M.D., and Paul D. Thompson, M.D.

Dr. Levine is a professor of cardiology and the director of the Institute for Exercise and Environmental Medicine, Presbyterian Hospital of Dallas, University of Texas Southwestern Medical Center at Dallas. Dr. Thompson is the director of the preventive cardiology program, Division of Preventive Cardiology, Hartford Hospital, Hartford, Conn.
work, was a big fan of the marathon and, ironically, first recognized his own heart disease because of angina that developed as he jogged over to the race venue to watch David McKenzie of New Zealand win the 1967 race.

Research interest in marathon participants during the first decades of the 20th century was driven by concern for their health. Little was known about cardiac adaptations to endurance exercise, and what was known was determined by auscultation and the use of the “trained finger” for palpation and percussion. Hallmarks of an athlete’s heart such as bradycardia, cardiac enlargement, and innocent flow murmurs, were, in the view of the clinicians of the day, possible signs of pathologic heart block, cardiomyopathy, and valvular obstruction. It was not until 1942 that White used electrocardiography to record markedly slow, but normal, sinus bradycardia in athletes. According to Tom Derderian, author of a history of the Boston Marathon, marathoners were the test pilots and astronauts of their time, running where none had run before — and possibly risking their health in the process. Concerns about the health of athletes ultimately abated with the growing understanding that these cardiac changes were normal physiological adaptations and that physical activity conferred multiple health benefits.

In actuality, marathoning is a reasonably safe sport, with less than one death per 50,000 participants. Deaths that occur during less extreme physical activity and in previously healthy persons are usually caused by cardiac disease — predominantly, congenital problems such as hypertrophic cardiomyopathy or coronary anomalies in young athletes and atherosclerotic coronary artery disease in persons older than 35 years of age.

Nontraumatic causes of death among marathoners and ultramarathoners, military recruits, and persons who labor in hot and humid conditions are more varied; historically, they have included heat stroke and exertional rhabdomyolysis. These conditions are mitigated by adequate hydration, and preventive efforts have led to widespread recommendations for aggressive fluid consumption during endurance events such as marathons. These recommendations stemmed from the argument that because thirst may not be a precise indicator of the state of the plasma volume, fixed (and large) quantities of fluids should be consumed by athletes during endurance events, regardless of fitness level, body size, and known amount or composition of sweat loss.

However in 1981, during the 90-km Comrades Ultramarathon in South Africa, two cases of hyponatremia developed; they were later reported by Timothy Noakes in a runners’ magazine called South African Runner. Although there has been vigorous debate about the relative importance of fluid overload as compared with sodium loss due to sweating in the development of hyponatremia in runners, an extensive literature has accumulated over the past 20 years documenting that the primary cause is water intake in excess of sodium loss. The relative importance of water loss and sodium loss depends on the type and duration of the race, weather conditions, and the rates of these losses (as well as the rate of replacement of water and sodium), which may vary widely among athletes.

It has become clear that the hormonal and renal mechanisms that correct for water intoxication in athletes constitute physiologic responses, but they can be overwhelmed by the dilution that occurs when one drinks excessive amounts of water — an effect that may be compounded by reductions in renal blood flow and glomerular filtration during exercise. It is important to recognize that currently available “sports drinks” are not protective: most are hypotonic and provide far more water than salt. Similarly, the infusion of large volumes of hypotonic intravenous solution into athletes who have an exercise-associated collapse, especially if it is performed without knowledge of the serum sodium concentration (as it often is by emergency medical personnel who assume that dehydration is the culprit), may exacerbate the underlying pathophysiology and do more harm than good.

Although it is common to see broad-based recommendations stating that vigorous hydration is
essential for preventing heat-related illness arising from participation in any prolonged exercise in the heat, it is now well recognized that excess hydration can lead to hyponatremia — for instance, during military operations or during desert hikes. Indeed, hyponatremia became so common among back-packers in desert areas of national parks that emergency rescue workers in Grand Canyon National Park began to use a “point of care” device for rapid field assessment of the serum sodium concentration in order to detect hyponatremia in collapsed hikers. These devices remain in use and, given the frequency with which hyponatremia appears to develop even during relatively short events such as marathons, should be considered standard equipment in the medical tents for all endurance races that take place in hot environments.

It should be emphasized that athletes of all types have been instructed that water consumption during exercise is necessary to prevent illness from heat and to maintain performance levels, which is undoubtedly true. However, it is also clear from the article by Almond et al. in this issue of the Journal (pages 1550–1556) that fixed, global recommendations for fluid replacement may not be optimal for individual athletes of different body types and with varying degrees of training and heat acclimatization, and varying rates of water and sodium loss. The fact that a slower pace during a race (and therefore a longer period when water can be consumed) seems to be a risk factor for this complication makes sense in the context of this discussion. The recommendation by Almond et al. that fluid-replacement schedules be individualized for athletes competing in such events is sensible and practical and should be considered seriously by all competitors.

In fact, many organizations are beginning to revise their recommendations that fixed, large volumes of dilute fluids be consumed during athletic competition. For example, USA Track and Field, the national governing body for track-and-field sports in the United States, now suggests that athletes use thirst as their guide for fluid replacement. This major change in guidelines (from “stay ahead of your thirst” to “replace sweat loss”) was released just before the 2003 Boston Marathon (the year after the one run by the participants studied by Almond et al.). Moreover, the International Olympic Committee Medical Commission has issued new recommendations regarding fluid intake during participation in sports that emphasize more limited fluid consumption in order to avoid weight gain; these recommendations were disseminated during the 2004 Olympic Games in Athens. Whether such changes have actually had an effect on the practices of individual athletes is unknown. Finally, an international consensus conference on exertional hyponatremia is convening in South Africa this spring and should contribute to the setting of new guidelines for fluid replacement during exercise — guidelines designed for the prevention of heat injury and the avoidance of the potentially devastating consequences of hyponatremia.

The article by Almond et al. continues the long tradition of using the Boston Marathon as a research laboratory. Williams, Arnold, DeMar, and White would be proud to know that the tradition lives on.

Daily versus As-Needed Corticosteroids for Mild Persistent Asthma

Homer A. Boushey, M.D., Christine A. Sorkness, Pharm.D., Tonya S. King, Ph.D., Sean D. Sullivan, Ph.D., John V. Fahy, M.D., Stephen C. Lazarus, M.D., Vernon M. Chinchilli, Ph.D., Timothy J. Craig, D.O., Emily A. Dimango, M.D., Aaron Deykin, M.D., Joanne K. Fagan, Ph.D., James E. Fish, M.D., Jean G. Ford, M.D., Monica Kraft, M.D., Robert F. Lemanske, Jr., M.D., Frank T. Leone, M.D., Richard J. Martin, M.D., Elizabeth A. Mauger, Ph.D., Gene R. Pesola, M.D., M.P.H., Stephen P. Peters, M.D., Ph.D., Nancy J. Rollings, M.Ed., Stanley J. Szefler, M.D., Michael E. Wechsler, M.D., and Elliot Israel, M.D., for the National Heart, Lung, and Blood Institute’s Asthma Clinical Research Network

ABSTRACT

BACKGROUND
Although guidelines recommend daily therapy for patients with mild persistent asthma, prescription patterns suggest that most such patients use these so-called controller therapies intermittently. In patients with mild persistent asthma, we evaluated the efficacy of intermittent short-course corticosteroid treatment guided by a symptom-based action plan alone or in addition to daily treatment with either inhaled budesonide or oral zafirlukast over a one-year period.

METHODS
In a double-blind trial, 225 adults underwent randomization. The primary outcome was morning peak expiratory flow (PEF). Other outcomes included the forced expiratory volume in one second (FEV₁) before and after bronchodilator treatment, the frequency of exacerbations, the degree of asthma control, the number of symptom-free days, and the quality of life.

RESULTS
The three treatments produced similar increases in morning PEF (7.1 to 8.3 percent; approximately 32 liters per minute; P=0.90) and similar rates of asthma exacerbations (P=0.24), even though the intermittent-treatment group took budesonide, on average, for only 0.5 week of the year. As compared with intermittent therapy or daily zafirlukast therapy, daily budesonide therapy produced greater improvements in pre-bronchodilator FEV₁ (P=0.005), bronchial reactivity (P<0.001), the percentage of eosinophils in sputum (P=0.007), exhaled nitric oxide levels (P=0.006), scores for asthma control (P<0.001), and the number of symptom-free days (P=0.03), but not in post-bronchodilator FEV₁ (P=0.29) or in the quality of life (P=0.18). Daily zafirlukast therapy did not differ significantly from intermittent treatment in any outcome measured.

CONCLUSIONS
It may be possible to treat mild persistent asthma with short, intermittent courses of inhaled or oral corticosteroids taken when symptoms worsen. Further studies are required to determine whether this novel approach to treatment should be recommended.
Treatment guidelines recommend daily antiinflammatory therapy to control mild persistent asthma. This recommendation for so-called controller therapy was prompted by studies reporting that such treatment improves physiologic measures of airway obstruction (peak expiratory flow [PEF] and forced expiratory volume in one second [FEV₁]), the severity of symptoms, the frequency of exacerbations, and the quality of life and was reinforced by reports that inhaled corticosteroid treatment may prevent progressive loss of pulmonary function. However, analysis of pharmacy records suggests that most patients infrequently renew their prescriptions for controller medications (inhaled corticosteroids and leukotriene-receptor antagonists).

We reasoned that patients with mild asthma may be using their treatment intermittently because they do not perceive the need for daily therapy. To analyze whether this strategy could be an acceptable approach to treatment in patients with mild persistent asthma, we modified a symptom-based action plan to guide the use of inhaled or oral corticosteroids when signs or symptoms of asthma worsened. In a three-way study — the Improving Asthma Control (IMPACT) Trial — we compared the level of asthma control obtained with the use of this intermittent-treatment approach with that obtained with use of the intermittent-treatment plan plus daily treatment with a controller medication, either an inhaled corticosteroid (budesonide) or a leukotriene-receptor antagonist (zafirlukast). Morning PEF, a widely used and robust indicator of airflow obstruction, was the primary outcome indicator. Secondary outcomes included the frequency of asthma exacerbations, the number of days lost from work or school, the number of symptom-free days, asthma-related quality of life, and a panel of physiological and biologic measures of asthma activity.

Methods

Patients

Patients were recruited between February 2000 and May 2002 at six centers with the use of methods and equipment described previously. The protocol was approved by the institutional review board of each center, and written informed consent was obtained from each participant. Inclusion criteria were physician-diagnosed asthma, an age of 18 to 65 years, and an FEV₁, measured more than four hours after the most recent use of a bronchodilator, that was at least 70 percent of the predicted value. All patients had an increase in the FEV₁ of at least 12 percent and at least 200 ml after the inhalation of albuterol or a fall in FEV₁ of at least 20 percent after inhaling a concentration of methacholine of less than 16 mg per milliliter (PC₂₀; lower concentrations indicate greater reactivity).

Exclusion criteria included cigarette smoking, respiratory tract infection or corticosteroid use in the previous six weeks, and hospitalization or two or more visits to the emergency department for asthma in the previous year. Patients qualifying at a screening visit were instructed in the use of an electronic peak flowmeter (AirWatch, ENACT Health Management Systems) and were given a diary to record morning and evening PEF, asthma symptoms, nocturnal awakenings related to asthma, and as-needed albuterol use. They were instructed to take one puff from a placebo-dispensing dry-powder inhaler (Turbuhaler, AstraZeneca), which was identical in appearance to the device used to dispense inhaled budesonide, and one placebo tablet (identical in appearance to zafirlukast) twice a day.

We enrolled patients only if their diary records and findings during visits in the next four weeks met the criteria for mild persistent asthma (self-treatment with a beta-agonist more than two days per week, nighttime awakenings related to asthma more than two days per month, or variability in the PEF of 20 to 30 percent). Apart from accepting a baseline FEV₁ as low as 70 percent of the predicted value, we excluded patients if they met any criteria for persistent moderate asthma (i.e., daily self-treatment with a beta-agonist, nighttime awakenings once a week, or more than 30 percent variability in PEF). Enrollment also required at least 70 percent adherence to diary keeping, Turbuhaler use (by counting the number of doses remaining in the inhaler), and tablet use (established by pill counts and electronic drug-exposure monitoring [eDEM, Aardex] of the time and date of each opening of the pill bottle). PEF measurements were made and diaries were kept for four weeks during the run-in period, at the midpoint of the study, and at the end of the study.

Protocol

On entry, all patients received 10 minutes of instruction in a symptom-based asthma treatment...
DAILY VS. AS-NEEDED THERAPY FOR MILD PERSISTENT ASTHMA

plan (details of the plan are provided in the Supplementary Appendix, available with the full text of this article at www.nejm.org). The plan called for patients to take open-label budesonide (800 µg twice daily) for 10 days or prednisone (0.5 mg per kilogram of body weight per day) for 5 days if their asthma symptoms worsened. The patients’ understanding of this plan was not formally evaluated, but they did receive a written summary of the plan, and the plan was reviewed briefly at each study visit.

After completing the run-in period, the patients were assigned to one of three parallel treatment groups: twice-daily oral placebo and inhalation of 200 µg of budesonide, twice-daily oral zafirlukast (20 mg) and inhalation of placebo, or twice-daily oral and inhaled placebo (intermittent treatment) (Fig. 1) (see the Supplementary Appendix for details of the procedures at visits). Treatment assignment was stratified according to center, and the use of an adaptive randomization scheme ensured balance with respect to PC_{20}, age, and racial or ethnic group.

Budesonide, zafirlukast, and matched placebos in identical delivery systems (pills or Turbuhaler) were donated by AstraZeneca. Representatives of the company reviewed and commented on the protocol but made no other contribution to its design, conduct, interpretation, or presentation.

The run-in and treatment phases both ended with a 10-to-14-day period of intense combined therapy, consisting of 0.5 mg of prednisone per kilogram per day, 800 µg of budesonide twice daily, and 20 mg of zafirlukast twice daily, plus treatment as needed with albuterol (540 to 720 µg), to eliminate any easily reversed causes of airflow obstruction affecting PEF or FEV₁.

At study visits, FEV₁ was measured, adherence to treatment was assessed, the degree of asthma control was assessed by means of a seven-item questionnaire (in which a score of 0 indicated no symptoms and a score of 6 severe symptoms), medication-related side effects were assessed, and symptom-related impairment or discomfort was evaluated by means of the Asthma Symptom Utility

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**Figure 1. Enrollment and Outcome.**

Reasons for exclusion during the run-in period were the need for inhaled budesonide therapy in 34 patients, excessive symptoms in 30, too few symptoms in 30, withdrawal of consent by 31, loss to follow-up of 19, failure to meet adherence criteria in 17, use of excluded medications by 6, presence of an excluded medical condition in 6, and other causes in 3. The run-in and treatment phases both ended with a 10-to-14-day period of intense combined therapy, consisting of 0.5 mg of prednisone per kilogram per day, 800 µg of budesonide twice daily, and 20 mg of zafirlukast twice daily, plus treatment with albuterol (540 to 720 µg), to eliminate any easily reversed causes of airflow obstruction affecting PEF or FEV₁.
The Asthma Symptom Utility Index is derived from a 10-item questionnaire completed by the patient. Scores range from 0 to 1, with higher scores indicating fewer symptoms. At visits during the periods in which diaries were maintained (four weeks during the run-in period and at the midpoint and end of the treatment periods, and during the two weeks of the two periods of intense combined treatment), peak flow was obtained from the diary records. Questionnaires about the number of symptom-free days, adverse events, and health care use were administered at all study visits and by means of telephone calls between visits. When these questionnaires identified patients who had had worsening of asthma symptoms, they were asked whether they had used the symptom-based action plan and this information was recorded.

The asthma-related quality of life was assessed by means of a questionnaire at enrollment and at the end of treatment; patients rate the degree of impairment caused by asthma during the preceding 14 days and respond to each of the 32 items using a 7-point scale on which a score of 1 indicates maximal impairment and a score of 7 no impairment. Changes in the score of 0.5, 1.0, and 1.5 correspond to small, moderate, and large differences, respectively. The questionnaire can be used to provide an overall score and scores in four areas: limitation of activities, asthma symptoms, emotional functioning, and symptoms arising from environmental exposures. Exhaled nitric oxide, the PC_{20}, and the percentage of eosinophils in sputum were measured at enrollment and at the end of treatment (Fig. 1).

**Outcome Variables**

The primary outcome variable was the change from baseline in two-week average morning PEF. Other objective outcome variables were the changes from baseline in the FEV_{1}, before bronchodilator use, the FEV_{1} after treatment with 540 to 720 µg of albuterol, and the morning PEF during the period of intense combined therapy and FEV_{1} after the period of intense combined therapy. We also measured the frequency of asthma exacerbations warranting the initiation of prednisone therapy according to the symptom-based action plan (whether initiated or not). Patients were instructed to notify their study center about these events, but we also identified such events by asking specific questions at study visits and during telephone contacts. Other patient-reported outcomes were responses to standard questionnaires on asthma control, asthma-related quality of life, symptom-free days, symptom-related impairment or discomfort, days missed from work or school, and adverse events (see above).

**Statistical Analysis**

The trial was designed to show the superiority of any one treatment over either of the other two. The primary outcomes were evaluated as the average percent change from the end of the run-in period to the end of treatment and were initially compared by means of analysis of variance. Pairwise comparisons between groups were evaluated if the P value for the overall test was less than 0.048 (by a two-sided test, adjusted for an interim analysis at the 0.005 level). These comparisons were then adjusted for baseline characteristics by including in an analysis-of-covariance model effects such as center, interaction between center and treatment, age, baseline PC_{20}, baseline FEV_{1}, duration of asthma, and other important baseline covariates listed in Table 1 (the list of covariates analyzed for each outcome is provided in the Supplementary Appendix). Repeated-measures analysis of covariance was also used on outcomes measured repeatedly throughout the study to evaluate correlated data.

The times to the first exacerbation of asthma were compared by means of Kaplan–Meier curves and the log-rank test. A repeated-measures proportional-hazards approach was used to compare groups, allowing for multiple exacerbations per patient. The patient-reported outcomes regarding asthma control and symptoms throughout the trial were evaluated with repeated-measures analysis of covariance.

The primary end point compared among the groups was the change in morning PEF from randomization to the end of the trial. Using the standard deviation for morning PEF of 36.6 liters per minute noted in a previous study, we calculated that a sample of 216 patients would provide a statistical power of 90 percent to detect the difference widely considered to be of clinical significance, 25 liters per minute, at a significance level of 4.8 percent, allowing a dropout rate of 15 percent. For the secondary end point — change in morning PEF from the first to the second period of intense combined therapy — we used the variability observed in the corticosteroid run-in period of a previous trial. We calculated that if 199 patients completed the study, the study would have a statistical power of 80 percent to detect a difference of 21 liters...
per minute in this morning PEF during the period of intense combined therapy between any two treatment groups. We further calculated that this sample would provide 80 percent power to detect a change of 13 liters per minute in morning PEF within groups.

### RESULTS

Of 411 patients who were enrolled after screening, 225 underwent randomization and 199 completed the study (Fig. 1). The treatment groups were well matched (Table 1). Twenty-six patients withdrew after randomization, 6 each from the budesonide and intermittent-treatment groups and 14 from the zafirlukast group (P=0.10). Reasons for withdrawal included loss to follow-up (in six patients), pregnancy (four patients), personal constraints (four patients), side effects possibly related to study medications (two patients), dissatisfaction with asthma control (one patient), and miscellaneous reasons (nine patients).

Adherence to study medication regimens, estimated from counting unused doses in the Turbuhaler and from pill counts and eDEM records, exceeded 90 percent and was similar among the groups. The use of open-label budesonide was no greater in the intermittent-treatment group than in the groups taking daily budesonide or zafirlukast (Fig. 2). Inhaled budesonide was taken for only 55 percent of the episodes of mild-to-moderate worsening of symptoms as defined by the asthma action plan (Supplementary Appendix). The average per-patient use of a daily controller medication over the year of the study was 47.8 weeks for the budesonide and zafirlukast groups (92 percent adherence × 52 weeks) and 0.48 week for the intermittent-treatment group.

The primary outcome, the change in morning PEF from the final two weeks of the run-in period to the final two weeks of the year of treatment, did not differ significantly among the groups, increasing about 7.8 percent (32 liters per minute) in all groups (P=0.90) (Table 2). The increases in average morning PEF from the first to the second period of intense combined therapy were also similar among the groups (3.5 to 5.7 percent, P=0.61) (Table 2), even after adjustment for center, age, minority status, and PC_{20}.

The pre-bronchodilator FEV₁ increased more in the budesonide group than in the other two groups (P=0.005) (Table 2), but the changes in

### Table 1. Baseline Characteristics of the Patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Daily Budesonide</th>
<th>Daily Zafirlukast</th>
<th>Intermittent Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(N=73)</td>
<td>(N=76)</td>
<td>(N=76)</td>
</tr>
<tr>
<td>Male sex — no. (%)</td>
<td>25 (34)</td>
<td>29 (38)</td>
<td>33 (43)</td>
</tr>
<tr>
<td>Minority — no. (%)†‡</td>
<td>13 (18)</td>
<td>26 (34)</td>
<td>22 (29)</td>
</tr>
<tr>
<td>Black race — no. (%)†</td>
<td>9 (12)</td>
<td>11 (14)</td>
<td>13 (17)</td>
</tr>
<tr>
<td>Age — yr</td>
<td>33.2±9.5</td>
<td>33.6±11.1</td>
<td>32.0±10.5</td>
</tr>
<tr>
<td>Duration of asthma — yr</td>
<td>17.1±11.0</td>
<td>20.9±13.1</td>
<td>19.5±11.8</td>
</tr>
<tr>
<td>Data missing — no. of patients</td>
<td>2</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>Height — cm</td>
<td>170.0±10.4</td>
<td>170.3±8.9</td>
<td>170.2±9.6</td>
</tr>
<tr>
<td>Weight — kg</td>
<td>74.3±15.3</td>
<td>77.1±16.6</td>
<td>74.6±15.4</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>25.7±4.4</td>
<td>26.5±5.0</td>
<td>25.7±4.6</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV₁ — Liters</td>
<td>3.2±0.8</td>
<td>3.2±0.8</td>
<td>3.2±0.7</td>
</tr>
<tr>
<td>% Predicted</td>
<td>90.5±12.6</td>
<td>88.2±14.4</td>
<td>87.8±12.7</td>
</tr>
<tr>
<td>Morning PEF, 2-wk average — liters/min</td>
<td>467±117</td>
<td>468±111</td>
<td>462±106</td>
</tr>
<tr>
<td>Exhaled nitric oxide — parts per billion</td>
<td>1.08±0.25</td>
<td>1.33±0.43</td>
<td>1.17±0.22</td>
</tr>
<tr>
<td>Data missing — no. of patients</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Median</td>
<td>16.8</td>
<td>16.8</td>
<td>16.4</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>11.7–25.1</td>
<td>10.5–24.9</td>
<td>10.3–23.5</td>
</tr>
<tr>
<td>Data missing — no. of patients</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Median</td>
<td>0.6</td>
<td>0.5</td>
<td>0.4</td>
</tr>
<tr>
<td>Interquartile range</td>
<td>0.2–2.0</td>
<td>0.0–1.3</td>
<td>0.0–1.3</td>
</tr>
<tr>
<td>Data missing — no. of patients</td>
<td>32</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Asthma Quality of Life score</td>
<td>5.8±0.7</td>
<td>5.8±0.6</td>
<td>5.9±0.6</td>
</tr>
<tr>
<td>Asthma-control score</td>
<td>1.1±0.6</td>
<td>1.1±0.5</td>
<td>1.1±0.5</td>
</tr>
<tr>
<td>No. of symptom-free days in past 14 days</td>
<td>5.9±4.4</td>
<td>5.5±4.2</td>
<td>6.1±4.3</td>
</tr>
<tr>
<td>Asthma Symptom Utility Index score**</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
<td>0.8±0.1</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD. To convert values for weight to pounds, multiply by 2.2. Body-mass index is the weight in kilograms divided by the square of the height in meters.
† Minority status and black race were self-reported.
‡ P=0.07 by the chi-square test.
§ Geometric means and coefficients of variation are given.
¶ Scores can range from 1 (totally limited) to 7 (not at all limited).
** Scores can range from 0 to 1, with higher scores indicating fewer symptoms.
post-bronchodilator FEV₁ did not differ significantly among the groups (P=0.29) (Table 2). The average changes in post-bronchodilator FEV₁ in the budesonide and intermittent-treatment groups were −1.7 percent and −1.0 percent, respectively, resulting in an average difference between groups of −0.7 percentage point. The 95 percent confidence interval for this difference was −2.1 percent (greater decrease in the budesonide group) to 0.7 percent (greater decrease in the intermittent-treatment group).

The change in post-bronchodilator FEV₁ in the 46 patients with an FEV₁ at entry that was 70 to 79 percent of the predicted value was not significantly different from that in the 144 patients with an FEV₁ at entry that was at least 80 percent of the predicted value (P=0.59) (data not shown). The FEV₁ measured after the period of intense combined therapy declined similarly in all groups. Patients treated with budesonide had greater improvements in the percentage of eosinophils in sputum, exhaled nitric oxide values, and PC_{20} values than did the patients in either of the other two groups (Table 2). As compared with intermittent treatment, treatment with zafirlukast produced no significantly greater improvement in any outcome.

Thirty exacerbations of symptoms warranting treatment with prednisone occurred in 25 (11.1 percent) patients, an overall rate of 0.13 per patient-year. The proportion of patients who had one or more exacerbations did not differ significantly among the groups (one exacerbation in eight patients in the budesonide group and three in two patients in this group; one exacerbation in six patients in the zafirlukast group; and one exacerbation in seven patients in the intermittent-treatment group and three in one patient in this group).

Kaplan–Meier curves showed no significant differences among the groups, whether they were plotted as the time to a first event (P=0.39 by the log-rank test) (Fig. 3) or allowed multiple events per patient (P=0.24). The 12-month Kaplan–Meier exacerbation rates for the budesonide and intermittent-treatment groups were 16.1 percent and 11.3 percent, respectively, resulting in an average difference (i.e., positive sign indicates more exacerbations in the budesonide group) of +4.8 percentage points. The 95 percent confidence interval for this difference was −7 percent (lower in the budesonide group) to 16 percent (higher in the budesonide group). Patients initiated prednisone treatment for only 36.7 percent of the episodes (5 of 14 episodes in the budesonide group, 2 of 6 in the zafirlukast group, and 4 of 10 in the intermittent-treatment group). Five exacerbations required a visit to the emergency department (three in the budesonide group and one each in the other two groups). None warranted hospitalization. Altogether, patients missed 13 days from work or school because of asthma (7 days in the budesonide group, 2 days in the zafirlukast group, and 4 days in the intermittent-treatment group; P=0.18).

Of the patient-reported outcomes, the improvements in the asthma control score and in the number of symptom-free days were significantly greater with budesonide treatment than with either zafirlukast or intermittent treatment, which did not differ significantly from each other (Table 2). The greater number of symptom-free days over a 2-week period with budesonide (9.9 days) than with zafirlukast (8.7 days) or intermittent treatment (8.8 days) translates to 26 additional symptom-free days per year (95 percent confidence interval, 1.8 to 48.5). This was not associated, however, with any difference in the changes in the scores for the asthma-related quality of life, which improved in all groups (Table 2).

Neither the overall frequency of adverse events nor the frequency of severe events (36 or 37 in each group) differed significantly among the groups; seven of the patients with severe events required hospitalization. In this blinded study, no hospital-
Our study of 225 patients with mild persistent asthma showed no clinically significant difference among the three treatment groups with respect to morning PEF. Although other objective measures of lung function and airway biology were improved and patients reported 26 more days free from symptoms of asthma per year when treated with budesonide on a daily basis than with the other treatments, the frequency of asthma exacerbations did not differ among the treatment groups.

**Table 2. Average Changes in Primary and Secondary Outcome Measures over a One-Year Period.**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Daily Budesonide</th>
<th>Daily Zafirlukast</th>
<th>Intermittent Treatment</th>
<th>Overall P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of Patients</td>
<td>Value</td>
<td>No. of Patients</td>
<td>Value</td>
</tr>
<tr>
<td>Morning PEF (%)</td>
<td>66</td>
<td>8.3±1.9</td>
<td>&lt;0.001</td>
<td>62</td>
</tr>
<tr>
<td>Morning PEF post-PICT (%)</td>
<td>66</td>
<td>5.7±1.7</td>
<td>0.002</td>
<td>62</td>
</tr>
<tr>
<td>FEV₁ (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-bronchodilator</td>
<td>67</td>
<td>4.0±1.2</td>
<td>0.001</td>
<td>62</td>
</tr>
<tr>
<td>Post-bronchodilator</td>
<td>67</td>
<td>−1.7±0.5</td>
<td>0.002</td>
<td>61</td>
</tr>
<tr>
<td>Post-PICT</td>
<td>67</td>
<td>−1.5±0.7</td>
<td>0.03</td>
<td>62</td>
</tr>
<tr>
<td>Exhaled nitric oxide (%)</td>
<td>63</td>
<td>0.75</td>
<td>0.02</td>
<td>60</td>
</tr>
<tr>
<td>Median</td>
<td>−14.4</td>
<td>12.4</td>
<td>26.6</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>−44.4 to 46.8</td>
<td>−24.6 to 82.8</td>
<td>−9.6 to 99.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Sputum eosinophils (%)</td>
<td>34</td>
<td>0.03</td>
<td>26</td>
<td>0.71</td>
</tr>
<tr>
<td>Median</td>
<td>−0.3</td>
<td>0.0</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Interquartile range</td>
<td>−1.6 to 0.2</td>
<td>−0.9 to 0.3</td>
<td>−0.1 to 1.5</td>
<td></td>
</tr>
<tr>
<td>PC_{20} (log₂)</td>
<td>63</td>
<td>1.8±0.2</td>
<td>&lt;0.001</td>
<td>58</td>
</tr>
<tr>
<td>Asthma Quality of Life score§</td>
<td>67</td>
<td>0.5±0.1</td>
<td>&lt;0.001</td>
<td>64</td>
</tr>
<tr>
<td>Asthma control score¶</td>
<td>70</td>
<td>−0.4±0.1</td>
<td>&lt;0.001</td>
<td>70</td>
</tr>
<tr>
<td>No. of symptom-free days¶</td>
<td>70</td>
<td>4.0±0.4</td>
<td>&lt;0.001</td>
<td>70</td>
</tr>
<tr>
<td>Asthma Symptom Utility Index¶/§</td>
<td>70</td>
<td>0.06±0.01</td>
<td>&lt;0.001</td>
<td>70</td>
</tr>
</tbody>
</table>

* Unless otherwise stated, values reflect mean (±SE) changes from baseline to the end of the treatment period (before the period of intense combined therapy [PICT]) (see Fig. 1). In each analysis of covariance model evaluated to confirm the unadjusted results above, the covariates used in the stratified randomization scheme of the study were included (center, age, minority status, and PC_{20} value). All other baseline covariates listed in Table 1 were then considered to see whether they added any significant explanatory power to the model. In the resulting main-effects models, the interaction between center and treatment and all pairwise interaction terms of the predictors in each model were also considered. The results based on the inclusion of these factors in each model did not differ significantly from the conclusions of the unadjusted results reported above.

† P values refer to differences among the groups with the use of analysis of variance or repeated-measures analysis of covariance.

‡ Results are mean changes from baseline averaged over all visits; P values are from longitudinal analysis (repeated-measures analysis of covariance).

§ Scores can range from 1 (totally limited) to 7 (not at all limited).

¶ Scores can range from 0 (no symptoms) to 6 (severe symptoms).

|| Scores can range from 0 to 1, with higher scores indicating fewer symptoms.

**Discussion**

Our study of 225 patients with mild persistent asthma showed no clinically significant difference among the three treatment groups with respect to morning PEF. Although other objective measures of lung function and airway biology were improved and patients reported 26 more days free from symptoms of asthma per year when treated with budesonide on a daily basis than with the other treatments, the frequency of asthma exacerbations did not dif-
The new england journal of medicine

1526

fer significantly among the groups. Although our study was not designed as a noninferiority trial and, thus, the findings must be considered preliminary, these data suggest that a novel approach to the treatment of persistent asthma — symptom-driven intermittent treatment with inhaled or oral corticosteroids — may be possible. Since the intermittent use of inhaled corticosteroids could decrease the adverse effects of these agents, our data provide the impetus for a large-scale trial to test this novel approach to asthma treatment.

Epidemiologic studies have reported that the use of inhaled corticosteroids reduces asthma-related hospitalizations and deaths, and some previous clinical studies of mild asthma have reported that inhaled corticosteroid therapy reduces the frequency of exacerbations and the rate of decline in the results of tests of airway caliber (PEF, FEV₁, or post-bronchodilator FEV₁). So the suggestion that a large subgroup of patients with asthma may not require daily controller treatment will arouse concern.

However, the protective effect in the epidemiologic studies was most apparent by far in patients using frequent doses of an inhaled beta-agonist, a pattern inconsistent with the criteria for mild persistent asthma. In our study of 411 patients who appeared on original screening to meet the criteria for mild persistent asthma, 64 were found to have asthma that was too severe and 30 asthma that was too mild to qualify. We believe that the lack of a difference among our treatment groups may reflect the low rate of exacerbations in patients who consistently meet the criteria for mild persistent asthma. This possibility is supported by our finding of a rate of exacerbations warranting prednisone treatment in our intermittent-treatment group (0.11 per patient-year) well below the rates reported in previous studies of mild asthma (0.21 to 0.77 per patient-year).

This difference in asthma severity could also account for the difference from previous studies reporting that continuous corticosteroid therapy prevented a decline in airway function in patients with mild asthma. We found no treatment-attributable difference in the change in post-bronchodilator FEV₁. Our study was shorter than the previous studies, but the preponderance of the differences between treatment groups in these studies occurred during the first year, and we noted no such effect. Our findings are consistent with those of the Childhood Asthma Management Program trial, a study of children with asthma, which showed no effect of five years of treatment with an inhaled corticosteroid on the change in post-bronchodilator FEV₁.

Furthermore, the robustness of our findings, as reflected by the confidence intervals for the differences in the decline in post-bronchodilator FEV₁ and in exacerbation rates, suggests that the treatment benefits that our study might have missed may be so small as not to justify the expense, potential adverse effects, and inconvenience of daily treatment with a controller therapy of all patients with mild persistent asthma.

We did find that budesonide (but not zafirlukast) improved markers of airway inflammation, such as bronchial reactivity, the percentage of eosinophils in sputum, and exhaled nitric oxide. It is noteworthy, however, that low-grade inflammation similar to that seen in our patients has been reported in patients with spontaneous, complete, sustained clinical remission of asthma, who are not now considered candidates for daily controller treatment. We found that daily treatment with budesonide (but not zafirlukast) was associated with a significant increase in the number of symptom-free days and a trend toward improvement in the scores for a weighted symptom utility index, but not in asthma-related quality of life. This lack of improvement in the quality of life may reflect the light burden of symptoms of mild asthma. In asthma of this severity, symptoms are occasional and are usually promptly relieved by treatment with an inhaled bronchodilator. Whether the increase in symptom-free days is worth the costs of treatment, both fiscal and
with respect to long-term side effects, may thus be an individual, subjective judgment best left to the patient and his or her health care provider. It is fair to ask whether the approach to treatment in our intermittent-treatment group could be practically applied outside of the artificial conditions of a clinical trial. We tried to mimic true clinical conditions by basing the action plan on symptoms, rather than on peak flow. All our patients were given an open-label budesonide inhaler, prednisone tablets, and 10 minutes of instruction in the symptom-based action plan. This instruction was reinforced by a written summary and by reminders of the plan at each visit and a telephone call (every six weeks). This attention to teaching an action plan might limit the generalizability of our findings. However, even under these conditions, patients took budesonide for only 55 percent of the episodes of mild-to-moderate worsening of symptoms and prednisone for only 37 percent of the episodes severe enough to warrant its use. We also found no significant difference in the rate of exacerbations warranting prednisone treatment in patients who should have but did not take budesonide (7 of 22) than in those who should have and did (15 of 21). Taken together, these observations suggest that close, formal adherence to the action plan may not have accounted for our findings.

In adults with long-standing, mild persistent asthma who were given medication and instructed to initiate corticosteroid therapy according to a symptom-based action plan, regularly scheduled controller treatment with either inhaled budesonide or oral zafirlukast had no significant effect on the rate of severe exacerbations, impairment in the quality of life, or the rate of loss of pulmonary function over a period of one year. These findings suggest that the novel approach of treating patients with mild persistent asthma with inhaled and oral corticosteroids as needed may be viable. Longer and larger studies will be needed before this approach to asthma treatment can be recommended.

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### References

9. StoloFF SW, Stempel DA, Meyer J, Stanford RH, Carranza Rosenzweig JR. Improved
DNA Topoisomerase II in Therapy-Related Acute Promyelocytic Leukemia

Anita R. Mistry, Ph.D., Carolyn A. Felix, M.D., Ryan J. Whitmarsh, B.A., Annabel Mason, B.Sc., Andreas Reiter, M.D., Bruno Cassinat, Pharm.D., Anne Parry, Ph.D., Christoph Walz, Joseph L. Wiemels, Ph.D., Mark R. Segal, Ph.D., Lionel Adès, M.D., Ian A. Blair, Ph.D., Neil Osheroff, Ph.D., Andrew J. Peniket, B.A., Marina Lafage-Pochitaloff, Ph.D., Nicholas C.P. Cross, Ph.D., Christine Chomienne, Ph.D., Ellen Solomon, Ph.D., Pierre Fenaux, Ph.D., and David Grimwade, Ph.D.

BACKGROUND
Chromosomal translocations leading to chimeric oncoproteins are important in leukemogenesis, but how they form is unclear. We studied acute promyelocytic leukemia (APL) with the t(15;17) translocation that developed after treatment of breast or laryngeal cancer with chemotherapeutic agents that poison topoisomerase II.

METHODS
We used long-range polymerase chain reaction and sequence analysis to characterize t(15;17) genomic breakpoints in therapy-related APL. To determine whether topoisomerase II was directly involved in mediating breaks of double-stranded DNA at the observed translocation breakpoints, we used a functional in vitro assay to examine topoisomerase II–mediated cleavage in the normal homologues of the PML and RARA breakpoints.

RESULTS
Translocation breakpoints in APL that developed after exposure to mitoxantrone, a topoisomerase II poison, were tightly clustered in an 8-bp region within PML intron 6. In functional assays, this “hot spot” and the corresponding RARA breakpoints were common sites of mitoxantrone-induced cleavage by topoisomerase II. Etoposide and doxorubicin also induced cleavage by topoisomerase II at the translocation breakpoints in APL arising after exposure to these agents. Short, homologous sequences in PML and RARA suggested the occurrence of DNA repair by means of the nonhomologous end-joining pathway.

CONCLUSIONS
Drug-induced cleavage of DNA by topoisomerase II mediates the formation of chromosomal translocation breakpoints in mitoxantrone-related APL and in APL that occurs after therapy with other topoisomerase II poisons.
Acute myeloid leukemia (AML) is commonly associated with reciprocal balanced chromosomal translocations that underlie the formation of chimeric proteins that have key roles in the development of leukemia.\textsuperscript{1,2} The most frequent translocation, (t(15;17)(q22;q21)), occurs in 10 to 15 percent of cases of AML\textsuperscript{3} and is the hallmark of acute promyelocytic leukemia (APL). This translocation creates the PML-RARA and RARAPML fusion genes.\textsuperscript{3} The resultant PML-RAR\textalpha fusion protein determines not only the phenotype of APL but also the response of APL to all-trans-retinoic acid and arsenic trioxide treatment.\textsuperscript{3,4}

The transforming function of leukemia-associated fusion proteins has been widely studied, but little is known about the mechanisms that cause the underlying translocations. Insights can be gained from investigations of therapy-related leukemias, all of which have counterparts in primary leukemias. Exposure to drugs that poison topoisomerase II — the anthracyclines daunorubicin, doxorubicin, and epirubicin; the anthracenedione mitoxantrone; and epipodophyllotoxins such as etoposide — predisposes patients to secondary leukemias with balanced chromosomal rearrangements, including MLL translocations involving band 11q23, t(8;21), inv(16), t(15;17), t(9;22), and NUP98 translocations involving band 11p15.\textsuperscript{5-7} The association of such chromosomal rearrangements with exposure to drugs that affect topoisomerase II suggests a role for topoisomerase II–mediated cleavage of DNA in forming translocations, but how this occurs remains to be established.

Topoisomerase II relaxes supercoiled DNA by cleaving and religating both strands of the double helix through the formation of a transient covalent cleavage intermediate.\textsuperscript{8} Chemotherapeutic drugs termed “topoisomerase II poisons” convert topoisomerase II into a DNA-damaging enzyme. They disrupt the cleavage–religation equilibrium and thereby increase the concentration of topoisomerase II–mediated cleavage complexes.\textsuperscript{8} Although the enzyme does not have a known DNA recognition sequence that is most likely to target, genomic sequencing studies have suggested possible binding sites for the enzyme at translocation breakpoints in primary and treatment-related leukemias with MLL, AML1-ETO, PML-RARA, and NUP98 rearrangements.\textsuperscript{9-15}

In early studies, less than 5 percent of APL cases were a consequence of chemotherapy.\textsuperscript{16,17} But more recently, the European APL group reported that therapy-related APL accounted for 22 percent of all cases.\textsuperscript{27} The rising incidence of therapy-related APL parallels the increased use of topoisomerase II poisons, particularly in the treatment of breast cancer. APL with t(15;17) is one of the most frequent secondary cancers that arise after the treatment of breast cancer.\textsuperscript{17-20} Mitoxantrone has been implicated in almost half these cases.\textsuperscript{17,19,20} In the present study, we examined genomic breakpoint regions in patients with APL after exposure to topoisomerase II poisons, particularly mitoxantrone, and used functional assays to gain further insight into mechanisms underlying the formation of the t(15;17) chromosomal translocation.

\textbf{Methods}

\textbf{Patients and Samples}

Genomic breakpoint locations in PML and RARA genes were studied in six patients with therapy-related APL, which arose after mitoxantrone treatment for breast carcinoma (in five) or multiple sclerosis (in one). To determine whether breakpoint clustering in PML intron 6 detected in mitoxantrone-related APL was statistically significant, a comparison was made with breakpoint locations in 7 patients with secondary APL arising after other types of exposure, mostly radiotherapy, and in 35 patients with primary APL. Chromosomal breakpoint mechanisms were subsequently investigated with the use of functional topoisomerase II cleavage assays in four of the patients with mitoxantrone-related cases (Patients 1 through 4) and an additional patient (Patient 5) with secondary APL that developed after exposure to doxorubicin and etoposide (Table 1). All patients gave written informed consent, in accordance with the Declaration of Helsinki.

\textbf{Characterization of Genomic Breakpoints}

Three breakpoint regions have been identified within the PML locus in APL: intron 3 (bcr3), exon 6 (bcr2), and intron 6 (bcr1); virtually all breakpoints in RARA occur in intron 2.\textsuperscript{21} The breakpoint pattern in PML was determined by nested reverse-transcriptase polymerase chain reaction (RT-PCR)\textsuperscript{22}; appropriate primers were used to amplify the sequences of genomic breakpoint junctions by long-range or “bubble” PCR, and the PCR products were sequenced.\textsuperscript{23} Breakpoint junction sequences obtained in this way were confirmed by a patient-specific breakpoint PCR with the use of a fresh aliquot of genomic DNA as template.
We examined DNA fragments of the normal homologues of \(PML\) (GenBank accession numbers S51489 and S57791) and \(RARA\) (GenBank accession numbers AF088889 and AJ297538) that encompassed the relevant translocation breakpoints using an in vitro topoisomerase II cleavage assay.

**Substrate DNA** was incubated with human topoisomerase II \(a\) in the presence of ATP and exposed to drugs that target topoisomerase II. Final concentrations of etoposide, etoposide catechol, etoposide quinone, and mitoxantrone were 20 \(\mu\)M; we selected a final concentration of doxorubicin of 25 nM on the basis of a titration using concentrations between 1 nM and 200 nM (data not shown). Cleavage complexes were irreversibly trapped on the addition of sodium dodecyl sulfate, and cleavage products were resolved in a gel containing 8 percent polyacrylamide and 7.0 M urea alongside a DNA-sequencing ladder. This procedure allowed us to map cleavage sites precisely at the sequence level and to analyze the positions of the cleavage sites with respect to translocation breakpoint sites. Cleavage products were visualized by means of autoradiography and quantified with the use of a Phosphorimager and IMAGEQUANT software (Molecular Dynamics).

**Statistical Analysis**

The significance of the putative mitoxantrone cluster in cases of therapy-related APL or primary plus therapy-related APL was assessed with the use of scan statistics, which have been used widely for the assessment of spatial and temporal clustering of events. Generally, they are based on the maximal number of events occurring in a prescribed re-
This statistic is then referenced against a uniform (null) distribution (over the entire region or period) reflecting the absence of clustering. In the case of translocation breakpoint clustering, the event is the occurrence of a breakpoint, the interval is the number of base pairs spanning the putative cluster, and the reference interval is the relevant intron length.

Because the distribution of the scan statistic is exceedingly complex, a number of approximations have been developed. Here, we used the accurate, end-point–corrected, large-deviation approximation to the one-dimensional scan statistic.

Identification of a translocation breakpoint hot spot in mitoxantrone-related APL

Genomic breakpoint junction sequences on the derivative (der) chromosomes 15 and 17 were characterized in APL that arose after mitoxantrone-based treatment for breast cancer in five patients and mitoxantrone treatment for multiple sclerosis in one patient. Remarkably, the der(15) and der(17) PML breakpoints in four of these patients (Patients 1, 2, 3, and 4) (Table 1) were tightly clustered in an 8-bp region (positions 1482 to 1489; GenBank accession number S57791) in PML intron 6 (Fig. 1 and 2), a result consistent with the presence of a hot spot of DNA damage. Scan statistics indicated that the clustered breakpoints within an intron longer than 1 kb were unlikely to have arisen by chance (P<0.001 for the comparison with 7 cases of APL related to other therapy, P<0.05 for the comparison with the 7 other therapy-related cases plus 35 cases of primary APL, and P<0.05 for the comparison with the 35 cases of primary APL alone).

In contrast to the clustering of the PML breakpoints in cases of mitoxantrone-related APL, the RARA breakpoints were dispersed (Table 1 and Fig. 2). Study of the der(15) and der(17) sequences in the four patients with mitoxantrone-related cases associated with the hot spot indicated that the breakpoint junctions were formed without the gain or loss of any bases relative to the native PML and RARA sequences (Fig. 2). The short sequence homologies between PML and RARA (underlined in Fig. 2) are characteristic of DNA repair by the non-homologous end-joining pathway (NHEJ), which requires minimal overlapping sequences between nonhomologous chromosomes to repair breaks in double-stranded DNA.

SITE OF FUNCTIONAL TOPOISOMERASE II–MEDIATED CLEAVAGE AT THE PML INTRON 6 TRANSLATION BREAKPOINT HOT SPOT

We evaluated topoisomerase II–mediated cleavage of the normal homologue of the PML translocation breakpoint hot spot in vitro using a 268-bp double-stranded DNA substrate encompassing the 8-bp hot spot in the presence of mitoxantrone, etoposide or its catechol, or quinone metabolites and in the absence of these agents. Few cleavage sites were observed in the absence of drug (Fig. 3A). Bands of various sizes and intensities showed where cleavage sites were enhanced by the different agents (Fig. 3A).

The 8-bp translocation breakpoint hot spot at positions 1482 to 1489 corresponded to a topoisomerase II–mediated cleavage site at position 1484, where the position indicates the base immediately 5’ to the cleavage (−1 position). Cleavage at position 1484 was detected in the absence of drug, but it was markedly enhanced by etoposide, both etoposide metabolites, and mitoxantrone. Position 1484 was a preferred site of cleavage by topoisom-
erase II in the presence of mitoxantrone, as evidenced by the intensity of the cleavage band (Fig. 3A). Mitoxantrone-induced cleavage was enhanced by a factor of 8.9 and 2.5, respectively, relative to cleavage at this site without drug or in the presence of etoposide. Cleavage at many sites decreased substantially or was eliminated after heating (Fig. 3A). By contrast, the mitoxantrone-induced cleavage at position 1484 remained detectable after heating (Fig. 3A), indicating stability of the cleavage complexes. These results show that the PML intron 6 translocation breakpoint hot spot in mitoxantrone-related APL is a preferred and stable mitoxantrone-induced site of cleavage by topoisomerase II.

We performed in vitro topoisomerase II cleavage assays on double-stranded DNA substrates spanning the normal homologues of the RARA translocation breakpoints in Patients 1, 2, 3, and 4 to determine whether topoisomerase II also mediated the breakage at the RARA locus (Fig. 3B and Table 1). In Patient 1 (Fig. 3B), topoisomerase II–mediated cleavage was observed at position 2695 of the RARA intron 2 proximal to the der(15) and der(17) RARA translocation breakpoints and was heat-stable (Fig. 3B). The RARA breakpoints on both derivative chromosomes in specimens from Patients 2, 3, and 4 were also at, or proximal to, sites of func-
tional mitoxantrone-induced cleavage by topoisomerase II (Table 1). Assays on all substrates were repeated, and the repeated assays confirmed these results.

**SITES OF MITOXANTRONE-INDUCED CLEAVAGE BY TOPOISOMERASE II AND t(15;17) BREAKPOINT JUNCTIONS**

We used the functional sites of topoisomerase II–mediated cleavage of DNA at the translocation breakpoints to generate a model for the formation of the t(15;17), incorporating known repair mechanisms of breaks in double-stranded DNA. Figure 4 shows how recombination of mitoxantrone-enhanced cleavage sites at PML position 1484 and RARA position 2695 would form the der(15) and der(17) genomic breakpoint junctions identified in the APL in Patient 1. The sites of topoisomerase II–mediated cleavage of each DNA strand are four bases apart, thereby creating 5' overhangs, as shown in Figure 4. In the model, repair of the overhangs in PML and RARA entails exonucleolytic digestion, pairing of complementary bases, and joining of the DNA free ends by means of the NHEJ pathway, with template-directed polymerization to fill in any gaps. Models were also generated showing that mitoxantrone-induced cleavage by topoisomerase II formed the der(15) and der(17) breakpoint junctions in the leukemias in Patients 2, 3, and 4 (data not shown).

**PML AND RARA TRANSLOCATION BREAKPOINTS IN ETOPOSIDE- AND DOXORUBICIN-RELATED APL**

A fifth patient (Patient 5) received a diagnosis of APL after being treated with etoposide and doxorubicin for laryngeal cancer (Table 1). Study of the breakpoint junction sequences indicated that the translocation occurred with the loss of a single G nucleotide from RARA (position 16089) (Fig. 1A in the Supplementary Appendix, available with the full text of this article at www.nejm.org). Short sequence homologies were observed in PML and RARA characteristic of DNA repair by the NHEJ pathway. In the in vitro assay, etoposide, its catechol and quinone metabolites, and doxorubicin induced topoisomerase II–mediated cleavage at the PML and RARA translocation breakpoints. Cleavage was shown to be heat-stable with each of these drugs at the PML breakpoint (Fig. 1B in the Supplementary Appendix) and with etoposide quinone at the RARA breakpoint (Fig. 1C in the Supplementary Appendix). A model (Fig. 1D in the Supplementary Appendix) indicated how recombination of cleavage sites at PML position 1239 and RARA position 16089 would form the der(15) and der(17) genomic breakpoint junctions in this case of APL.

**DISCUSSION**

Leukemia characterized by balanced translocations, including t(15;17), can be a complication of treatment with anticancer drugs that poison topoisomerase II. The mechanism by which these drugs predispose patients to leukemia remains in dispute.

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**Figure 3 (facing page). Functional Topoisomerase II Cleavage Sites at Mitoxantrone-Associated Translocation Breakpoints.**

Chromosomal breakpoint regions were examined by means of a functional in vitro assay, which identifies topoisomerase II–dependent cleavage of DNA induced by various chemotherapeutic agents and their metabolites. Cleavage products were fractionated according to size and compared with a DNA-sequencing ladder to allow precise mapping of sites of DNA cleavage. Panel A shows topoisomerase II–mediated cleavage of DNA substrate spanning positions 1284 to 1551 of PML intron 6 (GenBank accession number S57791) encompassing the 8-bp translocation breakpoint hot spot (positions 1482 to 1489). The cleavage products in 25 ng (30,000 cpm) of DNA labeled only at the 5' end were examined after 10 minutes' incubation at 37°C with 147 nM human topoisomerase IIa, 1 mM ATP, and the following drugs at final concentrations of 20 μM etoposide (VP16), etoposide catechol (VP16-OH), etoposide quinone (VP16-Q), and mitoxantrone (Mit). Cleavage complexes were irreversibly trapped on the addition of sodium dodecyl sulfate (SDS), and purified cleavage products were resolved in a gel containing 8 percent polyacrylamide and 7.0 M urea, alongside DNA sequencing reactions primed at the same 5' end. Although very few cleavage sites were visible in the absence of drug (indicated by the minus signs), cleavage sites were enhanced by exposure to the various topoisomerase II–targeted agents (indicated by the plus signs). Specified reactions were incubated for an additional 10 minutes at 75°C before the addition of SDS in order to examine the stability of the cleavage complexes formed. The nucleotide 1484 is on the 5' side of the cleavage site (−1 position), which corresponds to the der(15) and der(17) translocation breakpoints in four patients with mitoxantrone-related APL. Panel B shows DNA topoisomerase II–mediated cleavage of the normal homologue of the der(15) and der(17) RARA translocation breakpoints in APL in Patient 1. The substrate spanning positions 2603 to 2871 of RARA intron 2 (GenBank accession number A(297538) contained the translocation breakpoints. The nucleotide 2695 indicates the (−1) position of the cleavage site corresponding to the der(15) and der(17) translocation breakpoints.
**A** PML

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**B** RARA

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**Translocation Mechanism in Therapy-Related APL**

**Der(15) der(17)**

- Patient 1: 1482–6 1488–9
- Patient 2: 1483–5 1484–6
- Patient 3: 1488 1488–9
- Patient 4: 1485–7 1486–9
some evidence supports a direct role for topoisomerase II in causing the DNA damage that leads to chromosomal rearrangements. An indirect mechanism involving the induction of apoptosis-inducing nucleases has also been proposed.

Few genomic breakpoint junctions have been characterized in therapy-related APL. Our study of the der(15) and der(17) genomic breakpoint junctions in APL arising after mitoxantrone treatment revealed clustering of breakpoints in the PML gene within an 8-bp region in intron 6, a result consistent with the existence of a translocation breakpoint hot spot. The PML-RARA rearrangements occurred without the gain or loss of any bases relative to the native genes, indicating that the translocation breakpoint sites could recombine to form the der(15) and der(17) breakpoint junctions observed in this patient. These results suggest that topoisomerase II-mediated cleavage is a general mechanism causing DNA damage in APL that develops after treatment with various agents that target topoisomerase II.

Recent reports of treatment-related APL indicate that epirubicin and mitoxantrone are the most common antecedent drugs and that a substantial proportion of the patients had breast cancer. Although etoposide is implicated in some cases of treatment-related APL, this drug is more often associated with MLL translocations that disrupt band 11q23. These observations suggest that different chemotherapeutic agents predispose patients to different translocations. A key question is how such specificity is conferred. Our in vitro assays show that mitoxantrone and etoposide or its metabolites stimulate topoisomerase II to cleave different sites in PML and RARA, implying the existence of different genomic hot spots for topoisomerase II-mediated cleavage in APL.

The mitoxantrone-related PML translocation breakpoint hot spot corresponded with a preferred site of topoisomerase II–mediated cleavage that was not religated after heating. In vitro, mitoxantrone stimulated cleavage at the translocation breakpoint hot spot that was nine times that observed in the absence of the drug. The RARA translocation breakpoints in each of the four patients with mitoxantrone-related APL investigated by functional assays were also found to correspond to mitoxantrone-induced sites of cleavage of DNA by topoisomerase II. Models were devised in which recombination of broken DNA at sites of topoisomerase II–mediated cleavage formed the der(15) and der(17) breakpoint junctions. These studies indicate that mitoxantrone induces cleavage of PML and RARA by topoisomerase II and that this cleavage resulted in the observed translocation breakpoint junctions in mitoxantrone-related APL.

To determine whether topoisomerase II–mediated cleavage is relevant to other drugs in therapy-related APL, we evaluated a patient in whom APL developed after exposure to etoposide and doxorubicin. Etoposide and its metabolites and doxorubicin related APL investigated by functional assays show that mitoxantrone and etoposide or its metabolites stimulate topoisomerase II to cleave DNA at the PML and RARA translocation breakpoints. The cleavage sites could recombine to form the der(15) and der(17) breakpoint junctions observed in this patient. These results suggest that topoisomerase II-mediated cleavage is a general mechanism causing DNA damage in APL that develops after treatment with various agents that target topoisomerase II.

Recent reports of treatment-related APL indicate that epirubicin and mitoxantrone are the most common antecedent drugs and that a substantial proportion of the patients had breast cancer. Although etoposide is implicated in some cases of treatment-related APL, this drug is more often associated with MLL translocations that disrupt band 11q23. These observations suggest that different chemotherapeutic agents predispose patients to different translocations. A key question is how such specificity is conferred. Our in vitro assays show that mitoxantrone and etoposide or its metabolites stimulate topoisomerase II to cleave different sites in PML and RARA, implying the existence of different genomic hot spots for topoisomerase II-mediated cleavage in APL.

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erase II-mediated cleavage in the presence of the different drugs. It is likely that such hot spots occur throughout the genome but that only translocations that confer a proliferative or survival advantage in an appropriate hematopoietic progenitor lead to leukemia. The identification of this translocation mechanism has important implications for the chemotherapy of cancer.


aberrations of acute myeloid leukemia may
develop in different ways and may contrib-
ute differently to malignant transformation.
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The Effect of Cardiac Resynchronization on Morbidity and Mortality in Heart Failure

John G.F. Cleland, M.D., Jean-Claude Daubert, M.D., Erland Erdmann, M.D., Nick Freemantle, Ph.D., Daniel Gras, M.D., Lukas Kappenberger, M.D., and Luigi Tavazzi, M.D., for the Cardiac Resynchronization — Heart Failure (CARE-HF) Study Investigators

Background
Cardiac resynchronization reduces symptoms and improves left ventricular function in many patients with heart failure due to left ventricular systolic dysfunction and cardiac dyssynchrony. We evaluated its effects on morbidity and mortality.

Methods
Patients with New York Heart Association class III or IV heart failure due to left ventricular systolic dysfunction and cardiac dyssynchrony who were receiving standard pharmacologic therapy were randomly assigned to receive medical therapy alone or with cardiac resynchronization. The primary end point was the time to death from any cause or an unplanned hospitalization for a major cardiovascular event. The principal secondary end point was death from any cause.

Results
A total of 813 patients were enrolled and followed for a mean of 29.4 months. The primary end point was reached by 159 patients in the cardiac-resynchronization group, as compared with 224 patients in the medical-therapy group (39 percent vs. 55 percent; hazard ratio, 0.63; 95 percent confidence interval, 0.51 to 0.77; P<0.001). There were 82 deaths in the cardiac-resynchronization group, as compared with 120 in the medical-therapy group (20 percent vs. 30 percent; hazard ratio 0.64; 95 percent confidence interval, 0.48 to 0.85; P<0.002). As compared with medical therapy, cardiac resynchronization reduced the interventricular mechanical delay, the end-systolic volume index, and the area of the mitral regurgitant jet; increased the left ventricular ejection fraction; and improved symptoms and the quality of life (P<0.01 for all comparisons).

Conclusions
In patients with heart failure and cardiac dyssynchrony, cardiac resynchronization improves symptoms and the quality of life and reduces complications and the risk of death. These benefits are in addition to those afforded by standard pharmacologic therapy. The implantation of a cardiac-resynchronization device should routinely be considered in such patients.
Despite improvements in pharmacologic treatment, many patients with heart failure have severe and persistent symptoms, and their prognosis remains poor.\(^1,2\) Such patients commonly have regions of delayed myocardial activation and contraction, leading to cardiac dyssynchrony. In a series of trials lasting up to six months, cardiac resynchronization decreased symptoms and improved exercise capacity, the quality of life, and ventricular function.\(^3\) The Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) trial showed that cardiac-resynchronization therapy alone or combined with an implantable defibrillator reduced the risk of complications and death of standard pharmacologic treatment, many patients with heart failure have severe and persistent symptoms, and their prognosis remains poor.\(^1,2\) Such patients commonly have regions of delayed myocardial activation and contraction, leading to cardiac dyssynchrony. In a series of trials lasting up to six months, cardiac resynchronization decreased symptoms and improved exercise capacity, the quality of life, and ventricular function.\(^3\) The Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) trial showed that cardiac-resynchronization therapy alone or combined with an implantable defibrillator reduced the risk of complications and death of standard pharmacologic therapy for moderate or severe heart failure and cardiac dyssynchrony.

**Methods**

The Cardiac Resynchronization — Heart Failure (CARE-HF) trial was a multicenter, international, randomized trial comparing the effect of cardiac resynchronization on the risk of complications and death among patients who were receiving standard medical therapy for moderate or severe heart failure and cardiac dyssynchrony.

**Patients**

Eligible patients were at least 18 years of age, had heart failure for at least six weeks, and were in New York Heart Association (NYHA) class III or IV despite receipt of standard pharmacologic therapy, with a left ventricular ejection fraction of no more than 35 percent, a left ventricular end-diastolic dimension of at least 30 mm (indexed to height), and a QRS interval of at least 120 msec on the electrocardiogram. Patients with a QRS interval of 120 to 149 msec were required to meet two of three additional criteria for dyssynchrony: an aortic prejection delay of more than 140 msec, an interventricular mechanical delay of more than 40 msec, or delayed activation of the posterolateral left ventricular wall.\(^11,13\)

Patients who had had a major cardiovascular event in the previous six weeks, those who had conventional indications for a pacemaker or an implantable defibrillator, and those with heart failure requiring continuous intravenous therapy were excluded. Also excluded were patients with atrial arrhythmias, since such patients cannot benefit from the atrial component of resynchronization.\(^11,13\)

**Study Procedures**

Randomization was stratified according to the NYHA class and was carried out by Quintiles with the use of a minimization procedure. Patients who were randomly assigned to undergo cardiac resynchronization received a Medtronic InSync or InSync III device, which provided atrial-based, biventricular stimulation with the use of standard right ventricular and Attain (Medtronic) left ventricular leads. Investigators were asked to position the left ventricular lead to pace the lateral or posterolateral left ventricular wall transvenously and provide radiographic documentation. Backup atrial pacing was set at 60 beats per minute. The interventricular delay was set...
to zero, and the atrioventricular delay was echocardiographically optimized. Patients were monitored overnight after receiving the device. If the initial procedure failed, repeated attempts at implantation were encouraged, and expert assistance was provided.

**Follow-up**

Patients were evaluated at 1, 3, 6, 9, 12, and 18 months and every six months thereafter, and standard medications were adjusted as appropriate at these visits. Investigators were asked to report all adverse events, which were classified in a blinded fashion by an end-points committee or, if they were procedure-related or device-related, by an independent expert who was not blinded to the study-group assignments (see the Appendix).

The protocol required follow-up to continue for 18 months after the last patient had been enrolled, unless the data and safety monitoring board stopped the study earlier or fewer than 300 patients had reached a primary end point at that time, in which case the trial could be extended. On March 6, 2004, the board recommended extending the study until May 2005 without disclosing the reasons. However, since the prespecified criteria had been met, the steering committee decided to conclude the study as planned on September 30, 2004, and implemented, without knowledge of the results, an extension phase with death from any cause as the (nominal) primary outcome. On February 24, 2005, after this article had been submitted for publication, the data and safety monitoring board indicated that the main reasons for its recommendation were interim analyses showing a trend toward more cardiovascular events in the first 10 days after randomization among patients assigned to cardiac resynchronization than among those assigned to medical therapy alone and a trend toward a favorable effect of resynchronization on long-term mortality that they thought might fail to reach significance by the time of the planned closure date.

**Endpoints**

The primary end point was a composite of death from any cause or an unplanned hospitalization for a major cardiovascular event; only the first event in each patient was included in this analysis. Data on patients who underwent elective heart transplantation were censored seven days after the procedure. Emergency heart transplantation was counted as a death.
were also compared in the two groups at follow-up. \textsuperscript{11} No data other than NYHA class were imputed for patients who died.

\textbf{Statistical analysis}

All prespecified analyses were conducted according to the intention-to-treat principle. P values other than for the primary end point are nominal. The study had a statistical power\textsuperscript{17} of 80 percent to identify a 14 percent relative reduction or a 5.7 percent point reduction in the rate of events, given a conventional one-sided \( \alpha \) value of 0.025 and a predicted number of 300 events.\textsuperscript{11} The time to an event was calculated according to the Kaplan–Meier method and analyzed with the use of Cox proportional-hazards models, which included baseline NYHA class as a covariate.\textsuperscript{16} Continuous data were analyzed with the use of mixed models, which included baseline variables as patient-level covariates and study centers as random effects.\textsuperscript{19} Dichotomous outcomes were analyzed with the use of nonlinear mixed models, which included the NYHA class as a patient-level covariate and study centers as random effects. The rates of adverse events were compared between groups by means of Fisher’s exact test. Analyses were conducted with the use of SAS software (version 9.12, SAS Institute). The data and safety monitoring board conducted two planned interim analyses with the use of nonsymmetric stopping rules.\textsuperscript{20}

\section*{Results}

A total of 404 patients were assigned to receive medical therapy alone and 409 to receive medical therapy plus cardiac resynchronization. The mean duration of follow-up was 29.4 months (range, 18.0 to 44.7). By the end of the study, the survival status of all patients was known, 383 patients had reached the primary end point, and 202 patients had died.

\section*{Study population}

Baseline characteristics were similar in the two groups (Table 1). Patients had well-treated moderate or severe heart failure and major left ventricular systolic dysfunction. Only 43 percent were taking high doses of diuretics (defined as at least 80 mg of furosemide, at least 2 mg of bumetanide, or at least 20 mg of torsemide). Beta-blockers were taken at some time during the study by 85 percent of the patients in the medical-therapy group and 84 percent of those in the cardiac-resynchronization group.

Implantation of a device was attempted in 404 of the 409 patients assigned to undergo cardiac resynchronization. One patient died before undergoing the procedure, and in four instances, the patient or investigator decided not to proceed. A cardiac-resynchronization device was implanted and activated in 390 patients (95 percent), in 349 on the first attempt; the device was implanted a median of four days (interquartile range, two to seven) after randomization. The median duration of hospitalization for implantation was five days (interquartile range, two to eight). Before the device could be activated, six patients had an unplanned hospitalization for cardiovascular reasons that qualified as a primary end point. Eight patients assigned to undergo cardiac resynchronization had a device with an additional defibrillator function implanted during the study.

In the medical-therapy group, implantation of a cardiac-resynchronization device alone was attempted in 43 patients and implantation of a resynchronization device with a defibrillator was attempted in 23 patients (both approaches were attempted in 1 patient). The device was activated in 50 patients. In 10 instances, a device was successfully implanted but programmed to provide only standard pacemaker or defibrillator functions to avoid crossover. In five patients, the attempt at implantation was unsuccessful. The device was activated in 19 patients (5 percent) before they reached the primary end point. Eight of these patients subsequently reached a primary end point, six of whom died. Of 31 patients in whom the device was activated after they had reached the primary end point, 7 subsequently died.

\section*{Primary end point}

By the end of the study, the primary end point had been reached in 159 patients in the cardiac-resynchronization group, as compared with 224 patients who received medical therapy alone (39 percent vs. 55 percent; hazard ratio, 0.63; 95 percent confidence interval, 0.51 to 0.77; \( P<0.001 \) ) (Fig. 1A and Table 2). There were 384 unplanned hospitalizations for a major cardiovascular event in the control group and 222 in the cardiac-resynchronization group. Death was the primary event in 74 patients, and hospitalization in 309. Prespecified subgroup analyses for the primary end point revealed no heterogeneity in the effect of cardiac resynchronization (Fig. 2). Twelve patients in the cardiac-resynchronization group and 10 in the medical-therapy group
Table 1. Baseline Characteristics of the Patients.*

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Medical Therapy Alone (N=404)</th>
<th>Medical Therapy plus Cardiac Resynchronization (N=409)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>66</td>
<td>67</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>293 (73)</td>
<td>304 (74)</td>
</tr>
<tr>
<td>NYHA class IV (%)</td>
<td>27 (7)</td>
<td>23 (6)</td>
</tr>
<tr>
<td>Dilated cardiomyopathy (%)</td>
<td>193 (48)</td>
<td>177 (43)</td>
</tr>
<tr>
<td>Ischemic heart disease (%)</td>
<td>144 (36)</td>
<td>165 (40)</td>
</tr>
<tr>
<td>Heart disease of other causes (%)</td>
<td>67 (17)</td>
<td>67 (16)</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>70</td>
<td>69</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>110</td>
<td>110</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>100–125</td>
<td>100–125</td>
</tr>
<tr>
<td>N-terminal pro–brain natriuretic peptide (pg/ml)†</td>
<td>1806</td>
<td>1920</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume index (ml/m²)</td>
<td>117</td>
<td>121</td>
</tr>
<tr>
<td>QRS duration (msec)</td>
<td>160</td>
<td>160</td>
</tr>
<tr>
<td>Interventricular mechanical delay (msec)</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Mitral-regurgitation area‡</td>
<td>0.23</td>
<td>0.21</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min/1.73 m²)</td>
<td>61</td>
<td>60</td>
</tr>
<tr>
<td>Use of an ACE inhibitor or angiotensin-receptor blocker (%)</td>
<td>383 (95)</td>
<td>387 (95)</td>
</tr>
<tr>
<td>Use of a beta-blocker (%)</td>
<td>298 (74)</td>
<td>288 (70)</td>
</tr>
<tr>
<td>Use of spironolactone (%)</td>
<td>238 (59)</td>
<td>219 (54)</td>
</tr>
<tr>
<td>Use of a high-dose loop diuretic (%)§</td>
<td>177 (44)</td>
<td>175 (43)</td>
</tr>
<tr>
<td>Use of digoxin (%)</td>
<td>181 (45)</td>
<td>165 (40)</td>
</tr>
</tbody>
</table>

* NYHA denotes New York Heart Association, and ACE angiotensin converting enzyme.
† To convert values for N-terminal pro–brain natriuretic peptide to picomoles per liter, divide by 8.457.
‡ The area was calculated as the area of the color-flow Doppler regurgitant jet divided by the area of the left atrium in systole, both in square centimeters.
§ A high-dose loop diuretic consisted of furosemide at a dose of 80 mg or more, bumetanide at a dose of 2 mg or more, or torsemide at a dose of 20 mg or more.
had unplanned hospitalizations for a major cardiovascular event that occurred within 10 days after randomization and were therefore not counted as primary end points.

DEATHS
In the cardiac-resynchronization group, 82 patients died, as compared with 120 patients who had been assigned to medical therapy alone (20 percent vs. 30 percent; hazard ratio, 0.64; 95 percent confidence interval, 0.48 to 0.85; P<0.002) (Fig. 1B and Table 2). The principal cause of death was cardiovascular in 167 patients (83 percent), noncardiovascular in 34 patients (17 percent), and not classifiable in 1 patient (0.5 percent). The cause of death was attributed to worsening heart failure in 56 of the 120 patients who died in the medical-therapy group (47 percent) and in 33 of the 82 patients who died in the cardiac-resynchronization group (40 percent). The mode of death was classified as sudden in 38 of the 120 patients who died in the medical-therapy group (32 percent) and in 29 of the 82 patients who died in the cardiac-resynchronization group (35 percent). The mortality rate in the medical-therapy group was 12.6 percent at one year and 25.1 percent at two years, as compared with 9.7 percent and 18.0 percent, respectively, in the cardiac-resynchronization group.

There were three emergency and six elective heart transplantations in the medical-therapy group and one emergency and nine elective heart transplantations in the cardiac-resynchronization group. All the patients who underwent emergency heart transplantation died. None of the patients who underwent elective transplantation had died within seven days after transplantation, at which point their follow-up data were censored from the analysis.

OTHER SECONDARY END POINTS
As compared with medical therapy alone, cardiac resynchronization reduced the risk of the composite end point of death from any cause or hospitalization for worsening heart failure (hazard ratio, 0.54; 95 percent confidence interval, 0.43 to 0.68; P<0.001) (Table 2). There were 252 hospitalizations for worsening heart failure among 133 patients in the medical-therapy group (33 percent) and 122 such hospitalizations among 72 patients in the cardiac-resynchronization group (18 percent).

As compared with patients in the medical-therapy group, patients in the cardiac-resynchronization group had less severe symptoms (P<0.001) and a better quality of life (P<0.001) at 90 days (Table 2). At 90 days, 15 patients had died in the medical-therapy group and 12 patients had died in the cardiac-resynchronization group. At 18 months, 105 of the patients in the cardiac-resynchronization group were in NYHA class I, 150 were in NYHA class II, and 80 were in NYHA class III or IV; the respective values in the medical-therapy group were 39, 112, and 152.
Echocardiographic, Biochemical, and Hemodynamic Assessments

At both 3 months and 18 months, the left ventricular ejection fraction was significantly greater, the left ventricular end-systolic volume index was significantly lower, the area of mitral regurgitation was significantly smaller, and the interventricular mechanical delay was significantly shorter in the cardiac-resynchronization group than in the medical-therapy group (Table 3). By 18 months, plasma levels of N-terminal pro–brain natriuretic peptide were lower among patients in the cardiac-resynchronization group (Table 3). Systolic blood pressure was higher at both 3 months and 18 months among patients in the cardiac-resynchronization group.

Serious Adverse Events

There was one device-related death in each group: one patient in the cardiac-resynchronization group died of heart failure aggravated by lead displacement, and one patient in the medical-therapy group died of septicemia after receiving a device. The most common adverse device- or procedure-related events in the cardiac-resynchronization group were lead displacement (24 patients), coronary-sinus dissection (10 patients), pocket erosion (8 patients), pneumothorax (6 patients), and device-related infection (3 patients). Worsening heart failure was more common in the medical-therapy group (affecting 263 patients, as compared with 191 patients in the cardiac-resynchronization group; P<0.001), whereas atrial arrhythmias or ectopy was more com-
mon in the cardiac-resynchronization group (affecting 64 patients in that group, as compared with 41 in the medical-therapy group; \( P=0.02 \)). The frequencies of respiratory tract infections, hypotension, falls or syncope, acute coronary syndromes, renal dysfunction, ventricular arrhythmias or ectopy, and neurologic events were similar in the two groups.

**DISCUSSION**

We found that cardiac resynchronization substantially reduced the risk of complications and death among patients with moderate or severe heart failure owing to left ventricular systolic dysfunction and cardiac dyssynchrony. The benefits were similar among patients with ischemic heart disease and patients without ischemic heart disease and were in addition to those afforded by pharmacologic therapy. The data are consistent with a resynchronization-induced reduction in cardiac dyssynchrony, leading to a sustained increase in left ventricular performance and a diminution of mitral regurgitation and, in turn, a rise in perfusion pressure, a fall in cardiac filling pressure, and favorable left ventricular remodeling. These changes in function translate into improvements in well-being and decreases in symptoms, complications, and the risk of death.

The favorable effects of cardiac resynchronization on symptoms, the quality of life, ventricular function, and blood pressure in our trial are similar to those reported in previous trials. However, we also found that cardiac resynchronization significantly reduced the risk of death. Calculations based on hazard ratios suggest that, for every nine devices implanted, one death and three hospitalizations for major cardiovascular events are prevented. This effect is in addition to the benefits of pharmacologic therapy and is similar to the reduction in the risk of death associated with beta-blocker therapy as compared with placebo in a similar population.

The benefit of cardiac resynchronization therapy in our study was due, at least in part, to the adherence of patients and investigators to the protocol and to the increasing effect of cardiac resynchronization over a long follow-up period, but it was not due to the recruitment of patients at higher risk for events than those in other studies. Indeed, the mortality rate was lower than that in many other studies, possibly reflecting the high standard of care, the presence of less severe heart failure, or both.

The extent to which risk can be modified may be greater among patients with less severe disease. Cardiac resynchronization may be beneficial in patients with cardiac dyssynchrony even if their symptoms are not severe, although we excluded patients judged by the investigator to be in NYHA class I or II.

The hazard ratio for death among patients with a cardiac-resynchronization device, as compared with those receiving medical therapy alone (0.64; 95 percent confidence interval, 0.48 to 0.85; \( P<0.002 \)), was similar to that among patients who received both a resynchronization device and a defibrillator, as compared with medical therapy alone, in the COMPANION trial (0.64; 95 percent confidence interval, 0.48 to 0.86; \( P=0.003 \)).

The COMPANION trial was not designed to investigate differences between the use of a cardiac-resynchronization device alone and the combination of a resynchronization device and an implantable defibrillator, but much of the effect of the latter approach could be explained by the cardiac-resynchronization component. In our study, the cardiac-resynchronization group had a decreased incidence of sudden death and a decreased incidence of death from worsening heart failure, both of which may reflect an improvement in cardiac function. A defibrillator might further reduce the risk of sudden death.

Retarding the progression of cardiac dysfunction to prevent malignant arrhythmias may be a better strategy than treating malignant arrhythmias once they occur, because defibrillation is stressful to the patient and associated with an adverse prognosis.
### Table: Patients with Event/Total No. of Patients vs Hazard Ratio (95% CI)

<table>
<thead>
<tr>
<th>Group</th>
<th>Patients with Event/Total No. of Patients</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Overall</strong></td>
<td>383/813</td>
<td>0.63 (0.51–0.77)</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;66.4 yr</td>
<td>163/406</td>
<td>0.55 (0.40–0.75)</td>
</tr>
<tr>
<td>≥66.4 yr</td>
<td>220/407</td>
<td>0.68 (0.52–0.89)</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>290/597</td>
<td>0.62 (0.49–0.79)</td>
</tr>
<tr>
<td>Female</td>
<td>93/215</td>
<td>0.64 (0.42–0.97)</td>
</tr>
<tr>
<td><strong>NYHA class</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>349/763</td>
<td>0.64 (0.52–0.80)</td>
</tr>
<tr>
<td>IV</td>
<td>34/50</td>
<td>0.50 (0.25–1.01)</td>
</tr>
<tr>
<td><strong>Dilated cardiomyopathy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>238/443</td>
<td>0.68 (0.53–0.88)</td>
</tr>
<tr>
<td>Yes</td>
<td>145/370</td>
<td>0.51 (0.36–0.73)</td>
</tr>
<tr>
<td><strong>Systolic blood pressure</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;117 mm Hg</td>
<td>208/401</td>
<td>0.60 (0.46–0.80)</td>
</tr>
<tr>
<td>≥117 mm Hg</td>
<td>170/402</td>
<td>0.66 (0.48–0.89)</td>
</tr>
<tr>
<td><strong>NT-BNP</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;214.5 pg/ml</td>
<td>122/366</td>
<td>0.53 (0.36–0.76)</td>
</tr>
<tr>
<td>≥214.5 pg/ml</td>
<td>224/366</td>
<td>0.70 (0.54–0.91)</td>
</tr>
<tr>
<td><strong>Ejection fraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;24.7%</td>
<td>205/372</td>
<td>0.65 (0.49–0.86)</td>
</tr>
<tr>
<td>≥24.7%</td>
<td>152/373</td>
<td>0.62 (0.44–0.85)</td>
</tr>
<tr>
<td><strong>End-systolic volume index</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;119.2 ml/m²</td>
<td>156/366</td>
<td>0.71 (0.52–0.98)</td>
</tr>
<tr>
<td>≥119.2 ml/m²</td>
<td>193/366</td>
<td>0.54 (0.40–0.73)</td>
</tr>
<tr>
<td><strong>QRS interval</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;160 msec</td>
<td>152/290</td>
<td>0.74 (0.54–1.02)</td>
</tr>
<tr>
<td>≥160 msec</td>
<td>222/505</td>
<td>0.60 (0.46–0.79)</td>
</tr>
<tr>
<td><strong>Interventricular mechanical delay</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;49.2 msec</td>
<td>199/367</td>
<td>0.77 (0.53–1.02)</td>
</tr>
<tr>
<td>≥49.2 msec</td>
<td>147/368</td>
<td>0.50 (0.36–0.70)</td>
</tr>
<tr>
<td><strong>Mitral-regurgitation area</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.218</td>
<td>114/302</td>
<td>0.86 (0.60–1.25)</td>
</tr>
<tr>
<td>≥0.218</td>
<td>175/303</td>
<td>0.56 (0.41–0.75)</td>
</tr>
<tr>
<td><strong>Glomerular filtration rate</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;60.3 ml/min/1.73 m²</td>
<td>196/369</td>
<td>0.67 (0.50–0.89)</td>
</tr>
<tr>
<td>≥60.3 ml/min/1.73 m²</td>
<td>142/370</td>
<td>0.57 (0.40–0.80)</td>
</tr>
<tr>
<td><strong>Beta-blockers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>131/227</td>
<td>0.72 (0.51–1.02)</td>
</tr>
<tr>
<td>Yes</td>
<td>252/586</td>
<td>0.59 (0.46–0.76)</td>
</tr>
<tr>
<td><strong>Spironolactone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>166/356</td>
<td>0.58 (0.43–0.79)</td>
</tr>
<tr>
<td>Yes</td>
<td>217/457</td>
<td>0.67 (0.51–0.88)</td>
</tr>
<tr>
<td><strong>Loop diuretics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;80 mg of furosemide or equivalent</td>
<td>181/461</td>
<td>0.56 (0.42–0.76)</td>
</tr>
<tr>
<td>≥80 mg of furosemide or equivalent</td>
<td>202/352</td>
<td>0.69 (0.53–0.92)</td>
</tr>
<tr>
<td><strong>Digoxin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>218/467</td>
<td>0.66 (0.50–0.86)</td>
</tr>
<tr>
<td>Yes</td>
<td>165/346</td>
<td>0.59 (0.43–0.81)</td>
</tr>
</tbody>
</table>

**Resynchronization Better** vs **Medical Therapy Better**
Table 3. Hemodynamic, Echocardiographic, and Biochemical Assessments. *

<table>
<thead>
<tr>
<th>Variable</th>
<th>Difference in Means at 3 Mo (95% CI)</th>
<th>P Value</th>
<th>Difference in Means at 18 Mo (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate (beats/min)</td>
<td>+1.1 (-1.2 to 3.4)</td>
<td>0.33</td>
<td>+1.0 (-1.5 to 3.6)</td>
<td>0.43</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>+5.8 (3.5 to 8.2)</td>
<td>&lt;0.001</td>
<td>+6.3 (3.6 to 8.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>+1.5 (0.1 to 2.9)</td>
<td>0.03</td>
<td>+1.3 (-1.8 to 4.4)</td>
<td>0.42</td>
</tr>
<tr>
<td>Interventricular delay (msec)</td>
<td>-21 (-25 to -18)</td>
<td>&lt;0.001</td>
<td>-21 (-25 to -17)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left ventricular ejection fraction (%)</td>
<td>+3.7 (3.0 to 4.4)</td>
<td>&lt;0.001</td>
<td>+6.9 (5.6 to 8.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Left ventricular end-systolic volume index (mL/m²)</td>
<td>-18.2 (-21.2 to -15.1)</td>
<td>&lt;0.001</td>
<td>-26.0 (-31.5 to -20.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mitral regurgitation area†</td>
<td>-0.051 (-0.073 to -0.028)</td>
<td>&lt;0.001</td>
<td>-0.042 (-0.070 to -0.014)</td>
<td>0.003</td>
</tr>
<tr>
<td>N-terminal pro–brain natriuretic peptide (pg/ml)‡</td>
<td>-225 (-705 to 255)</td>
<td>0.36</td>
<td>-1122 (-1815 to -429)</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

* Differences were not adjusted for the higher mortality rate in the medical-therapy group. A plus sign indicates a greater value, and a minus sign a smaller value, in the cardiac-resynchronization group than in the medical-therapy group. CI denotes confidence interval.
† The area was calculated as the area of the color-flow Doppler regurgitant jet divided by the area of the left atrium in systole, both in square centimeters.
‡ To convert the values for N-terminal pro–brain natriuretic peptide to picomoles per liter, divide by 8.457.

Owing either to the cause of the arrhythmia or to the effects of the shock. Assuming that the combination of a cardiac-resynchronization device and a defibrillator could prevent two thirds of sudden deaths, a future study would require 1300 patients per group and a follow-up period similar to ours to have a statistical power of 90 percent to detect an absolute reduction in the risk of death from any cause of 5 percent with the use of combination therapy, as compared with the use of cardiac resynchronization alone.

In summary, we found that cardiac resynchronization is an effective therapy for patients with left ventricular systolic dysfunction and cardiac dyssynchrony who have moderate or severe heart failure and who are in sinus rhythm.

Supported by a grant from Medtronic.

Dr. Cleland reports having served as a consultant for Medtronic, Amgen, Menarini, and Pfizer and having received speakers’ honoraria from Medtronic, Takeda, AstraZeneca, and Pfizer and grant support from the Medical Research Council (United Kingdom), Hull and East Yorkshire Cardiac Trust, Medtronic, Vasomedical, and Abbott. Dr. Daubert reports having served as a consultant for and having received speakers’ honoraria from Medtronic and St. Jude Medical. Dr. Erdmann reports having served as a consultant for and having received speakers’ honoraria from Takeda, Merck (Darmstadt), Medtronic, and Guidant. Dr. Freemantle reports having served as a consultant for Medtronic and Pfizer and having received speakers’ honoraria from Medtronic and grant support from Medtronic, Aventis, Amgen (United Kingdom), and the British Heart Foundation. Dr. Gras reports having served as a consultant for and having received speakers’ honoraria from Medtronic and Guidant. Dr. Kappenberger reports having served as a consultant for Medtronic and Pfizer, having received speakers’ honoraria from Medtronic and grant support from Medtronic, Aventis, Amgen (United Kingdom), and the British Heart Foundation. Dr. Tavazzi reports having served as a consultant for Medtronic, Menarini, Servier, and Pfizer and having received speakers’ honoraria from Medtronic, Novartis, and Takeda.

This article is dedicated to the memory of Werner Klein, professor of cardiology at the University Hospital of Graz (Austria), a member of the steering committee who died in 2004. He contributed substantially to the design and conduct of the study but did not live to see it completed. His wise counsel is greatly missed by his colleagues.
EFFECT OF CARDIAC RESYNCHRONIZATION ON HEART FAILURE


REFERENCES

Hyponatremia among Runners in the Boston Marathon

Christopher S.D. Almond, M.D., M.P.H., Andrew Y. Shin, M.D., Elizabeth B. Fortescue, M.D., Rebekah C. Mannix, M.D., David Wypij, Ph.D., Bryce A. Binstadt, M.D., Ph.D., Christine N. Duncan, M.D., David P. Olson, M.D., Ph.D., Ann E. Salerno, M.D., Jane W. Newburger, M.D., M.P.H., and David S. Greenes, M.D.

From the Departments of Medicine (C.S.D.A., A.Y.S., E.B.F., R.C.M., B.A.B., C.N.D., D.P.O., A.E.S., D.S.G.) and Cardiology (D.W., J.W.N.) and the Clinical Research Program (D.W.), Children’s Hospital; the Department of Pediatrics, Harvard Medical School (C.S.D.A., A.Y.S., E.B.F., R.C.M., D.W., B.A.B., C.N.D., D.P.O., A.E.S., J.W.N., D.S.G.); and the Department of Biostatistics, Harvard School of Public Health (D.W.) — all in Boston. Address reprint requests to Dr. Almond at the Department of Cardiology, Children’s Hospital, Bader 2, 300 Longwood Ave., Boston, MA 02115, or at christopher.almond@childrens.harvard.edu.

Background

Hyponatremia has emerged as an important cause of race-related death and life-threatening illness among marathon runners. We studied a cohort of marathon runners to estimate the incidence of hyponatremia and to identify the principal risk factors.

Methods

Participants in the 2002 Boston Marathon were recruited one or two days before the race. Subjects completed a survey describing demographic information and training history. After the race, runners provided a blood sample and completed a questionnaire detailing their fluid consumption and urine output during the race. Prerace and postrace weights were recorded. Multivariate regression analyses were performed to identify risk factors associated with hyponatremia.

Results

Of 766 runners enrolled, 488 runners (64 percent) provided a usable blood sample at the finish line. Thirteen percent had hyponatremia (a serum sodium concentration of 135 mmol per liter or less); 0.6 percent had critical hyponatremia (120 mmol per liter or less). On univariate analyses, hyponatremia was associated with substantial weight gain, consumption of more than 3 liters of fluids during the race, consumption of fluids every mile, a racing time of >4:00 hours, female sex, and low body-mass index. On multivariate analysis, hyponatremia was associated with weight gain (odds ratio, 4.2; 95 percent confidence interval, 2.2 to 8.2), a racing time of >4:00 hours (odds ratio for the comparison with a time of <3:30 hours, 7.4; 95 percent confidence interval, 2.9 to 23.1), and body-mass-index extremes.

Conclusions

Hyponatremia occurs in a substantial fraction of nonelite marathon runners and can be severe. Considerable weight gain while running, a long racing time, and body-mass-index extremes were associated with hyponatremia, whereas female sex, composition of fluids ingested, and use of nonsteroidal antiinflammatory drugs were not.
A s marathon running has surged in popularity during the past quarter-century, reports have emerged of serious illness and death from hyponatremia, as in the case of a 28-year-old woman who died after the 2002 Boston Marathon. The incidence of hyponatremia among marathon runners is unknown, since previous studies have been small and limited to runners presenting for medical attention.

Excessive fluid intake is believed to be the primary risk factor for hyponatremia, on the basis of observations of marathon runners who have collapsed and studies of elite athletes. However, other risk factors have also been suggested, including the composition of fluids consumed (e.g., plain water, rather than sports drinks that contain electrolytes), relatively low body-mass index, long racing time, lack of marathon experience, use of nonsteroidal antiinflammatory drugs (NSAIDs), and female sex. We undertook the present study to estimate prospectively the incidence of hyponatremia among marathon runners and to identify the principal risk factors involved.

**METHODS**

**STUDY POPULATION**

Marathon runners were recruited prospectively at an exposition one or two days before the Boston Marathon, in April 2002. All registered participants 18 years of age or older were eligible, regardless of whether they registered for the marathon on the basis of a competitive qualifying time or on behalf of a charitable organization — a mechanism for which no previous marathon experience was required. Subjects were approached at random in an area adjacent to race registration and invited to participate. Written informed consent was obtained from all subjects. The study protocol was approved by the Committee on Clinical Investigation at Children’s Hospital in Boston.

**STUDY DESIGN**

Before running the marathon, subjects completed a survey describing baseline demographic and training information, medical history, and anticipated hydration strategies for the race. At the finish line, runners provided a blood sample and completed a questionnaire detailing their fluid consumption and urine output during the race. Blood samples were centrifuged on site and frozen at −70°C until analyzed. With the use of a digital balance, the prerace and postrace weights were recorded for each runner.

**OUTCOME MEASURES**

The primary hypothesis of the study was that excessive consumption of hypotonic fluids is associated with hyponatremia in marathon runners. Hyponatremia was defined as a serum sodium concentration of 135 mmol per liter or less. Severe hyponatremia and critical hyponatremia were defined as serum sodium concentrations of 130 and 120 mmol per liter or less, respectively. Independent variables analyzed for association with hyponatremia included weight change during the race and self-reported fluid intake including volume, frequency, and type. Both water and a sports drink containing electrolytes were offered at each milepost, and runners were asked to estimate the proportion of their intake from each. Other predictors that we considered included sex (a dichotomous variable), body-mass index (the weight in kilograms divided by the square of the height in meters), training pace, number of previous marathons (dichotomized at a median of five), duration of the marathon in hours and minutes, use or nonuse of NSAIDs in the past week (a dichotomous variable), age, and race (a dichotomous variable [white vs. nonwhite]). Race was self-reported by the runners.

**STATISTICAL ANALYSIS**

Descriptive statistics were used to estimate the incidence of hyponatremia and to characterize the demographic information supplied by the runners. Unless otherwise specified, t-tests and Fisher’s exact test were used to identify univariate predictors associated with hyponatremia, at a level of statistical significance of P≤0.05. Logistic regression (SAS software, version 9.0) and generalized additive models† (S-Plus software, version 6.1 for Windows) were used in the multivariate analysis to identify independent predictors of hyponatremia.

Table 1 summarizes the baseline demographic and training characteristics of the study population. Of 766 runners enrolled, 511 (67 percent) reported to the finish-line research station. Of these, 489 provided a blood sample (constraints such as plane flights precluded 22 runners from providing a sample). One sample was considered of insufficient quantity, leaving a total of 488 subjects for analysis. Overall, among all 766 runners enrolled, female runners were younger than male runners (mean [±SD] age, 36.1±8.8 vs. 40.4±9.7 years; P<0.001) and had a lower prerace weight (58.8±6.8 vs.
The new england journal of medicine

1552

Table 1. Baseline Characteristics of the 2002 Boston Marathon Study Population.†

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Male Runners (N=473)</th>
<th>Female Runners (N=293)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Reporting at Finish Line (N=336)</td>
<td>Not Reporting at Finish Line (N=137)</td>
</tr>
<tr>
<td>Age — yr</td>
<td>40.4±9.6</td>
<td>40.4±10.0</td>
</tr>
<tr>
<td>Nonwhite race — %</td>
<td>9</td>
<td>10</td>
</tr>
<tr>
<td>Prerace weight — kg</td>
<td>74.6±9.5</td>
<td>76.6±10.7</td>
</tr>
<tr>
<td>Body-mass index†</td>
<td>23.7±2.6</td>
<td>24.5±2.7</td>
</tr>
<tr>
<td>Training pace — min:sec/mi</td>
<td>7:53±1:02</td>
<td>8:04±1:09</td>
</tr>
<tr>
<td>Previous marathons — median no. (interquartile range)</td>
<td>5 (2–12)</td>
<td>4 (1–12)</td>
</tr>
<tr>
<td>Self-reported water loading — %‡</td>
<td>75</td>
<td>79</td>
</tr>
<tr>
<td>Self-reported use of NSAIDs — %§</td>
<td>51</td>
<td>54</td>
</tr>
<tr>
<td>Race duration — hr:min¶</td>
<td>3:37±0:42</td>
<td>3:46±0:40</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD. The temperature and humidity at noon, at the start of the race, were 53°F (12°C) and 96 percent, respectively; at 2 p.m. at the finish line, they were 55°F (13°C) and 83 percent.
† The body-mass index is the weight in kilograms divided by the square of the height in meters.
‡ Water loading was defined as an increase in fluid consumption above baseline specifically in preparation for running the Boston Marathon.
§ NSAIDs denotes nonsteroidal antiinflammatory drugs. Use of NSAIDs was defined as any use within the week before the Boston Marathon.
¶ Race times of runners who did not report at the finish line were obtained by means of the Boston Marathon tracking Web site.

75.2±9.9 kg, P<0.001), lower body-mass index (21.4±2.1 vs. 24.0±2.7, P<0.001), a slower training pace (8:40±1:01 vs. 7.56±1:04 minutes per mile, P<0.001), less marathon experience (median of three vs. five previous marathons, Wilcoxon P<0.001), and longer racing time (4:02±0:35 vs. 3:40±0:42 hours, P<0.001). Runners who appeared for follow-up studies at the finish line had characteristics similar to runners who did not, except that women who reported for follow-up had completed one more previous marathon than women who did not report for follow-up (P=0.008) and were less likely to report water loading than women who were not followed up (P=0.04). Men who reported for follow-up had a lower body-mass index than men who did not report for follow-up (P=0.004) and completed the race nine minutes faster than men who were not followed up (P=0.04).

At the finish line, the runners had a mean serum sodium concentration of 140±5 mmol per liter (range, 114 to 158). Thirteen percent (62 of 488) had hyponatremia, including 22 percent of women (37 of 166) and 8 percent of men (25 of 322). Three runners (0.6 percent) had critical hyponatremia (serum sodium concentrations, 119, 118, and 114 mmol per liter).

Table 2 summarizes univariate and multivariate predictors of hyponatremia. Univariate predictors included female sex, a body-mass index of less than 20, longer racing time, consumption of fluids every mile, consumption of more than 3 liters of fluids during the race, and an increased frequency of voiding during the race. Hyponatremia was strongly correlated with weight gain during the race (Fig. 1). There were no differences between the runners with and those without hyponatremia in age, composition of fluid consumed, or self-reports of water loading and use of NSAIDs.

In the multivariate analysis, hyponatremia was associated with weight gain, longer racing time, and a body-mass index of less than 20. In selecting covariates for inclusion in the final model, we did not include variables for fluid consumption because of colinearity with weight gain, which we considered to be a stronger and more objective measure of fluid intake. Also excluded was training pace, which was colinear with race duration. Additional adjustment for the composition of ingested fluid, number of previous marathons, and reported use of NSAIDs was not statistically significant; their inclusion did not appreciably alter the coefficients of the remaining variables in the model.

Generalized additive models were used to assess the effects of a change in weight, race duration, and

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Table 2. Univariate and Multivariate Predictors of Hyponatremia.*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Univariate Predictors</th>
<th>Multivariate Predictors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hyponatremia (N=62)</td>
<td>No Hyponatremia (N=426)</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (yr)</td>
<td>38.1±9.5</td>
<td>39.0±9.4</td>
</tr>
<tr>
<td>Nonwhite race (%)</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>60</td>
<td>30</td>
</tr>
<tr>
<td>Body-mass index</td>
<td>22.8±3.7</td>
<td>23.0±2.5</td>
</tr>
<tr>
<td>Category of body-mass index</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>&lt;20 (%)</td>
<td>25</td>
<td>8</td>
</tr>
<tr>
<td>20–25 (%)</td>
<td>54</td>
<td>73</td>
</tr>
<tr>
<td>&gt;25 (%)</td>
<td>21</td>
<td>19</td>
</tr>
<tr>
<td>Training and performance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous marathons (no.)</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Training pace (min:sec/mi)</td>
<td>8:52±1:11</td>
<td>8:02±1:01</td>
</tr>
<tr>
<td>Race duration (hr:min)</td>
<td>4:12±0:47</td>
<td>3:42±0:42</td>
</tr>
<tr>
<td>Category of race duration (hr:min)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>&lt;3:30 (%)</td>
<td>13</td>
<td>44</td>
</tr>
<tr>
<td>3:30–4:00 (%)</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>&gt;4:00 (%)</td>
<td>52</td>
<td>25</td>
</tr>
<tr>
<td>Fluids and electrolytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-reported fluid intake</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency (%)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Every mile</td>
<td>75</td>
<td>54</td>
</tr>
<tr>
<td>Every other mile</td>
<td>25</td>
<td>36</td>
</tr>
<tr>
<td>Every third mile or less often</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Volume, &gt;3 liters (%)</td>
<td>42</td>
<td>26</td>
</tr>
<tr>
<td>Composition, 100% water (%)</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Self-reported water loading (%)‡</td>
<td>82</td>
<td>73</td>
</tr>
<tr>
<td>Self-reported frequency of voiding during race (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>Once</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td>Twice</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Three times or more</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>Postrace weight &gt; prerace weight (%)</td>
<td>71</td>
<td>29</td>
</tr>
<tr>
<td>Self-reported use of NSAIDs (%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Hyponatremia was defined as a serum sodium concentration of 135 mmol per liter or less. Plus–minus values are means ±SD. CI denotes confidence interval, and NSAIDs nonsteroidal antiinflammatory drugs. Dashes indicate not applicable. Percentages may not sum to 100 because of rounding.
† For the univariate analysis, all continuous variables were analyzed with the use of t-tests, all categorical data were analyzed with the use of Fisher’s exact test, and the number of previous marathons was analyzed with the use of the Wilcoxon rank-sum test. For the multivariate analysis, P values were determined by Wald tests, and profile-likelihood confidence intervals were determined with the use of logistic regression.
‡ Race was self-reported.
§ This group served as the reference group in the multiple logistic-regression analysis.
¶ Water loading was defined as increasing fluid consumption above baseline specifically in preparation for running the Boston Marathon.
|| Use of NSAIDs was defined as any use within the week before the Boston Marathon.
body-mass index as continuous predictors of hyponatremia (Fig. 2). There were no significant departures from linearity for weight change (P=0.47) or race duration (P=0.40), but body-mass index had a strong nonlinear (approximately quadratic) relation with hyponatremia (P=0.002). A 1-kg increase in weight conferred an odds ratio of 2.0 (95 percent confidence interval, 1.6 to 2.6; P<0.001), and a 30-minute increase in running time conferred an odds ratio of 1.6 (95 percent confidence interval, 1.3 to 2.1; P<0.001). Additional adjustment for female sex (P=0.20) or drinking 100 percent water (P=0.89) was not statistically significant and did not appreciably alter the coefficients of the remaining variables in the model.

Given the strength of weight gain as a predictor of hyponatremia, we performed a secondary analysis to identify the determinants of weight gain. Thirty-five percent of the runners gained weight during the race (range, 0.1 to 4.1 kg). In a multivariate analysis, intake of 3 or more liters of fluid, fluid intake every mile, longer racing time, female sex, and body-mass index of less than 20 were associated with weight gain.

**DISCUSSION**

We observed that hyponatremia occurs in a substantial fraction of marathon runners and can be severe. The strongest single predictor of hyponatremia was considerable weight gain during the race, which correlated with excessive fluid intake. Longer racing time and body-mass-index extremes were also associated with hyponatremia, whereas the composition of fluids consumed (plain water, rather than sports drinks that contain electrolytes), female sex, and reported use of NSAIDs were not.

These results are consistent with earlier reports that suggested a link between excessive fluid consumption and hyponatremia. However, earlier studies were limited by a small sample size, a retrospective study design, or a focus on elite or ultraendurance runners whose risk of the development of hyponatremia seems to be substantially lower than the risk among nonelite runners. In contrast, our study focused on a large, athletically diverse cohort of marathon runners followed prospectively to estimate the incidence of hyponatremia.

These observations suggest that hyponatremia — and particularly severe hyponatremia — may be a greater problem than previously recognized. If our sample was representative of the overall 2002 Boston Marathon field of runners, we would estimate that approximately 1900 of the nearly 15,000 finishers had some degree of hyponatremia, and that approximately 90 finishers had critical hyponatremia. Substantial weight gain appeared to be the most important predictor of hyponatremia and correlated with increased fluid intake. Our finding of greater frequency of voiding among runners with hyponatremia suggests that most runners gain weight as a result of excessive fluid consumption, although inappropriate fluid retention may also have a role. Most reported cases of serious illness have involved runners in the United States. Our findings indicate that the problem of excessive hydration is not an isolated occurrence but may be part of a tendency among many U.S. marathon runners, especially those in the nonelite category, in which most of the growth in running has occurred.

We could find no association between the composition of fluids consumed and hyponatremia. This finding probably reflects the relative hypotonicity of most commercial sports drinks, which have a typical sodium concentration of 18 mmol per liter.
HYponatremia AMONG RUNNERS IN THE BOSTON MARATHON

Figure 2. Adjusted Odds Ratios for Weight Change (Panel A), Race Duration (Panel B), and Body-Mass Index (Panel C) as Predictors of Hyponatremia among Runners in the 2002 Boston Marathon.

Results from a logistic-regression model showing the linear relationships of weight gain and race duration with hyponatremia, and the quadratic relationship of body-mass index with hyponatremia, were overlaid on the plot of the generalized additive model, demonstrating that the simpler parametric model adequately described the covariate effects. Dashed lines represent the fit of the generalized additive model. Solid lines represent the parametric logistic-regression fit (quadratic for body-mass index and linear for race duration and weight change). Dotted lines represent pointwise 95 percent confidence limits for the parametric fits. P values denote the overall effect of the covariate in predicting hyponatremia in the parametric logistic-regression fit. Tick marks above the odds-ratio curve represent runners with hyponatremia (defined as a serum sodium concentration of 135 mmol or less), whereas tick marks below the odds-ratio curve represent runners without hyponatremia. All models were constrained to cross at an odds ratio of unity.

less than one fifth the concentration of normal saline. Although it is difficult to rule out some effect of the type of fluid consumed on the risk of hyponatremia, our findings suggest that the contribution of the type of fluid is small as compared with the volume of fluid ingested.

Hyponatremia developed in more female than male runners, but this difference was not statistically significant after adjustment for body-mass index, racing time, and weight change. Female runners remain a readily identifiable risk group, and our observations suggest that this may be because of body size and longer racing time, rather than sex per se. However, the influence of sex on weight change during exercise merits further study. It is not clear why both high and low body-mass indexes are associated with hyponatremia. Low body-mass index may be associated with hyponatremia because smaller runners may drink larger volumes of fluids in proportion to their size than larger runners. Conversely, in proportion to their size, larger runners may lose less free water than smaller runners through evaporation (by means of sweat), as a result of a lower ratio of surface area to volume.

The data from the present study suggest that hyponatremia associated with the running of marathons — and more broadly, with high-endurance exercise — may be a preventable condition. One relatively simple strategy to reduce the risk would be for runners to weigh themselves before and after training runs to gauge the effectiveness of their overall hydration strategy and adjust their fluid intake accordingly. This could be particularly useful during long training runs in which the distance and duration most closely approximate those of an actual marathon. Because runners vary considerably in size and in rates of perspiration, general recommendations regarding specific volumes of fluids and frequencies of intake are probably unsafe and have been superseded by recommendations favoring thirst or individual perspiration rates as a primary guide. Sporadically checking their weight could be a relatively easy way for runners to deter-
mine whether their current hydration strategy puts them at undue risk for the development of hyponatremia.

The present study must be interpreted within the context of certain limitations. First, follow-up in our study population was 67 percent, which may have skewed results if differential follow-up occurred. However, female and slower runners, who appear to be at higher risk for hyponatremia, seemed to be less likely to follow up with the researchers at the finish line, suggesting that our observations underestimated the overall incidence of hyponatremia. Second, baseline measurements of serum sodium concentration were not obtained before the marathon, raising the possibility that electrolyte derangements present at the end of the marathon were present before the start of the race. However, we could find no data to suggest that baseline concentrations of serum sodium in athletes would be different from those in nonathletes. Finally, we relied on runners’ self-reports of fluid intake during the marathon, which may be an imprecise estimate of intake. However, runners’ self-reports of fluid intake correlated well with weight change, which is a more objective and widely accepted clinical measure of fluid balance.

In summary, we observed that a substantial proportion of runners have abnormally low serum sodium concentrations after completing a marathon. Excessive consumption of fluids, as evidenced by substantial weight gain while running, is the single most important factor associated with hyponatremia. Efforts to monitor and regulate fluid intake may lead to a reduction in the frequency and severity of this condition, which, in rare cases, can be fatal.

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APPENDIX


REFERENCES

Modification of Human Hearing Loss by Plasma-Membrane Calcium Pump PMCA2

Julie M. Schultz, Ph.D., Yandan Yang, Ph.D., Ariel J. Caride, Ph.D., Adelaida G. Filoteo, B.S., Alan R. Penheiter, Ph.D., Ayala Lagziel, Ph.D., Robert J. Morell, Ph.D., Saidi A. Mohiddin, M.D., Ph.D., Lameh Fananapazir, M.D., Anne C. Madeo, M.S., John T. Penniston, Ph.D., and Andrew J. Griffith, M.D., Ph.D.

From the Section on Human Genetics (J.M.S., A.L., R.J.M.), the Section on Gene Structure and Function (Y.Y., A.J.G.), and the Hearing Section (A.C.M., A.J.G.), National Institute on Deafness and Other Communication Disorders, and the Cardiovascular Branch, National Heart, Lung, and Blood Institute (S.A.M., L.F.), National Institutes of Health, Rockville and Bethesda, Md.; the Department of Biochemistry and Molecular Biology (A.J.C., A.G.F.) and the Department of Anesthesiology (A.R.P.), Mayo Foundation, Rochester, Minn.; and the Neuroscience Center, Massachusetts General Hospital and Harvard Medical School, Boston (J.T.P.). Address reprint requests to Dr. Griffith at the NIDCD, National Institutes of Health, 5 Research Ct., Rm. 2A-01, Rockville, MD 20850, or at griffita@nidcd.nih.gov.

Five adult siblings presented with autosomal recessive sensorineural hearing loss: two had high-frequency loss, whereas the other three had severe-to-profound loss affecting all frequencies. Genetic evaluation revealed that a homozygous mutation in CDH23 (which encodes cadherin 23) caused the hearing loss in all five siblings and that a heterozygous, hypofunctional variant (V586M) in plasma-membrane calcium pump PMCA2, which is encoded by ATP2B2, was associated with increased loss in the three severely affected siblings. V586M was detected in two unrelated persons with increased sensorineural hearing loss, in the other caused by a mutation in MYO6 (which encodes myosin VI) in one and by noise exposure, suggesting that this variant may modify the severity of sensorineural hearing loss caused by a variety of factors.
loss. We undertook this study to identify the cause of their sensorineural hearing loss and a potential genetic modifier of its severity. We then sought to determine whether this same modifier might account for variations in the severity of sensorineural hearing loss caused by other factors in unrelated persons.

**METHODS**

**SUBJECTS**

This study was approved by the institutional review board of the National Institute of Neurological Disorders and Stroke and the National Institute on Deafness and Other Communication Disorders, National Institutes of Health. All the participants gave written informed consent before participation. The participants were members of the LMG132 family, which is descended from European ancestors. Medical-history interviews, physical examinations, video nystagmography with caloric testing, and pure-tone and speech audiometry were performed. The Usher syndrome was ruled out by funduscopy and electroretinography.

**GENETIC ANALYSIS**

Genomic DNA was extracted from venous-blood samples (Puregene, Gentra Systems). DNA samples were genotyped for short tandem-repeat markers flanking known nonsyndromic recessive deafness loci, and all exons of CDH23 were sequenced essentially as described. Primer sequences and polymerase-chain-reaction amplification and sequencing conditions for ATP2B2, which encodes the plasma-membrane calcium pump PMCA2, are provided in Table 1 of the Supplementary Appendix (available with the full text of this article at www.nejm.org). Control DNA samples were obtained from Coriell Cell Repositories and consisted of Human Variation Panels HD01 through HD09, HD027, and HD100CAU (described by the repository as a panel of samples from “self-declared Caucasians”). Our laboratory collected additional normal control DNA samples from 14 unrelated persons with a variety of self-reported European ancestries.

**PMCA2 FUNCTIONAL ASSAY**

Expression vectors were constructed for the PMCA2a splice isoform of PMCA2, since the former is the predominant PMCA isoform expressed in hair bundles in bullfrogs and inner-ear neurosensory cells in rats. Full-length human complementary DNA fragments encoding wild-type PMCA2a or PMCA2a with the V586M variant (in which methionine replaces valine at amino acid position 586) were sequenced in their entirety and subcloned into baculovirus expression vector pVL1392 (Invitrogen). Details of the cloning procedures are provided in the Methods section of the Supplementary Appendix. Preparation and amplification of recombinant baculovirus, expression of PMCA2a in Sf9 cells, preparation of microsomes, and measurement of ATPase activity were performed as described elsewhere. Equivalent amounts of the expressed wild-type and mutant proteins were used in their respective reactions. The free calcium concentration was calculated with the Maxchelator program (www.stanford.edu/~cpatton/maxc.html).

**CALCULATION OF ATP2B2V586M FREQUENCIES**

Some of the samples used to calculate ATP2B2V586M frequencies were derived from members of the same families. To avoid duplicative counting of alleles that were identical by descent among members of the same family, we examined each pedigree to deduce the numbers of independent wild-type and ATP2B2V586M alleles. If we could not determine whether two sampled alleles were identical or not identical by descent, we defined minimum and maximum possible values, respectively, which were used to calculate high and low composite estimates of the frequency of ATP2B2V586M in the entire group of samples. Since the frequency of the ATP2B2V586M allele was low and no homozygotes were detected, carrier frequency was approximated by doubling the allele frequency.

**CASE REPORT**

Five affected offspring (42 to 55 years of age) of a consanguineous union in Family LMG132 had autosomal recessive, nonsyndromic sensorineural hearing loss, with normal vestibular and retinal function (Fig. 1A). All five siblings had severe-to-profound high-frequency sensorineural hearing loss that had begun, according to their recollection, in the first decade of life, after the initial development of speech and language, and that had steadily progressed to current levels during the subsequent decade. However, there were two different phenotypes among the siblings: Subjects II-4 and II-6 had normal low-tone hearing, whereas Subjects II-1, II-9, and II-10 had severe-to-profound low-frequency loss that had begun in the first or second decade.
Brief Report

Figure 1. CDH23 Genotypes and Phenotypes of Members of Family LMG132.
Panel A shows the pedigree of five affected siblings, the offspring of a consanguineous union (double line), who were homozygous for the F1888S mutation (S) of CDH23. The 25-year-old child of a first cousin of the siblings had nonsyndromic, congenital, severe-to-profound sensorineural hearing loss affecting all frequencies (not shown). She was a compound heterozygote for F1888S and a novel frame-shift mutation (8882_8883insT) in CDH23. Solid symbols indicate persons with nonsyndromic sensorineural hearing loss, and symbols with a slash indicate deceased family members. Panel B shows electropherograms of wild-type (Subject II-5), heterozygous (Subject II-2), and homozygous (Subject II-10) genomic nucleotide sequences with respect to the missense mutation F1888S in exon 42 (arrows). Panel C shows the alignment of cadherin 23 amino acid sequences including and flanking F1888 (arrowhead) from Homo sapiens (Hs), Mus musculus (Mm), Rattus norvegicus (Rn), Gallus gallus (Gg), Danio rerio (Dr), and Tetraodon nigroviridis (Tn) (GenBank accession numbers AAG27034, AAG52817, NP_446096, XP_421595, NP_999974, and CAG04741, respectively). The alignment program ClustalW was used. Identical residues are indicated by dark shading and conservatively substituted residues by light shading. Amino acids are denoted by their single-letter codes. Panel D shows pure-tone air-conduction thresholds for the better-hearing ear of Subjects II-1 through II-11. Bone-conduction thresholds were consistent with the presence of sensorineural hearing loss (data not shown). Arrows indicate that there was no response to a stimulus at the highest tested level. Normative 90th percentile pure-tone thresholds are from International Organization for Standardization publication ISO 7029.13 dB HL denotes decibels hearing level. Audiometric profiles are grouped according to CDH23 and ATP2B2 genotypes, where S denotes the F1888S mutation and M the V586M variant.
of life and that had been followed by steady progression to current levels during the subsequent few decades (Fig. 1D). The latter group relied on sign language, lip-reading, hearing aids, or a cochlear implant for communication, in contrast to the two siblings whose intact low-frequency and midfrequency hearing permitted oral and auditory communication without hearing aids. There was no history of exposure to aminoglycoside antibiotics, ototoxic noise levels, head trauma, or systemic or otic infections that could account for the sensorineural hearing loss in the five affected siblings.

RESULTS AND DISCUSSION

CDH23 DEAFNESS IN FAMILY LMG132

All five affected siblings were homozygous for short tandem-repeat markers linked to CDH23 on chromosome 10q22.1 (data not shown). Genomic nucleotide-sequence analysis of CDH23 exons in the affected siblings revealed homozygosity for a point mutation (5663T→C; GenBank accession number, AVO10111) in exon 42, predicted to result in the substitution of serine for phenylalanine at amino acid position 1888 (F1888S; GenBank accession number, AY010111) in the extracellular domain of cadherin 23 (Fig. 1B). This phenylalanine residue is conserved in mouse, rat, and chicken cadherin 23 (Fig. 1B). This phenylalanine residue is conserved in mouse, rat, and chicken cadherin 23 (Fig. 1C) but is not located within the motifs involved in calcium-mediated intermolecular associations among cadherins.14 The CDH23F1888S/F1888S genotype cosegregated with sensorineural hearing loss in Family LMG132, and the CDH23F1888S mutation was not detected in 108 European (“Caucasian”) control samples.

ATP2B2 AS A MODIFIER OF CDH23 DEAFNESS IN FAMILY LMG132

A variety of recessive mutations of Cdh23 cause profound deafness and vestibular dysfunction in homozygous waltzer mice,15 whereas another allele of Cdhh3, calledahl, underlies less severe, age-related hearing loss in many inbred mouse strains.16 The severity of this age-related hearing loss is significantly increased by heterozygosity for the dfwu/Defwaddler allele of Atp2b2,16 which encodes PMCA2, the predominant PMCA of hair bundles. This interaction has been attributed to a reduction in PMCA2 activity that results in a decrease in extracellular calcium concentrations around hair bundles, where calcium-dependent, cadherin-mediated adhesion is thought to occur.17,18

We hypothesized that one or more alleles of ATP2B2 modify the severity of sensorineural hearing loss caused by CDH23F1888S/F1888S. DNA samples from Subjects II-1, II-4, II-6, II-9, and II-10 were genotyped for short tandem-repeat markers linked to ATP2B2. The resulting haplotypes were consistent with a model in which a dominant allele of ATP2B2 (Fig. 2A, black haplotype bar) exacerbates sensorineural hearing loss in a manner analogous to the interaction between the dfwu/Defw allele of Atp2b2 and the ahl allele of Cdh23 in mice. Nucleotide-sequence analysis of ATP2B2 exons in CDH23F1888S...
homozygotes revealed that heterozygosity for a point mutation in exon 12 (2075G→A; GenBank accession number, NM_001683) was linked to this haplotype. The 2075G→A mutation is predicted to result in the substitution of methionine for valine at amino acid position 586 (V586M; GenBank accession number, NP_001674) in the T4–T5 intracellular catalytic loop of PMCA2 (Fig. 2B). Molecular modeling of V586M based on the three-dimensional structure of the closely related sarcoplasmic reticulum calcium pump predicts that substitution with the sterically larger methionine side chain distorts packing underneath the ATP-binding interface or increases its projection from the external solvent-exposed surface of the nucleotide-binding domain (data not shown).

The valine residue at position 586 is completely conserved among mouse, rat, and fish PMCA2 orthologues, and either valine or a conservatively substituted residue (isoleucine) is present at this position in all known PMCA1 and PMCA3 amino acid sequences (Fig. 2C). Mouse and rat PMCA4 has methionine at this residue, but PMCA2 has a faster calcium-activation time than PMCA4. Since up-regulation and relocation of PMCA1 and PMCA4 to stereocilia do not rescue auditory function in dfw^2j,

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![Diagram showing genotype analysis](image)

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**D**

![Graph showing calcium ATPase activity](image)

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of hearing loss
of-function alleles (Subjects II-5 and II-11 of Family LMG132 [Fig. 1D] and three affected relatives (Fig. 3A). We also identified of sensorineural hearing loss as a function of age in olds, whereas mice that are heterozygous for loss-
metric phenotypes consistent with noise-induced exposure and high-frequency sensorineural hearing loss (data not shown). All carriers with normal hearing (Sub-
by Mohiddin et al. ATP2B2V
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deaf-waddler mice, the faster calcium-activation time of PMCA2 may be required for normal hearing. When expressed as a recombinant baculovirus protein in Sf9 cells, human PMCA2aV586M has approximately 50 percent of the calcium ATPase activity of wild-type PMCA2a (Fig. 2D).

Studies of heterozygous deaf-waddler mice demonstrate that partial loss of PMCA2 activity is expected for an allele that modifies, but is not itself sufficient to cause, hearing loss. The dfu allele product contains a pathogenic amino acid substitution in the T2–T3 cytoplasmic loop; this product retains approximately 30 percent of wild-type PMCA2 activity. Atp2b2dfu/+ mice have normal hearing thresholds, whereas mice that are heterozygous for loss-of-function alleles (dfu2j and dfu3j) of Atp2b2 have functionally significant sensorineural hearing loss on the same genetic background. Analogous to Atp2b2dfu, Atp2b2V586M is not itself a dominant deafness-causing allele, since two siblings with normal hearing in Family LMG132 (Subjects II-5 and II-11) were Atp2b2V586M heterozygotes (Fig. 1D and 2A).

ATP2B2V586M AS A MODIFIER OF OTHER FORMS OF HEARING LOSS

To explore the phenotypic consequences of the ATP2B2V586M allele further, we screened 57 affected members of unrelated families with various progressive hearing-loss phenotypes and identified ATP2B2V586M in 1 subject. The affected ATP2B2V586M heterozygote (Subject IV-6 described by Mohiddin et al.21) had age-related hearing loss associated with a dominant missense substitution of MYO6. She had low-frequency (0.25-, 0.5-, and 1-KHz) hearing loss that was more severe than would be predicted by linear regression estimates of sensorineural hearing loss as a function of age in her affected relatives (Fig. 3A). We also identified three ATP2B2V586M heterozygotes, all of European ancestry, among 128 (119 self-reported European) unaffected members of families with a variety of other phenotypes. Two of these ATP2B2V586M carriers had normal hearing (Fig. 3B and 3C), but the third had a history of occupational and recreational noise exposure and high-frequency sensorineural hearing loss that was highly characteristic of noise-induced ototoxicity (Fig. 3D). Four of the 125 subjects who did not carry the ATP2B2V586M allele also had audiometric phenotypes consistent with noise-induced sensorineural hearing loss (data not shown). All four ATP2B2V586M carriers with normal hearing (Subjects II-5 and II-11 of Family LMG132 [Fig. 1D] and the two carriers described above [Fig. 3B and 3C]) reported that they had no history of noise exposure. Although we cannot conclusively correlate the sensorineural hearing loss in the carrier with noise exposure (Fig. 3D) with the ATP2B2V586M+ genotype, it has been reported that heterozygosity for a null allele of Atp2b2 predisposes mice to noise-induced sensorineural hearing loss.

The allele frequency of ATP2B2V586M was cumulatively estimated in the European members of Family LMG132 and these cohorts. The lowest and highest estimates of allele frequency for the entire group were 4 of 258 (1.6 percent; 95 percent confidence interval, 0.6 to 3.9 percent) and 5 of 218 (2.3 percent; 95 percent confidence interval, 1.0 to 5.2 percent), respectively. The differing estimates arose from ambiguities in the segregation, and thus the independence, of alleles within some pedigrees. The corresponding low and high heterozygous carrier frequencies were deduced to be 4 of 129 (3.1 percent; 95 percent confidence interval, 1.3 to 7.7 percent) and 5 of 109 (4.6 percent; 95 percent confidence interval, 2.0 to 10.3 percent), respectively. In agreement with these findings, we also detected ATP2B2V586M in 4 of 87 normal “Caucasian” control samples from an independent source (Coriell Cell Repositories) (4.6 percent; 95 percent confidence interval, 1.9 to 11.2 percent). We did not detect ATP2B2V586M in 87 normal Pakistani control samples, but we did detect ATP2B2V586M in 3 of 84 DNA samples from a Human Diversity Panel (Coriell Cell Repositories) representing 10 ethnic backgrounds. All three ATP2B2V586M+ samples were from a subgroup of five Pima Indian samples in this panel, although we could find no literature on hearing loss in Pima Indians. These carrier frequencies are consistent with a potential role for ATP2B2V586M or other alleles of ATP2B2 in the etiology of presbycusis. Although the interaction of a heterozygous dfu allele with a hypomorphic Cdh23 allele inahl strains of mice suggests that ATP2B2V586M could act as a dominant modifier allele in humans, our results do not formally rule out a model in which it is a recessive modifier allele that, in combination with another haplotype in Family LMG132 (Fig. 2A, green haplotype bars), exacerbates sensorineural hearing loss. It is possible that important sequence variants within noncoding regions of this allele were missed by our genomic sequencing protocol. Nonetheless, our study indicates that ATP2B2V586M or other alleles of ATP2B2 may be general modifiers of a variety of human hearing-loss phenotypes that are due
Figure 3. Phenotypes of ATP2B2Heterozygotes.
Panel A shows the pure-tone air-conduction threshold responses as a function of age and grouped according to stimulus frequency for affected persons with a mutation of MYO6 that results in the substitution of arginine for histidine at amino acid position 246 (H246R). Open circles represent the hearing thresholds of a five-year-old affected person (Subject VI-6 described by Mohiddin et al.21), who also carried ATP2B2Heterozygous, and closed circles the hearing thresholds of her affected relatives who were also carriers of H246R but who had wild-type ATP2B2. Cross-sectional age-related progression of sensorineural hearing loss among the H246R carriers who had wild-type ATP2B2 was approximated by linear regression analysis (dashed lines) of the thresholds.22 The arrow indicates an 8-kHz response threshold that is a 90-dB HL hearing level. dB HL denotes decibels hearing level. Panels B and C show pure-tone air-conduction thresholds for a 9-year-old girl and a 41-year-old woman, respectively, who were ATP2B2Heterozygous; both had normal hearing and no significant history of noise exposure. Open circles indicate the right-side air-conduction threshold, and crosses indicate the left-side air-conduction threshold. Dotted lines indicate sex- and age-matched 90th-percentile air-conduction thresholds from International Organization for Standardization publication ISO 7029; normative threshold data are not available for children. Panel D shows pure-tone air-conduction thresholds for a 37-year-old man who was ATP2B2Heterozygous and who had mild and moderate-to-severe sensorineural hearing loss in his left and right ears, respectively. The notched configuration characteristic of noise ototoxicity is evident. Bone-conduction thresholds were consistent with sensorineural hearing loss (data not shown).
to genetic determinants, environmental factors, or combinations of these influences. Since CDH23 and MYO6 mutations and ototoxic noise directly affect sensory hair cells of the inner ear,2,4-23 the effects of ATP2B2\(^{\text{V586M}}\) may be confined to sensorineural hearing loss characterized by pathologic processes affecting primarily the hair cell. Although audiometric differences in ATP2B2\(^{\text{V586M}}\) carriers are most obvious with respect to low-frequency hearing in Family LMG132 (Fig. 1D) and in the family with a MYO6 mutation (Fig. 3A), the lack of detectable high-frequency hearing in ATP2B2\(^{\text{V586M}}\) carriers in Family LMG132 (Subjects II-1, II-9, and II-10) and the sensorineural hearing loss in the ATP2B2\(^{\text{V586M}}\) carrier with noise exposure (Fig. 3D) raise the possibility that ATP2B2\(^{\text{V586M}}\) can modify hearing loss at all frequencies. Additional studies are needed to address these questions and to provide accurate genetic, prognostic, lifestyle, and occupational (i.e., noise avoidance) counseling as well as communication-rehabilitation counseling based on ATP2B2 genotype results.

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The opening of the Panama Canal in 1914 initiated an era in which efforts to control malaria were aimed at the anopheline mosquito. The World Health Organization’s Global Malaria Eradication Campaign in the 1950s and 1960s marked the apex of these efforts. The use of dichlorodiphenyltrichloroethane (DDT) rid vast areas of endemic malaria virtually everywhere except in sub-Saharan Africa. The eradication strategy was abandoned in 1969, because it came to be considered logistically, socially, and politically impractical, especially given public concern about the effects of DDT on the environment. It took two decades and a global resurgence of malaria before a new strategy emerged, one that was focused on treatment rather than prevention. This treatment strategy is currently being debated, and its efficacy is unproved.

Both the broad collapse of preventive efforts and the waning efficacy of standard antimalarial drugs account for the global resurgence of malaria. New therapies are available, but the use of older drugs persists for social, economic, and clinical reasons, despite resistance of the organisms to the older drugs. Efforts to use new antimalarial drugs may be hampered by regulatory requirements or economic obstacles as well as by important questions about safety for the large numbers of patients who treat themselves. Public health agencies continue to support the distribution of older therapies, despite strong criticism from scientists conducting research on the disease and its treatment and prevention.

In addition to inadequate drug efficacy, new therapies may fail because of inappropriate use, inadequate absorption, poor adherence, contraindications, intolerability, the use of counterfeit drugs or improper manufacture of drugs, or prohibitive cost. Chloroquine and sulfadoxine–pyrimethamine, which for several decades were the foundation of malaria therapy, had a low risk with respect to this array of problems. However, the emergence and consolidation of resistance to these drugs eroded their clinical usefulness.

The coccidian genus plasmodium contains 172 species that infect birds, reptiles, and mammals. Four species infect humans — Plasmodium falciparum, P. malariae, P. ovale, and P. vivax. P. falciparum and P. vivax account for the vast majority of the 300 million to 500 million infections that occur each year. Endemic transmission takes place in most tropical latitudes and reaches into temperate zones seasonally (Fig. 1). Although each type of infection causes debilitating febrile illness, only P. falciparum carries a substantial risk of death; 1 million to 3 million deaths occur each year in regions of holoendemic infection in sub-Saharan Africa, most of them among infants and young children.

For plasmodia, humans are not the “definitive host,” a concept defined by parasitologists as the site where sexual recombination occurs. Instead, many species in the genus anophelines have that distinction. From the perspective of the parasite, humans are...
simply a means of getting into mosquitoes, where sexual recombination can occur. A full description of the extraordinary complexity of this cycle is beyond the scope of this review. Figure 2 illustrates the essential features of the cycle and the terminology of antimalarial therapies.

**AVAILABILITY**
Most developing nations license and make available only the antimalarial drugs that are provided through national health programs. This approach often excludes relatively expensive or risky therapies, even for patients who may be able to afford a given drug and have access to medical supervision. The main factor affecting availability is economic — the ability or inability to purchase a drug for broad distribution and the potential reluctance to use an agent because of an inability either to screen users or to monitor its quality.

Chloroquine and sulfadoxine–pyrimethamine cost less than 15 percent of the cost of the least expensive alternative agents and approximately 1 to 2 percent of the cost of many of the agents marketed in the developed world (Table 1). The developing world requires distribution strategies for effective therapies that overcome the availability of cheap but ineffective drugs. The U.S. National Academies made a core recommendation that governments and international financial institutions should commit $300 million to $500 million per year within five years to subsidize artemisinin-combined therapies (an emerging family of antimalarial drugs, including artemether, artesunate and dihydroartemisinin) to achieve prices for the end user in the range of 10 to 20 cents. Unless this recommendation is followed, market forces will continue to drive the use of ineffective therapies.

**ADHERENCE**
Most people taking antimalarial drugs live in rural regions of the developing world and are not supervised by health professionals. A study conducted among 1640 febrile patients with malaria in Burkina Faso showed that 69 percent were self-treated, and in a study in Ethiopia, among 630 febrile patients with malaria, 67 percent were self-treated. Complex, inconvenient, or poorly tolerated antimalarial regimens carry a substantial risk of inadequate adherence. Among 414 Brazilian patients, the risk of
recurrent malaria correlated with self-reported poor adherence, whereas among 632 Nigerian children strict adherence correlated with clinical recovery. Convenient and easily understood packaging and education of the patients alleviate poor adherence. Owing to lengthy and complex regimens, currently used therapies such as quinine and primaquine and new combined therapeutic strategies challenge the ease of adherence.

COUNTERFEIT AND SUBSTANDARD DRUGS
Counterfeit antimalarial drugs pose a serious threat in regions where the trade in pharmaceuticals is not rigorously regulated. A survey conducted in Cameroon found insufficient or inactive ingredients in 38 percent of preparations labeled chloroquine, 78 percent of those labeled quinine, and 12 percent of tablets labeled as an antifolate agent. A survey in Southeast Asia involving 104 purchases of artesunate tablets found that 38 percent of the tablets contained no drug. The trade in counterfeit drugs undoubtedly results in many deaths, but it is lucrative and carries little risk of imprisonment. The inadvertent marketing of substandard pharmaceuticals poses another threat. In a survey of eight authorized wholesalers in Tanzania selling combined sulfadoxine–pyrimethamine tablets, 11 percent of the tablets failed industry standards for content, and 44 percent failed dissolution testing.

INTRINSIC DETERMINANTS OF DRUG EFFECTIVENESS

STAGE SPECIFICITY
Plasmodia pass through distinct stages of form, function, location, clinical consequence, and susceptibility to antimalarial drugs (Fig. 2 and Table 1). Drug activity ranges from narrow (e.g., the activity of quinine against asexual blood stages) to broad (e.g., the activity of primaquine against sexual and asexual forms in the blood and liver). Moreover, the range of stage-specific susceptibility differs among species of plasmodia — for example, chloroquine kills the gametocytes of P. vivax but exerts no effect against those of P. falciparum. These intrinsic properties define the recommended uses of antimalarial drugs.

PARASITE BURDEN
Most patients with malaria carry a burden of $10^8$ to $10^{13}$ parasites. Effective chemotherapy induces a constant fractional decline with each asexual cycle, at a rate that varies according to the susceptibility of the parasite to a given drug. For example, artemisinin derivatives induce reductions of $10^4$, whereas tetracycline achieves a reduction by only a factor of 10 with each cycle. The duration of exposure to a drug that is needed to eliminate infection hinges on the intrinsic rate of decline and, more important, on the initial parasite burden. High levels of parasitemia, as compared with a low burden, require longer exposure to effective drug levels and have a relatively higher risk of treatment failure.

PHARMACOKINETICS
A variety of factors affect the pharmacokinetics of antimalarial drugs. Variant alleles of the human
The gene for cytochrome P-450 2C19 (CYP2C19), for example, correlate with slow or rapid metabolism of some antimalarial agents. For example, chloroguanide, metabolized through the activity of CYP2C19, was studied in 126 healthy, unrelated Nigerian subjects, of whom 5 percent had slow metabolism of the drug (8 percent of the normal rate). A study of Thai subjects taking oral contraceptives reported that metabolism of chloroguanide was decreased by 34 percent, a finding that may be explained by inhibition of CYP2C19 activity by estrogen. Certain foods may also profoundly affect the bioavailability (and toxicity) of certain antimalarial drugs — for example, fatty foods affect the bioavailability of halofantrine and of the combination of lumefantrine and artemether.

SAFETY AND TOLERABILITY
In rural health care centers, staff with relatively little formal training may be responsible for taking care of patients with malaria. The patients often have no written medical history, and the centers have minimal capacity for laboratory screening for contraindications to particular medications. Worse still, most of these people with malaria treat themselves with antimalarial agents acquired over the counter or passed from household to household.

Any antimalarial drugs that are to be distributed in such regions need to be safe and well tolerated, and the risks associated both with the indicated use and with contraindicated or inappropriate uses should be considered. Clinical studies supporting the licensing of new antimalarial drugs rarely include highly vulnerable groups such as infants, young children, and pregnant women. Thus, safety issues often drive the decision to continue distributing chloroquine or sulfadoxine–pyrimethamine, despite substantial parasite resistance. Key data with regard to the safety and tolerability of the most commonly used antimalarial drugs are summarized in Table 2.

IMMUNITY
The intersection of immunity to plasmodia and antimalarial drug activity is poorly understood. In a study of Brazilian patients, self-reported poor adherence correlated with a risk of recurrence among nonimmune persons (i.e., those with a first infection) but not among semi-immune persons (i.e., those with >4.8 years’ exposure to plasmodia). Among 80 Lao patients, the level of humoral immunity to *P. falciparum* and the risk of therapeutic failure were correlated with the parasite count on admission to the hospital and the length of time to

### Table 1. Cost, Convenience, and Primary Clinical Application of Antimalarial Therapies.

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Cost ($)</th>
<th>No. of Doses</th>
<th>Duration of Therapy</th>
<th>Application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>0.11</td>
<td>3</td>
<td>48 hr</td>
<td>Blood-stage schizonticide</td>
</tr>
<tr>
<td>Sulfadoxine–pyrimethamine</td>
<td>0.14</td>
<td>1</td>
<td>Single dose</td>
<td>Blood-stage schizonticide</td>
</tr>
<tr>
<td>Quinine</td>
<td>0.97</td>
<td>21</td>
<td>7 days</td>
<td>Blood-stage schizonticide</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>2.55</td>
<td>1</td>
<td>Single dose</td>
<td>Blood-stage schizonticide</td>
</tr>
<tr>
<td>Atovaquone–chloroguanide</td>
<td>48.00†</td>
<td>3</td>
<td>48 hr</td>
<td>Blood-stage schizonticide</td>
</tr>
<tr>
<td>Artemether–lumezantrine</td>
<td>9.12‡</td>
<td>6</td>
<td>48 hr</td>
<td>Blood-stage schizonticide, gametocytocide</td>
</tr>
<tr>
<td>Artesunate–mefloquine</td>
<td>5.00§</td>
<td>6</td>
<td>48 hr</td>
<td>Blood-stage schizonticide, gametocytocide</td>
</tr>
<tr>
<td>Artesunate–sulfadoxine–pyrimethamine</td>
<td>2.40¶</td>
<td>3</td>
<td>48 hr</td>
<td>Blood-stage schizonticide, gametocytocide</td>
</tr>
<tr>
<td>Artesunate–amodiaquine</td>
<td>2.00¶</td>
<td>3</td>
<td>48 hr</td>
<td>Blood-stage schizonticide, gametocytocide</td>
</tr>
<tr>
<td>Primaquine</td>
<td>1.68</td>
<td>7–14</td>
<td>7 days–8 wk</td>
<td>Tissue-stage schizonticide, gametocytocide</td>
</tr>
</tbody>
</table>

*Unless otherwise indicated, the cost shown is the cost, in 2003 U.S. dollars, of medication for one adult treatment regimen, purchased in bulk, according to the International Drug Price Indicator Guide (IDPIG) (http://erc.msh.org/mainpage.cfm?file=1.0.htm&module=dmp&language=English).
† U.S. commercial sources were surveyed; the cost is not available from the IDPIG.
‡ The cost shown is from the IDPIG; the combination is available through the World Health Organization (WHO) to qualified purchasers at a cost of $2.40 per adult treatment regimen.
§ The cost shown is from the WHO.
¶ The cost shown is from Arrow et al. 8
the resolution of fever. Djimde et al. showed that the clearance of \( P. falciparum \) organisms carrying a mutant transporter gene, \( pfcr \) K76T, that was linked with resistance to chloroquine correlated with acquired immunity in subjects in sub-Saharan Africa. In subjects with relatively little immunity who were infected with mutant parasites, the chloroquine therapy failed more frequently than in those infected with the same genotype of \( P. falciparum \) organisms who had clinical immunity. However, such correlations do not directly link immune effectors with drug activity; acquired immunity independently reduces the parasite burden, which is a well-known determinant of antimalarial effectiveness.

The acquired immunity that occurs throughout regions of holoendemic infection in sub-Saharan Africa may explain the relatively late increase in resistance in that region. Most people at risk in South America and Asia lack immunity and consequently carry substantially higher parasite burdens than do most Africans. Moreover, their symptomatic infections are generally treated far more often than are asymptomatic infections among Africans. In Asia and South America, these factors substantially increase the probability of the selection of resistant genotypes.

### RESISTANCE

The parasites causing malaria exhibit a range of susceptibility to antimalarial agents. Several distinct phenomena explain this range, including species-specific innate resistance (e.g., asexual blood stages of \( P. falciparum \) lack susceptibility to primaquine, whereas those of \( P. vivax \) appear to be sensitive to it); strain-specific innate resistance (e.g., that of asexual liver stages of \( P. vivax \) from the island of New Guinea against primaquine); and acquired resistance. Of these, acquired resistance is the most important, because failure may occur even in the presence of complete adherence to the recommended therapies. Studies in Senegal suggest that resistance to chloroquine contributed to an increase by a factor of 2 to 11 in mortality from malaria. In western Kenya, case fatality rates were markedly higher among children receiving chloroquine than among those receiving other therapies.

Most of the data on the responses of \( P. falciparum \) to therapy reflect this emphasis on acquired resistance. Nonetheless, \( P. vivax \) profoundly affects public health outside Africa, and data are also available that cover its resistance to chloroquine. \( P. malariae \) and \( P. ovale \) infect relatively few people, and little is known about acquired resistance in these organisms.

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**Table 2. Safety and Tolerability of Available Antimalarial Drugs.**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Adverse Effects</th>
<th>Contraindications</th>
<th>Severe Adverse Events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloroquine</td>
<td>Gastrointestinal upset, itching, dizziness</td>
<td>Epilepsy</td>
<td>Death from overdose</td>
</tr>
<tr>
<td>Sulfadoxine–pyrimethamine</td>
<td>—</td>
<td>Pregnancy, renal disease</td>
<td>Stevens–Johnson syndrome</td>
</tr>
<tr>
<td>Quinine</td>
<td>Tinnitus, vertigo, headache, fever, syncope, delirium, nausea</td>
<td>G6PD deficiency, pregnancy, optic neuritis, tinnitus, thrombocytopenic purpura, blackwater fever</td>
<td>Hemolytic anemia, coma, respiratory arrest, renal failure</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>Vomiting, headache, insomnia, vivid dreams, anxiety, dizziness</td>
<td>Depression, schizophrenia, anxiety disorder, any psychosis, irregular heartbeat</td>
<td>Psychiatry</td>
</tr>
<tr>
<td>Atovaquone–chloroguanide</td>
<td>Gastrointestinal upset, headache, stomatitis</td>
<td>Weight of &lt;11 kg in children, pregnancy, breast-feeding, renal impairment</td>
<td>None known</td>
</tr>
<tr>
<td>Artemether–lumefantrine</td>
<td>Dizziness, palpitations</td>
<td>Pregnancy, severe malaria</td>
<td>Impaired hearing</td>
</tr>
<tr>
<td>Artemisate–mefloquine</td>
<td>Vomiting, anorexia, diarrhea</td>
<td>Depression, schizophrenia, anxiety disorder, any psychosis, irregular heartbeat</td>
<td>None known</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>Gastrointestinal upset, prolonged QTc</td>
<td>Conduction abnormalities, pregnancy, breast-feeding, infancy, use of mefloquine</td>
<td>Cardiac arrest</td>
</tr>
<tr>
<td>Primaquine</td>
<td>Gastrointestinal upset, elevated levels of methemoglobin</td>
<td>Pregnancy, G6PD deficiency, breast-feeding</td>
<td>Hemolytic anemia</td>
</tr>
</tbody>
</table>

*Data in the table are from Taylor and White, Centers for Disease Control and Prevention, Phillips-Howard and Wood, and Wernsdorfer. G6PD denotes glucose-6-phosphate dehydrogenase, and QTc QT interval corrected for heart rate.
species. Resistance to chloroquine in *P. malariae* was reported from Indonesia but is apparently debatable. Genetic mutations among strains of plasmodia have been linked with phenotypes of clinical resistance. Some studies challenge specific links between genotype and phenotype in certain settings, but putative markers have emerged (Table 3); the extensive literature on this topic has been reviewed. Validated genetic determinants of resistance would vastly improve surveillance and, thus, the effectiveness of resources for the treatment and control of malaria.

### DRUG RESISTANCE

The risk of resistance varies according to species, strain, and drug. Estimates of risk are often confounded by the determinants already described. Methods of estimating risk also vary. The following estimates of the risk of treatment failure were distilled from many studies and should not be construed as quantitative region-specific risks, because patterns of resistance vary tremendously, even within nations. Protocols that evaluate efficacy generally follow the guidelines of the World Health Organization, which recommend a follow-up period of 7, 14, or 28 days. The timing of recurrent parasitemia or disease reflects the degree of resistance — earlier failure represents higher-grade resistance. Late recurrence may be confounded by reinfection, especially in regions of intense transmission. Investigators in sub-Saharan Africa thus favor an in vivo test with a duration of 7 or 14 days, whereas those in other regions tend to favor a 28-day test or even 63-day test. Comparison of genotypes of the strains causing original and recurrent parasitemias can be used to address confounding by reinfection, but relatively few reporting clinics or laboratories have the capacity to conduct such testing. Tests conducted in Africa tend to report both parasitologic failure (i.e., recurrent parasitemia after treatment, independent of clinical presentation) and the clinical failure of treatment, whereas investigators elsewhere focus on parasitologic failure.

### CHLOROQUINE

After an analogue that had been developed in Germany was captured in 1943, during World War II, chloroquine quickly came into universal use as therapy for and prophylaxis against malaria. Chloroquine was highly effective, easily administered, and inexpensive and had good safety and tolerability. However, resistance in *P. falciparum* appeared in the late 1950s in Thailand and Colombia and emerged in the 1970s in New Guinea and eastern sub-Saharan Africa. Today, resistance to chloroquine in malaria caused by *P. falciparum* occurs everywhere except in Central America (and Hispaniola) and in some regions of southwestern Asia.

In sub-Saharan Africa, the risk of parasitologic treatment failure with the use of chloroquine was almost uniformly greater than 40 percent, whereas the risk of clinical treatment failure tended to be higher in the eastern and central portions of the African continent (typically >30 percent and often >50 percent, respectively) than in the west (typically <20 percent). Elsewhere, the rates of parasitologic failure at day 28 were 57 percent (of 209 evaluations) in southwestern Asia, 46 percent (2280 evaluations) in southern Asia, 85 percent (223 evaluations) in Southeast Asia, and 66 percent (137 evaluations) in South America. Prescribing chloroquine monotherapy against this parasite in any setting, except one in which its effectiveness has recently been demonstrated, should be considered irresponsible.

Resistance to chloroquine by *P. vivax* has emerged, apparently having originated in New Guinea, where failure rates now approach 100 percent. In contrast, surveys in Thailand reveal uniformly sensitive vivax malaria (not shown in Fig. 1). Chloroquine-resistant *P. vivax* may be characterized as endemic to the Indonesian archipelago, especially in the east (including New Guinea), sporadic in the rest of Asia, and rare in South America.

### SULFADOXINE–PYRIMETHAMINE

Sulfadoxine–pyrimethamine, which has potent efficacy against chloroquine-resistant and pyrimethamine-resistant *P. falciparum*, became available in 1971 and became the standard second-line therapy against chloroquine-resistant *falciparum* malaria. This combination acts synergistically against folate synthesis, inhibiting dihydropteroate synthase and dihydrofolate reductase. Although rare, idiosyncratic allergic reactions have occurred among users of sulfa drugs, sulfadoxine–pyrimethamine has otherwise offered superior safety and tolerability, along with the advantage of single-dose therapy. However, resistance to sulfadoxine–pyrimethamine was recognized at the Thai–Cambodian border in the 1960s, and failures occurred in refugee camps in Thailand in the 1970s.
The rates of parasitologic failure of treatment with sulfadoxine–pyrimethamine in sub-Saharan Africa were relatively high in southern regions of holoendemic infection (>50 percent) and low elsewhere (<5 percent, except in Cameroon, where the rate of failure was 10 percent). The rates of clinical treatment failure in sub-Saharan Africa were similarly distributed (<5 percent in the west and 8 to 34 percent in the east and south). Studies in Southeast Asia indicated that the rates of parasitologic failure at day 7 and day 28 were 36 percent and 49 percent, respectively. Good efficacy (80 percent) persisted elsewhere — in southwestern Asia and on the Horn of Africa, where no parasitologic failures were reported among 362 evaluations; in southern Asia, where the failure rate was 18 percent (of 339 evaluations) by day 28; and in South America, where the failure rate was 18 percent (of 339 evaluations) by day 28 and 49 percent by day 28. The risk of resistance to sulfadoxine–pyrimethamine is relatively high in Southeast Asia and eastern Africa.

Mefloquine
Mefloquine emerged as a successor to chloroquine in the 1980s. Resistance appeared in regions at the border between Thailand and Cambodia within a few years, perhaps owing to widespread use of quinine, to which it is structurally related. Resistance in that border region remains high. However, in nearby regions resistance to mefloquine remains relatively low. Smithuis et al. reported that, of 75 patients, more than 90 percent in the region of the western border of Myanmar (Burma) responded to mefloquine (at a dose of 15 mg per kilogram of body weight). In another study, of 79 patients hospitalized in Bangkok and treated with mefloquine (25 mg per kilogram), 68 (86 percent) remained free of parasitemia after 28 days. Similar efficacy was observed in Bangladesh. However, a group of Dutch marines in Cambodia who were receiving mefloquine prophylaxis during the 1990s had high attack rates of falciparum malaria. In contrast, the protective efficacy of mefloquine among Indonesian soldiers in western New Guinea — where the efficacy of chloroquine against P. falciparum and P. vivax approaches zero — was 100 percent. The risk of the prophylactic or therapeutic failure of mefloquine in Southeast Asia appears to be low outside the shared borders of Thailand, Myanmar, and Cambodia.

Few studies have evaluated the effectiveness of mefloquine against falciparum malaria in Africa. In the mid-1990s, Lobel et al. examined mefloquine prophylaxis among 140 Peace Corps volunteers who were infected by P. falciparum. Poor adherence explained most of the infections, but in five cases the resistance appeared to be genuine (i.e., parasitemia was present with >620 ng of mefloquine per milliter in plasma). Despite sporadic, well-documented failures of mefloquine among travelers to Africa, the drug remains effective there.

Studies conducted in coastal Peru and in the Amazon Basin among 153 subjects with P. falciparum malaria who were receiving mefloquine (at a dose of 15 mg per kilogram) revealed complete sensitivity. Despite occasional reports of resistance in the Amazon basin, the available evidence shows a low risk of resistance throughout South America.

Mefloquine has received adverse attention in the media in recent years. The drug has been blamed for suicides, homicides, and other personal tragedies on the basis of anecdotal accounts, which, by their
nature, cannot establish cause and effect. Well-con-
trolled trials consistently indicate that mefloquine
given as prophylaxis is as well tolerated as other an-
timalarial drugs. Nonetheless, the drug has been
linked with a higher risk of insomnia, fatigue, and
adverse neuropsychiatric effects (e.g., depression
and anger) than other antimalarial drugs. The
risk appears highest among women, especially
those taking the drug for the first time and those
with a low body-mass index.

QUININE

Jesuit priests in Peru in the 1500s learned from the
Incas that powder from the bark of the cinchona tree
relieved shivering with cold, and they supposed it
would also offer relief from the chills of malaria. Its
activity against the parasite, later shown to be due
to quinine, was unknown to them. Resistance to
quine appears sporadically, and a moderate risk
of treatment failure appears to be limited to some
regions of Southeast Asia and New Guinea. Zalis et
al. suggested that there might be a link between
poor in vitro responses to quinine and diminished
clinical responsiveness in the Amazon basin, but
without supporting clinical data. Recent well-con-
trolled trials of quinine monotherapy showed vari-
able rates of efficacy against *P. falciparum*: 92 percent
in a group of 49 patients in Bangladesh, 67 percent
among 54 patients in western Thailand, and 80 percent
among 30 patients in a clinic in Bangkok. A seven-day regimen was more than 95 percent
efficient in Venezuela and Equatorial Guinea.

Rare reports of failure of intravenous
quine for the treatment of severe or complicated
malaria have appeared sporadically.

Oral quinine is used to treat uncomplicated ma-
laria, generally over a period of three to seven days
in combination with another blood-stage schizonti-
cide, typically tetracycline or doxycycline. Although
one study of 86 patients in Thailand showed the
superiority of a seven-day regimen combined with
tetracycline (100 percent efficacy rate) as com-
pared with a five-day regimen combined with tet-
racycline (87 percent), studies performed else-
where have shown complete efficacy with shorter
regimens combining quinine, doxycycline, and
primaquine.

Poor adherence carries a high risk of treatment
failure, particularly because quinine causes a syn-
drome of adverse effects known as cinchonism,
including primarily tinnitus, nausea, and vertigo.
A randomized trial in Thailand recorded a 71 per-
cent rate of adherence. However, in Cambodian
villages, the rate of compliance with the same regi-
men was far lower — 11 to 20 percent — even after
an intervention to raise awareness about the need
for compliance.

PRIMAQUINE

Developed during World War II, primaquine re-
mains the only licensed tissue-stage schizonticide
(Fig. 2) for the prevention of relapse after infec-
tion (standard therapy) or as presumptive therapy
against relapse after exposure to the risk of infection,
without evidence of infection (terminal prophylax-
is). Recently recommended for use as prophylax-
,

Despite more than 50 years of use in millions of
people per year, primaquine is still shrouded in con-
fusion and genuine mystery. At least four different
regimens, some prescribed for only one week and
others for as long as eight weeks, are aimed at the
same objective of preventing relapse. Three fac-
tors largely explain the lack of uniformity: first, the
reluctance of health care programs to accept therapy
that has a duration of two weeks; second, per-
ceived issues of toxicity and tolerability; and third,
the use of a total dose, rather than a dosing sched-
ule, as the primary determinant of efficacy. How the
same total dose of a rapidly eliminated drug kills
organisms irrespective of whether it is delivered over
a period of 7, 14, or 56 days defies explanation.

Resistance is not fully understood either. Resis-
tance in asexual blood stages of *P. vivax* has long
been known but is of no clinical consequence.

Reports of resistance to tissue-stage schizontical
activity fail to consider or describe patients’ adher-
ance, to exclude the possibility of reinfection, or to
address the possible recrudescence of chloroquine-
resistant strains. Lack of evidence of resistance to
primaquine in liver stages probably reflects a heavy
burden of proof rather than an absence of resis-
tance.

COMBINED THERAPIES AGAINST RESISTANCE

Combined therapies, which constitute a widely
practiced strategy in the treatment of diseases such
as leprosy, tuberculosis, and infection with the hu-
man immunodeficiency virus and which have long been known to be effective against malaria, have rarely been used in its treatment until recently. The use of more than one agent successfully requires separate mechanisms of action against the same stage of the parasite. Thus, because both sulfadoxine and pyrimethamine are folate antagonists, they would not be considered a combined therapy. Similarly, combining a blood-stage schizonticide with primaquine is not considered combined therapy, because the drugs attack different stages of the parasite. The fixed combination of atovaquone–chloroguanide, which affects mitochondrial electron transport and folate metabolism in asexual blood stages, represents true combination therapy.

Combined antimalarial therapies include old and new drugs — old drugs in new combinations (chloroquine and sulfadoxine–pyrimethamine), an old drug combined with a new drug (amodiaquine and artesunate), and new drugs in combination (lumefantrine and artemether). Combination therapy that includes artemisinin appears to be potent and particularly useful in endemic regions; extracted from the weed *Artemisia annua*, the artemisinins (primarily artemether, artesunate, and dihydroartemisinin) were developed in China during the 1960s.

The artemisinins act very rapidly, reducing parasitemia by a factor of $10^4$ with each cycle. Thus, for a parasite burden in the range of $10^{12}$, only three cycles are required to abolish parasitemia. The artemisinins are rapidly eliminated, and daily administration for a period of seven days (three cycles) is required. Episodes of recrudescence follow briefer regimens, but a seven-day regimen is considered to be impractical. Therefore, treatment for a period of three days with artemisinin combined with a slowly eliminated companion blood-stage schizonticide has been adopted. This three-day regimen of artemisinin reduces the parasite burden by a factor of $10^8$ (leaving only 0.000001 percent of parasites surviving to be abolished by mefloquine) (Fig. 3). The artemisinins also exert activity against gametocytes, reducing the probability of transmission.

The use of combination therapies with artemisinin does not preclude the onset of drug resistance, particularly since patients may not take the medication as directed. Thus, the multiple doses needed with such combined therapies (typically six doses over a period of three days) represent an important potential pitfall. The emergence of resistant strains may already be in progress. In vitro studies indicate

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**Figure 3. Combination Therapies with Artemisinin Derivatives.**

The figure illustrates the rationale behind the use of an artemisinin derivative combined with a slowly excreted drug such as mefloquine for therapy against *falciparum* malaria over a period of five weeks. Panel A shows plasma levels of mefloquine (blue line) and relatively high levels of parasitemia caused by strains resistant to mefloquine (solid red line) and sensitive to mefloquine (dashed red line). Prescribed therapy eliminates the sensitive but not the resistant infection. Panel B shows the importance of parasite burden as a determinant of drug effectiveness, indicating the elimination of both sensitive and resistant infections at lower levels of burden. Panel C shows the quick inhibitory effect of a short regimen of an artemisinin derivative (black line), but, owing to its short plasma half-life, the drug cannot completely eradicate the parasite. Panel D shows the effect of combining an artemisinin derivative (black line) with a drug such as mefloquine (blue line): a quick “knockdown” of the parasite burden by the artemisinin derivative allows the slower-acting and more slowly excreted mefloquine to exert activity against a greatly diminished parasite burden.
diminished susceptibility to artesunate among Asian isolates.\textsuperscript{41} Isolates from western Cambodia, where combination therapies with artemisinin derivatives are widely used, show diminished susceptibility to the combination of mefloquine and artesunate, as compared with isolates from eastern Cambodia.\textsuperscript{115} In contrast, in the 1990s in some regions bordering western Thailand, combination therapies with artemisinin derivatives were deployed as first-line therapy with excellent efficacy (more than 90 percent), and this success appears to be stable.\textsuperscript{116} Where more resources are available, including medications, resistant strains have been slower to develop. Where both medication and an effective health care infrastructure are present, resistance can be controlled. Such economic and personnel issues may explain the contrasting findings with regard to rates of resistance to combination therapies with artemisinin derivatives.\textsuperscript{117} Thus, supplying these therapies in the setting of an adequate health care infrastructure would seem the best way to prevent the onset of resistance.

Patients with severe or complicated malaria often cannot take oral medication, and parenteral quinine has been the standard approach to therapy. Rectal artesunate may radically improve the care of such patients, especially across the geographic expanses of regions of endemic infection where parenteral medications are neither available nor practical. Clinical trials indicated that rectal artesunate (combined with another blood-stage schizonticide) cleared parasitemia more quickly than parenteral quinine and with equal efficacy.\textsuperscript{118,119}

\section*{REFERENCES}

14. Basco LK. Molecular epidemiology of A global public health threat due to the resurgence of malaria stems from a general collapse of vector-control operations and from resistance to chloroquine or sulfadoxine–pyrimethamine. Recent surveys show rates of treatment failure higher than 50 percent for chloroquine in most affected regions, as well as poor efficacy of sulfadoxine–pyrimethamine in sub-Saharan Africa and Southeast Asia. Quinine and mefloquine remain effective therapies everywhere except in some regions bordering Thailand. Resistance to primaquine — the only drug for preventing relapse — probably occurs but has not yet been confirmed. New drugs should be effective among the poor, self-treating rural populations in regions of endemic disease and should be provided through programs that address issues of availability and cost, convenience and adherence, safety and tolerability, and quality assurance. The combination therapies with artemisinin derivatives represent the present best efforts toward providing such therapeutic agents. These drugs deliver an inhibitory effect that substantially reduces the probability of selection for resistant parasites, as compared with traditional monotherapies. However, widespread distribution without complementary capabilities in the delivery of health care places the clinical usefulness of these critical drugs in doubt.

The views expressed here are those of the author and do not necessarily reflect or represent those of the U.S. Navy or the Department of Defense.

I am indebted to Dr. Stephen L. Hoffman (Rockville, Md.) and Dr. Chansuda Wongrenchamai (Bangkok) for thoughtful reviews of this manuscript, and to Zoila Pretell at the Naval Medical Research Center Detachment, Lima, Peru, for research resources.
An Unusual Case of Pulmonary Embolism

Tom Routledge, M.R.C.S.
David Jenkins, F.R.C.S.
Papworth Hospital
Papworth Everard, Cambridge,
United Kingdom

A 33-YEAR-OLD WOMAN HAD DYSPNEA FOR SIX MONTHS, WITH INTERMITTENT FEVER. She was admitted to the hospital with worsening dyspnea, hypoxia, and patchy bilateral pulmonary consolidations. Computed tomography showed large, central pulmonary filling defects (Panel A, arrows). The patient was treated with anticoagulation and antibiotics. Her condition deteriorated, and she received an intravenous thrombolytic agent, with no improvement. She then underwent urgent pulmonary thromboendarterectomy, during which she was found to have pale, lobulated tissue completely filling both pulmonary arteries. A complete resection was performed, but a reperfusion lung injury developed, and the patient died. Histologic examination of the resected specimen showed a pleomorphic sarcoma (Panel B). At autopsy, the primary tumor site was found to be in the inferior vena cava. The tumor had embolized to the pulmonary artery and grown in situ to form the casts shown.

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Airway Dilatation after Inhalation of a Beta-Agonist

A 37-YEAR-OLD WOMAN WITH A 25-YEAR HISTORY OF ASTHMA that had been managed with inhaled budesonide and albuterol (salbutamol) as needed underwent high-resolution multislice helical computed tomographic scanning. Thirty minutes after the inhalation of albuterol, a cross-sectional multiplanar reconstruction of the right upper lobe, obtained at maximal inspiration, revealed an increase in the airway caliber. Each panel of images from these two videos consists of the view before the inhalation on the left and the corresponding view after the inhalation on the right. Panel A shows the segmental, or third-generation, bronchus (arrows); Panel B the fourth-generation bronchus at the branching point (arrows); and Panel C more of the periphery of the fourth-generation bronchus, with full wall visualization (arrows). Panels D, E, F, and G show bronchial divisions from the fifth to the eighth generation (arrows); the same bronchial divisions are shown in the video clip. Panel H shows preinhalation airway occlusions (left-hand image), which opened up after the inhalation (right-hand image). The forced expiratory volume in one second, initially 1.27 liters (49.8 percent of the predicted value), increased by 0.49 liter after the inhalation of albuterol. It is important to note that airway resistance is an inverse function of the airway radius to the fourth to fifth power.

Gen Tamura, M.D.
Tohoku University Hospital
Sendai 980-8574, Japan

Yuji Suda, M.D.
Sendai City Medical Center
Sendai 983-0824, Japan
A 32-year-old woman in the 17th week of her first pregnancy was referred for genetic counseling after her fetus had been found to have an abnormal karyotype. She had had an uneventful first trimester. In the 14th week, transabdominal and transvaginal ultrasonography, performed to evaluate an ovarian cyst, revealed a single intrauterine gestational sac containing a normal fetus with a normal heart rate. The area of nuchal translucency was 3.7 mm in diameter (normal, ≤2.5 mm) (Fig. 1 shows an ultrasound study of a similar fetus). There were two cysts within the left ovary; a clear cyst, 4.1 cm in diameter, and a second cyst, 4.5 cm in diameter, that was consistent with an endometrioma. It was recommended that amniocentesis be performed at 15 weeks of gestation. In a meeting with a genetic counselor at that time, the patient declined to provide a detailed family history.

Two weeks later, a follow-up ultrasound study demonstrated a regular fetal heartbeat and fluid within the fetal stomach. The cranium and spine appeared to be well formed for gestational age. There were bilateral nuchal hygromas. An amniocentesis was performed. The analysis of amniotic fluid revealed a normal alpha-fetoprotein level for gestational age. The amniotic fluid karyotype was 46,XY, add(18)(p11.2), indicating the presence of additional material on the short arm of chromosome 18 at band 11.2 (Fig. 2).

Two weeks later, the parents returned to the Prenatal Diagnosis Unit. They were told that the fetus could be affected by an unbalanced chromosomal translocation. The mother reported that her paternal aunt had had two sons with birth defects. The father had no relatives with known birth defects or mental retardation. No other major risk factors were identified. The mother had smoked in the past but had stopped smoking before she conceived. She drank two to three alcoholic beverages per week but had stopped when she learned she was pregnant.

A diagnostic test was performed.

**DIFFERENTIAL DIAGNOSIS**

*Dr. Lewis B. Holmes: Dr. Nadel, would you show us the ultrasound studies?*
Dr. Allan S. Nadel: In the late first and early second trimester, fetal edema is usually most prominent in the subcutaneous tissue of the neck; this produces an area of translucency in the nuchal soft tissues. Mild nuchal edema is quantified by measuring the nuchal translucency, the sonolucent space in the skin of the posterior neck, as seen on a midsagittal image (Fig. 1). Mild nuchal edema before 14 weeks is associated with an increased risk of chromosomal abnormalities, structural anomalies, and a number of genetic syndromes. Therefore, when excessive nuchal edema is detected, evaluation of the fetal karyotype (by chorionic-villus sampling or amniocentesis) should be offered to the parents and followed by a detailed structural survey with special attention to the heart and great vessels. If all the findings are normal, the fetus is probably completely healthy, although a slightly increased risk of a genetic syndrome remains. This fetus had mild nuchal edema in the 14th week, and the mother was counseled to undergo amniocentesis after 15 weeks, when the risk of procedure-related complications is thought to be minimal. At that time, the structural survey of the fetus was limited by its early gestational age. Amniocentesis was performed.

Dr. Holmes: After the first ultrasound study, the parents were asked to meet with a genetic counselor to discuss the amniocentesis procedure, including its potential risks and the benefits. As part of that session, the genetic counselor asked about the medical histories of their families, but the parents were distracted by the news of the fetal abnormalities and preferred not to review the family history at that time.

A chromosomal abnormality was identified on amniocentesis, with additional chromosomal material on the short arm of chromosome 18, possibly reflecting an unbalanced translocation (Fig. 2A). The parents returned for a discussion of this finding and its potential clinical significance and interpretation. I showed them the karyotype and pointed out the chromosomal abnormality identified on amniocentesis and told them that an unbalanced chromosomal translocation was one possible explanation of the finding. I explained that one of them could be a carrier of a balanced translocation, which, in turn, had led to their fetus’s having an unbalanced chromosomal translocation. When we began to construct the family tree, the mother said that she must have the chromosomal translocation, because of her family’s medical history. Both her parents, her brother and sister, and six nieces and nephews were healthy. However, she knew that one of her father’s sisters had had two children with birth defects. The parents agreed to blood testing to determine whether one of them had a chromosomal translocation. We agreed to wait for the results of the chromosomal analysis before the woman asked her relatives for more information.

The couple returned two weeks later to review the findings. The genetic team described the process of chromosomal analysis and showed them images of normal (Giemsa-banded) karyotypes. We then showed them their karyotypes; the father’s was normal 46,XY, and the mother’s contained a balanced translocation (Fig. 2B). A color-coded diagram (Fig. 2C) was used to show the derived chromosome 1; the distal portion of the long arm (q42.1–ter) was
absent and had been replaced by the distal portion (p11.2-ter) of one copy of the short arm of chromosome 18. The derived chromosome 18 had the translocated portion of chromosome 1. The mother’s karyotype was 46,XX,t(1;18)(q42.1;p11.2), indicating that material from the long arm (q) of chromosome 1 at band 42.1 had switched positions with material from the short arm (p) of chromosome 18 at band p11.2, resulting in a balanced translocation — that is, no chromosomal material was missing. That of the fetus was 46,XY,der(18)t(1;18)(q42.1;p11.2)mat — indicating that the derivative (der) chromosome 18 was the result of the maternal (mat) balanced translocation. Since this fetus inherited the normal chromosome 1 from the mother, the translocation was unbalanced, and the fetus effectively had trisomy for the translocated portion of 1q and monosomy for the terminal portion of chromosome 18.

The parents chose to terminate this pregnancy because of the serious prognosis associated with the unbalanced translocation. The mother said that she would contact relatives for more information about the medical history of her cousins with birth defects. The pregnancy was terminated at 18 weeks. The parents returned to the genetics clinic to review the risk that future pregnancies would be abnormal. They were told that a future pregnancy could have one of three outcomes: a healthy child, a healthy child with the same translocation as the mother’s, or a child with either of two unbalanced translocations.

The mother had brought with her a copy of an article in a genetics journal, published in 1979, in which the same chromosomal translocation had been described in several of her father’s relatives. She had not been told about the risk of this genetic abnormality because she was related to these people through her father; since only females had been identified as carriers, her relatives believed that her father was not at risk and that she was therefore not at risk either.

When we contacted the first author of the report, Ruth M. Liberfarb, M.D., Ph.D., she recalled that the first contact with the family occurred as a result of the evaluation of a child with multiple congenital anomalies and developmental delay. Both the mother of the child (Fig. 3; II-2), a maternal aunt of the child (II-5), and the maternal grandmother (I-2) had had multiple spontaneous abortions and stillbirths, as well as live-born children who died in infancy with congenital anomalies. Chromosomal analysis identified the unbalanced translocation in the child; studies of his parents showed that his mother had a balanced translocation. Screening of the parents’ phenotypically normal son and daughter, the maternal grandparents, and all the mother’s siblings was recommended to determine whether they were carriers of this translocation. The mother of the child with birth defects invited her relatives to come to her home for a discussion with Dr. Liberfarb about the risk of carrier status and the option of undergoing chromosomal analysis. Her brother (II-6), the father of the woman whose pregnancy is discussed here, did not attend this meeting or undergo genetic testing. The pedigree of the family described by Liberfarb et al., with the addition of information about the patient’s generation (the patient is III-15) and offspring, is shown in Figure 3.

During the discussion at our genetics center, the mother of the affected fetus asked about the new method of prenatal diagnosis called preimplantation genetic diagnosis. She was eager to know whether this approach would allow her to have a

![Figure 3. Pedigree of the Mother’s Family.](https://example.com/figure3.png)
healthy infant and to avoid having to consider the difficult and traumatic option of termination of pregnancy if another fetus had an unbalanced chromosomal translocation. Since this procedure is not performed at this hospital, I referred the couple to our colleagues at Brigham and Women’s Hospital, who are using preimplantation genetic diagnosis to monitor pregnancies at risk for an unbalanced translocation.

**DISCUSSION OF MANAGEMENT**

*Dr. Antonio R. Gargiulo:* The couple saw Dr. Louise Wilkins-Haug in the Maternal–Fetal Medicine Division. At that time, the patient was again pregnant. Dr. Wilkins-Haug provided genetic counseling during a second pregnancy and performed chorionic-villous sampling, which disclosed abnormal material on the short arm of chromosome 18. After the termination of their second consecutive pregnancy, the couple came to the Center for Reproductive Medicine at Brigham and Women’s Hospital to explore options for preimplantation detection of fetal chromosomal aberrations.

The couple’s reproductive history disclosed profound subfertility, with unprotected timed intercourse practiced for four years before their first conception. The results of the diagnostic workup for infertility included a normal semen analysis and elevation of the wife’s serum estradiol level to 62 pg per milliliter on day 3 of her menstrual cycle, with a normal serum level of follicle-stimulating hormone (FSH) of 7.6 mIU per milliliter.

Carriers of reciprocal translocations, such as this woman, produce both balanced and unbalanced gametes. The chromosomal constitution of the gametes formed depends on the type of segregation after pairing of homologous chromosomes at meiosis in a quadriradial figure. Typically, a chromosomal segregation results in the formation of two chromosomally balanced gametes and four unbalanced gametes. When fertilization occurs with a normal haploid gamete, the balanced gametes will produce a phenotypically normal conceptus with either normal chromosomes or the same balanced translocation carried by a parent. Some translocations can affect implantation and thus fertility; however, the amount of chromosomal material involved in this case would be unlikely to explain this couple’s infertility.

The woman had been followed by her gynecologist by means of serial pelvic ultrasonography for a persistent complex left ovarian cyst. In preparation for assisted reproduction, we performed a combined laparoscopy and hysteroscopy to evaluate the ovarian cyst, as well as to survey the anatomical features of the endometrial cavity, looking for abnormalities that might explain her subfertility. We found a left ovarian endometriotic cyst, which we excised, and otherwise normal pelvic anatomy, with patent fallopian tubes and a normal endometrial cavity. This degree of pelvic endometriosis probably caused her infertility.

The couple was counseled about the technical aspects of in vitro fertilization and preimplantation genetic diagnosis for the identification of embryos with unbalanced chromosomes.

*Dr. Catherine Racowsky:* Preimplantation genetic diagnosis entails the screening of embryos produced by in vitro fertilization for a variety of genetic abnormalities. Genetic analysis of the embryos involves delicate micromanipulation techniques to remove cellular material. One or two blastomeres are removed from the embryo at the 6-to-8-cell cleavage stage. Removal of two blastomeres instead of one has been shown to improve the accuracy of preimplantation genetic diagnosis, especially in couples with balanced chromosomal translocations, in which a high incidence of blastomere mosaicism is encountered.

Once the cellular material is obtained, it can be analyzed by different techniques: fluorescence in situ hybridization, polymerase chain reaction, or comparative genomic hybridization. Fluorescence in situ hybridization is used to determine numerical or structural abnormalities of specific chromosomes, as well as for determination of sex chromosomes. It is the technique best suited to screening embryos before implantation to identify carriers of balanced translocations. Its application has been shown to reduce drastically the chance of chromosomally abnormal pregnancies in couples with such translocations.

*Dr. Gargiulo:* Fluorescence in situ hybridization studies with use of DNA probes for the subtelomeric regions of the short arm of chromosome 18 (18pter) and the long arm (18qter) were performed initially on chromosomes in metaphase and on nuclei in interphase from peripheral-blood lymphocytes obtained from the woman and her husband; the results were compared with those for matched controls. Two hybridization signals specific for the
found 18pter probe and two hybridization signals specific for the 18qter probe were observed in 90 percent of nuclei obtained from the wife’s peripheral-blood sample, in 94 percent of nuclei obtained from the husband’s peripheral-blood sample, and in 92 percent of nuclei that were examined from a control specimen.

Once this preliminary test was completed, the couple began a cycle of in vitro fertilization. Controlled ovarian hyperstimulation was induced with high doses of recombinant human FSH (300 IU given subcutaneously twice a day), and pituitary down-regulation was achieved by administration of the gonadotropin-releasing–hormone (GnRH) agonist leuprolide acetate at a daily dose of 0.5 mg, given subcutaneously, starting in the luteal phase preceding the stimulation cycle. In spite of this aggressive stimulation protocol, only six measurable follicles were present after 11 days of gonadotropin administration, when dimension criteria (two leading follicles with a mean diameter of 18 mm) for final oocyte maturation were reached. A single dose of human chorionic gonadotropin (hCG) (10,000 IU) was administered intramuscularly, and follicular aspiration guided by transvaginal ultrasonography was performed after 36 hours. All clinical embryology procedures were carried out by the Brigham and Women’s In Vitro Fertilization Laboratory.

Seven cumulus–oocyte complexes were identified. Four of these contained metaphase II oocytes (mature oocytes that have extruded the first polar body and can be fertilized normally). Intracytoplasmic sperm injection was performed on the four mature oocytes, all of which were fertilized. Given the low number of mature oocytes and subsequently of embryos obtained, as well as the expected 1:3 ratio of embryos with the balanced translocation to those with the unbalanced translocation, the couple was counseled to have all embryos frozen at the pronuclear stage of development (i.e., about 18 hours after intracytoplasmic sperm injection) and to undergo another cycle of in vitro fertilization to obtain more embryos. Reports of successful preimplantation genetic diagnosis with cryopreserved embryos had recently appeared, proving that this genetic analysis was still possible after embryos had been thawed.7

A second cycle of controlled ovarian hyperstimulation was started after a rest cycle. This time, an even more aggressive stimulation protocol was adopted, with daily doses with recombinant human FSH (300 IU given subcutaneously) and purified urinary luteinizing hormone (LH) with FSH (300 IU given subcutaneously). Moreover, leuprolide acetate was administered together with gonadotropins at doses of 0.05 mg given subcutaneously twice a day. This protocol allows a mild “flare” of endogenous secretion at the beginning of the cycle, while still providing sufficient pituitary suppression to prevent the LH surge later in the cycle. In spite of this adjustment in the protocol, the ovarian response was again low. Six cumulus–oocyte complexes were identified, all of which contained mature oocytes; however, the fertilization rate with intracytoplasmic sperm injection was lower than expected, and only two embryos were obtained. For the reasons described above, these embryos were also cryopreserved for future analysis.

A third cycle of controlled ovarian hyperstimulation was begun after a rest cycle. In this case, we used the same gonadotropin regimen as in the second cycle but substituted the GnRH antagonist cetrorelix for the GnRH agonist leuprolide acetate. Cetrorelix (250 µg given subcutaneously per day) was started after six days of gonadotropin treatment to achieve rapid pituitary suppression of the LH surge with no compromise of FSH levels during the early part of the stimulation cycle. Once again, six cumulus–oocyte complexes were identified, all of which contained mature oocytes and on which intracytoplasmic sperm injection was performed; four embryos were obtained and again frozen at the pronuclear stage.

A fourth and final cycle of controlled ovarian hyperstimulation was started after two rest cycles. In this cycle the same stimulation protocol was used as in the preceding cycle, and the same number of mature oocytes resulted. Three of the four oocytes were successfully fertilized with intracytoplasmic sperm injection.

Dr. Racowsky: At this point, the decision was made to proceed with preimplantation genetic diagnosis, and the 10 previously cryopreserved embryos were thawed in order to synchronize their development with that of the freshly produced embryos. All 10 cryopreserved embryos survived the thawing process. Blastomere biopsy was performed on day 3 of embryo development. Blastomeres were obtained from 9 embryos that had developed to the stage of having six or more blastomeres — that is, from the 3 fresh embryos and from 6 of the 10 thawed embryos. In six of the nine biopsied embryo-
os, it was technically feasible to obtain two blastomeres. Blastomeres were then processed for chromosome spreading and fixation by a modification of the Tarkowski method. They were then submitted to the cytogenetics laboratory for analysis by fluorescence in situ hybridization. The results are shown in Table 1.

In two of the nine embryos, the nuclei of the fixated blastomeres could not be located by fluorescence in situ hybridization. Results were therefore available for seven embryos on the day of embryo transfer (day 4 of embryo development). Three chromosomally balanced embryos (which were either chromosomally normal or had a balanced chromosomal translocation) and four chromosomally unbalanced embryos (two with partial monosomy 18 and two with partial trisomy 18) were identified (Fig. 4A and 4B and Table 1). One of the balanced embryos had developed appropriately to the morula stage, whereas in the other two balanced embryos growth had been arrested after the blastomere biopsy. One of these two appeared strikingly abnormal, with multiple large fragments and cytoplasmic vacuoles. After thorough consultation, the couple elected to transfer the two normal-appearing, chromosomally balanced embryos (Fig. 5) and to discard all the other embryos.

Dr. Gargiulo: Transcervical embryo transfer with use of a soft-tip catheter was performed without difficulty. Luteal-phase progesterone supplementation was provided by daily intramuscular injections of progesterone in oil (50 mg). The serum level of the beta subunit of hCG two weeks after embryo transfer (649 mIU per milliliter) indicated early implantation, with a normal increase after 48 hours (to 1818 mIU per milliliter). The first clinical evidence of pregnancy was obtained at 5.9 weeks of gestation, when ultrasonography showed a single intrauterine pregnancy with a fetal heart rate of 111 beats per minute. A follow-up ultrasound examination at eight weeks of gestation confirmed an intrauterine pregnancy with a live single fetus, with normal interval growth and a normal fetal heart rate of 153 beats per minute. The patient was referred to her obstetrician for routine prenatal care. Given an expected rate of accuracy of 90 to 95 percent for preimplantation genetic diagnosis, depending on the condition analyzed and the technique used, amniocentesis is strongly recommended for all women whose embryos have undergone this selection procedure before transfer. This patient declined both so-called triple screening (measurement of estriol, the beta subunit of hCG, and alpha-fetoprotein) and amniocentesis.

The woman had an uneventful pregnancy. Labor was induced at 41 weeks of gestation, and delivery was eventually accomplished by primary low transverse cesarean section because of failed induction and a nonreassuring fetal heart rate. A healthy baby boy, weighing 3420 g and with one-minute and five-minute Apgar scores of 9 and 9, was evaluated by the pediatrician and discharged home with his mother. Dr. Gargiulo: The largest study done to assess the developmental steps of children born after preimplantation genetic diagnosis followed some such children until two years of age and found no developmental abnormalities. However, unlike in vitro fertilization or intracytoplasmic sperm injection, for which we now have cohorts of children followed all the way into school age and young adulthood, we do not at present have any published developmen-

### Table 1. Characteristics of All Embryos Produced by the Couple.

<table>
<thead>
<tr>
<th>Type of Embryo</th>
<th>No. of Cells Day 3</th>
<th>Result of FISH†</th>
<th>No. of Blastomeres Extracted</th>
<th>Outcome</th>
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<tr>
<td>Fresh</td>
<td>8</td>
<td>Morula</td>
<td>Unbalanced</td>
<td>1</td>
</tr>
<tr>
<td>Fresh</td>
<td>8</td>
<td>Morula</td>
<td>Balanced with abnormal morphology</td>
<td>2</td>
</tr>
<tr>
<td>Fresh</td>
<td>8</td>
<td>Morula</td>
<td>Balanced</td>
<td>3</td>
</tr>
<tr>
<td>Thawed</td>
<td>4</td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Thawed</td>
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<td>3</td>
<td>Balanced</td>
<td>2</td>
</tr>
<tr>
<td>Thawed</td>
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<td>14</td>
<td>Anucleate</td>
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<tr>
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<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Thawed</td>
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<td>4</td>
<td>Unbalanced</td>
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</tr>
<tr>
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<td></td>
<td>0</td>
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<tr>
<td>Thawed</td>
<td>8</td>
<td>9</td>
<td>Unbalanced</td>
<td>1</td>
</tr>
</tbody>
</table>

† FISH denotes fluorescence in situ hybridization. "Balanced" indicates that an embryo was chromosomally normal or had a balanced translocation. "Unbalanced" indicates the presence of a chromosomal imbalance.

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* The number of cells on day 3 denotes the number of blastomeres in each embryo on the day of the biopsy. On day 4, the number of blastomeres or the stage of development (morula) on the day of embryo transfer is shown.

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Dr. Racowsky: There is an uneven distribution of regulatory proteins even as early as the one-cell stage in the human embryo, and this uneven distribution becomes more apparent as the embryo undergoes cleavage to the morula stage. During preimplantation genetic diagnosis, critical proteins that are currently unidentified might be removed with the biopsied blastomere and might affect development down the road. The European Society of Human Reproduction preimplantation genetic diagnosis consortium follows babies born after use of this procedure throughout the world, and to date there do not appear to be any differences in the developmental timelines of these babies and babies whose births did not follow preimplantation genetic diagnosis. However, these are still early days with regard to the application of this invasive technique, and we must counsel our patients appropriately. Larger studies are needed to assess the long-term outcomes of these children.

Dr. Holmes: At the last follow-up, the infant was nine months old and progressing normally.

Anatomical diagnosis

Balanced maternal chromosomal translocation — 46,XX,t(1;18)(q42.1;p11.2) — resulting in an unbalanced fetal translocation — 46,XY,der(18)t(1;18)(q42.1;p11.2)mat.
REFERENCES


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SLIDE SETS FOR THE CASE RECORDS AVAILABLE IN DIGITAL FORMAT

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Insights into Leukemogenesis from Therapy-Related Leukemia

Jens Pedersen-Bjergaard, M.D., Ph.D.

Therapy-related acute myeloid leukemia (AML), often presenting as therapy-related myelodysplasia, is the most serious long-term complication of cancer chemotherapy. This disease offers a unique opportunity to study leukemogenesis by relating specific cytogenetic and genetic abnormalities to the biologic effects of cytostatic agents. Two types of drugs, alkylating agents and topoisomerase II inhibitors, have been shown to induce therapy-related leukemia.

High risks of therapy-related myelodysplasia and AML were first reported in patients with multiple myeloma who had been treated with melphalan. Subsequently, almost all other alkylating agents in clinical use have been shown to be leukemogenic in patients treated for a wide spectrum of diseases (Table 1). The actuarial risk of therapy-related AML varied from study to study, but an increase in the risk of AML of 0.25 to 1.0 percent per year, beginning two years after the start of chemotherapy and lasting five to seven years after its cessation, was generally observed. The risk was dose-dependent and increased exponentially with age after the age of 40 years, paralleling the risk of primary AML in the general population. Differences in the ages of the patients and in the cumulative dose of alkylating agents can explain the wide variations in risk from study to study.

In addition to alkylating agents, topoisomerase II inhibitors have been shown to be leukemogenic, as first demonstrated in children who received epipodophyllotoxins for acute lymphoblastic leukemia. Subsequently, the anthracyclines, mitoxantrone, and the diospiroperazine derivatives razoxane and bimolane, all of which also inhibit topoisomerase II, were found to be associated with AML after...
Table 1. Diseases and Drugs with a Reported Risk of Treatment-Related Myelodysplasia or AML.

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<tr>
<th>Hematologic neoplasias</th>
<th>Alkylation agents</th>
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<td>Multiple myeloma</td>
<td>Melphalan</td>
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<td>Hodgkin’s disease</td>
<td>Mechlorethamine</td>
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<td>Non-Hodgkin’s lymphoma</td>
<td>Cyclophosphamide</td>
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<td>Polycythemia vera</td>
<td>Busulfan</td>
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<td>Essential thrombocytopenia</td>
<td>Dihydroxybusulfan</td>
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<td>Acute lymphoblastic leukemia</td>
<td>Chlorambucil</td>
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<td>Gastrointestinal cancer</td>
<td>Carmustine</td>
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<td>Testicular cancer</td>
<td>Dacarbazine</td>
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<tr>
<td>Gastrointestinal cancer</td>
<td>Cisplatin</td>
</tr>
<tr>
<td>Childhood tumors</td>
<td>Carboplatin</td>
</tr>
<tr>
<td>Nonmalignant diseases</td>
<td>Topoisomerase II inhibitors</td>
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<td>Rheumatoid arthritis</td>
<td>Etoposide</td>
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<tr>
<td>Psoriasis</td>
<td>Teniposide</td>
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<tr>
<td>Wegener’s granulomatosis</td>
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<td>Bimolane</td>
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The chromosomal abnormalities of therapy-related myelodysplasia and AML characterize at least eight genetic pathways. Cases with the abnormality 7q−/−7 but normal chromosomes 5, belong to the first pathway, and cases with 5q−/−5, with or without abnormalities of chromosome 7, belong to the second pathway; hematopoietic cells from these two subtypes have different gene-expression profiles. The abnormal 5q−/−5 karyotype is closely associated with a point mutation of the p53 gene, and in such cases, there are many additional chromosomal abnormalities and highly rearranged chromosomes, sometimes with gene amplification. The abnormality 7q−/−7, by contrast, is associated with a less complicated karyotype, and a point mutation of the AML1 gene is common. Despite considerable research, the critical molecular events underlying 5q−/−5 and 7q−/−7 have not been identified.

Balanced chromosomal aberrations involving the MLL, AML1/ CBFB, RARA, or NUP98 genes characterize four other genetic pathways of therapy-related myelodysplasia and AML. As in primary AML, these abnormalities result in rearrangements between genes coding for hematopoietic transcription factors and their various partner genes. The result is a loss of function and the development of oncogenes.

About 1 m of double-stranded DNA is packed into the nucleus of a normal cell in a form called supercoiled DNA. To make this intricate tangle accessible to transcription factors and other DNA-binding proteins, supercoiled DNA must be unwound and unknotted by topoisomerases. Topoisomerase II is an ATP-dependent enzyme that cuts both strands of supercoiled DNA, making double-stranded breaks and thereby changing the topology of DNA (Fig. 1). The breaks are ultimately repaired when homologous chromosome fragments realign.

An important question is whether the balanced chromosomal aberrations of primary AML develop when topoisomerase II–induced double-stranded breaks are incorrectly repaired by crossover recombination — illegitimate joints made between two unrelated (nonhomologous) chromosomes. A related question is whether treatment with topoisomerase II inhibitors, which interfere with ligation of the enzyme-induced breaks in DNA, enhances this mechanism of inducing chromosomal abnormalities.

Answers to these questions have been sought by examining the genomic breakpoints of the balanced...
chromosomal aberrations. The breakpoints for translocations to chromosome band 11q23 with rearrangements of the MLL gene,7-9 the t(8;21) with rearrangement between the AML1 and the ETO genes,10 and the t(11;20) with rearrangement between the NUP98 and the TOP1 genes11 have been examined, but no specific motifs in nucleic acid sequences were identified at these junctions. However, all studies showed that the breakpoints of the involved genes correlated with cleavage sites in DNA for topoisomerase II.7-11 In two of these studies, the cleavage sites colocalized with DNase-hypersensitive sites that have an open chromatin structure.9,10

In this issue of the Journal, Mistry et al. report on studies of acute promyelocytic leukemia (APL) characterized by t(15;17), which rearranges the PML and RARA genes. The studies included cases of APL that were related to treatment with mitoxantrone or other agents and primary cases of APL.12 With the addition of the study by Mistry et al., all four genetic pathways with balanced chromosomal aberrations have been investigated. The researchers elegantly demonstrated microhomologies at the translocation breakpoints in mitoxantrone-related APL,12 which indicates that nonhomologous end-joining of the broken DNA strands occurred (Fig. 1E). In therapy-related AML involving translocations to 11q23, the breakpoints within the MLL gene cluster differently from those of primary AML.9 Likewise, in mitoxantrone-related APL, Mistry et al. observed a clustering of breakpoints within a short 8-bp region of intron 6 of the PML gene, whereas the breakpoints for other therapy-related cases of APL and cases of primary APL were more dispersed within the intron.12 The specific 8-bp re-
region of PML was a particular “hot spot” for mitoxantrone-induced topoisomerase II cleavage.

The development of balanced chromosomal aberrations in AML has turned out to be a complicated process. Despite the results of the new studies, many questions remain. Is topoisomerase II inevitably involved? To what extent are regions of the chromosomal scaffold implicated (the scaffold consists of protein fibers that remain after removal of histones)? Do apoptosis-inducing DNases participate? Are the many cases of therapy-related AML with translocations to 11q23 arising in children who received epipodophyllotoxins and the many cases of APL in women who received mitoxantrone for breast cancer the results of a different targeting of DNA by the two drugs, as suggested by Mistry et al., or are they related to other factors — the patient’s age, for instance? After more than 30 years of comprehensive research on the risk of, risk factors for, and pathological and cytogenetic findings in therapy-related myelodysplasia and AML, these diseases now provide new insights into the molecular biology of myelodysplasia and AML in general.

From the Cytogenetics Laboratory, Rigshospitalet, Copenhagen.

7. Stanulla M, Wang J, Chervinsky DS, Thandla S, Aplan PD. DNA cleavage within the MLL breakpoint cluster region is a specific event which occurs as part of higher-order chromatin fragmentation during initial stages of apoptosis. Mol Cell Biol 1997;17:4070-9.

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**Resynchronizing Ventricular Contraction in Heart Failure**

John A. Jarcho, M.D.

One quarter to one third of patients with congestive heart failure have some form of intraventricular conduction abnormality that is manifested as an increased QRS duration on the electrocardiogram. The most common pattern is left bundle-branch block. In patients with left bundle-branch block, electrical activation of the lateral aspect of the left ventricle can be substantially delayed in relation to that of the right ventricle and interventricular septum. Dyssynchronous electrical activation results in dyssynchronous contraction, which is mechanically inefficient. Regional myocardial workload is distributed unequally, and regional myocardial blood flow and metabolism may be altered. As a result, the left ventricular ejection fraction and cardiac output decrease, and congestive heart failure becomes more severe. A similar mechanical dyssynchrony also occurs in some patients with right bundle-branch block or nonspecific intraventricular conduction delay.

The idea that optimal timing of left and right ventricular contraction with respect to one another might be of fundamental clinical importance arose both from evidence that intraventricular conduction abnormalities are associated with a worse prognosis in patients with congestive heart failure and from studies showing that conventional right ventricular pacing, which creates an artificially induced intraventricular conduction delay, actually impairs ventricular function, even in patients with-
out congestive heart failure. These and similar observations led to trials of pacing techniques to synchronize ventricular contraction.

The use of cardiac pacing to coordinate the contraction of the left and right ventricles is called cardiac-resynchronization therapy (see Video Clips 1 and 2 of the Supplementary Appendix, available with the full text of this article at www.nejm.org). In most patients, this technique requires the simultaneous pacing of both ventricles (i.e., biventricular pacing). A right ventricular pacing lead is inserted in a standard fashion. Pacing of the left ventricle, however, is more problematic. The approach that is conventionally used involves inserting the left ventricular lead into the mouth of the coronary sinus (in the right atrium) and advancing it posteriorly around the atrioventricular valve ring, toward the left ventricle (Fig. 1). The lead is then passed into a venous branch running along the free wall of the left ventricle. Echocardiography can be used to guide device programming to achieve optimal filling and forward output.

Biventricular-pacemaker implantation is technically more demanding than implantation of a conventional, dual-chamber pacemaker, and complications of insertion are more frequent. The most common problem is inability to implant the left ventricular lead successfully, usually because of unfavorable coronary venous anatomy. Proximity to the left phrenic nerve, and the resulting uncomfortable diaphragmatic stimulation during pacing, limits the acceptable pacing sites in some patients. In addition, subsequent lead dislodgement occurs in as many as 10 percent of patients in whom implantation is initially successful. Other complications include coronary-sinus dissection and cardiac perforation. Serious sequelae of these complications are infrequent, but given the current state of the art, patients are best served if implantation is performed by an electrophysiologist with considerable experience in the technique.

Many patients who are appropriate candidates for cardiac-resynchronization therapy are also candidates for implantation of a cardioverter–defibrillator. Initially, there was the technical concern that, in a patient receiving both devices, interactions could occur, leading to unintended defibrillator firing. This issue was resolved by the development of integrated devices capable of performing both functions.

Initial randomized trials of cardiac-resynchronization therapy demonstrated improvements in ventricular function, exercise tolerance, and quality of life, as well as a reduction in the frequency of hospitalizations. The subsequent Comparison of Medical Therapy, Pacing, and Defibrillation in Heart Failure (COMPANION) trial, a larger and longer study, also evaluated the effect of cardiac-resynchronization therapy, with or without an implantable cardioverter–defibrillator, on survival.

Figure 1. Biventricular Pacing for Cardiac Resynchronization Therapy.
The right atrial and right ventricular pacing leads are inserted in the standard fashion. The left ventricular pacing lead is inserted into the coronary sinus and advanced into a cardiac vein on the lateral wall of the left ventricle. Because the coronary venous anatomy is variable, the location and accessibility of a suitable vein differ from patient to patient.
Several areas of uncertainty remain. It has not been established whether cardiac-resynchronization therapy is beneficial in patients with mild congestive heart failure (NYHA class II). It is also not clear whether an increased QRS duration is the best criterion for benefit from cardiac-resynchronization therapy. Some uncontrolled studies have suggested that echocardiographic evidence of ventricular dyssynchrony, which does not always correlate precisely with electrocardiographic findings, is a more reliable determinant of efficacy. The benefit of cardiac resynchronization in patients with atrial fibrillation is also still under study. Finally, cardiac-resynchronization therapy does not benefit all recipients, for reasons that are not always clear; in some cases, unsuccessful lead positioning may be responsible, whereas in others, the severity of dyssynchrony before the procedure may have been overestimated.

The authors of the CARE-HF report raise an interesting question about the benefit of an implantable cardioverter–defibrillator in the setting of cardiac-resynchronization therapy. They note that the survival benefit in the COMPANION trial in the group that received cardiac-resynchronization therapy along with a cardioverter–defibrillator (hazard ratio for death, 0.64) was similar to the benefit in the CARE-HF study with cardiac resynchronization alone (hazard ratio, 0.64) and not markedly different from the benefit in COMPANION with cardiac resynchronization alone (hazard ratio, 0.76). Although implantation of a cardioverter–defibrillator might have further reduced mortality in CARE-HF, only 7 percent of the patients in the cardiac-resynchronization group died suddenly.

Cleland et al. make the provocative suggestion that “retarding the progression of cardiac dysfunction to prevent malignant arrhythmias may be a better strategy than treating malignant arrhythmias once they occur” and offer a calculation for the size and duration of the trial necessary to prove an incremental benefit with implantable cardioverter–defibrillator implantation in addition to cardiac-resynchronization therapy. This issue is worthy of discussion because implantable cardioverter–defibrillators are larger and substantially more expensive than pacemakers and because they introduce the potential for inappropriate shocks from supraventricular tachycardias or device malfunction.

However, it seems unlikely that a definitive trial comparing biventricular pacing with and without
use of an implantable cardioverter–defibrillator will be performed, and it is more likely that combination-device therapy will become the preferred intervention. After all, sudden deaths accounted for 35 percent of all deaths in the cardiac-resynchronization group in CARE-HF; some, at least, of those 29 deaths would most likely have been prevented with a defibrillator.

In addition, it is noteworthy that the beneficial effect of cardiac-resynchronization therapy on mortality is gradual and probably due to the effects of reverse ventricular remodeling, whereas the benefits of defibrillator therapy are immediate. This difference is suggested by detailed examination of the survival curves in the COMPANION trial: the curves for pharmacologic therapy alone and for cardiac-resynchronization therapy do not begin to diverge until about eight months, whereas the curve for cardiac resynchronization plus implantation of a cardioverter–defibrillator begins to diverge much earlier. Waiting for the protective effect of cardiac-resynchronization therapy to “kick in,” rather than implanting a cardioverter–defibrillator at the outset, will probably not be a very attractive option for most patients. The data from CARE-HF do provide support for the implantation of a pacing device alone in those patients in NYHA class IV who do not desire an implantable cardioverter–defibrillator but who do desire a treatment to improve their quality of life without the risk of painful shocks from a cardioverter–defibrillator.

The issue of cost-effectiveness is not addressed in CARE-HF, but modeling studies suggest that cost-effectiveness is highly dependent on the patient population selected.14 As with studies of implantable cardioverter–defibrillators, trials demonstrating a benefit with cardiac-resynchronization therapy have led to the establishment in the United States of carefully crafted criteria for reimbursement from the Centers for Medicare and Medicaid Services. These criteria tend to become the de facto criteria for use of the device and are therefore the target of intense scrutiny by electrophysiologists, device manufacturers, and government accountants. In an era of resource limitation, it is the payer who ultimately decides how an expensive therapy will be used clinically. Careful clinical trials such as CARE-HF are physicians’ best guarantee that such decisions will be made on the basis of sound data.
Listening to Genetic Background Noise

Joseph H. Nadeau, Ph.D.

Since the early days of modern genetics, researchers have largely shut their ears to the “background noise” of genetic modifiers that modulate the expression of mendelian traits. Because modifier genes complicate regular patterns of inheritance and because their identification can be difficult, their importance has been recognized, but they have rarely been the focus of genetic studies. However, attention is beginning to focus on these genetic background effects, in part because they are the entry into the genetics and systems biology of more complex and common diseases, and in part because they have great potential as powerful and effective ways to treat and perhaps prevent disease.

Keeping things simple was the key to discovering the rules of inheritance. With insight and luck, Mendel carefully selected traits, emphasizing those that showed a virtually invariant phenotype. His crosses provided segregation ratios for these traits, from which the fundamental and universal rules of inheritance were inferred. Mendel’s landmark discovery would have been impossible if he, like others before him, had selected traits that show more complex patterns of inheritance. And yet, remarkably few traits are truly mendelian.

Most traits vary, sometimes in simple ways and sometimes in profound ways. Perhaps the most important discovery from the study of spontaneous, engineered, and chemically induced genetic variants in model organisms is the fact that their phenotypes depend strongly on the genetic background. For example, a specific genetic variant may lead to embryonic lethality on some genetic backgrounds but to full viability and no obvious phenotype on other backgrounds. Background genes that can suppress or exacerbate detrimental phenotypes are ubiquitous and highly polymorphic. They are easiest to detect when a single genetic variant is the target of modification, but they are probably involved in both genetically simple and complex traits.

In general, phenotypic noise may result from allelic heterogeneity, variable environments, or stochastic effects, as well as from modifier genes. In humans, distinguishing among these sources of variability can be difficult. With model organisms, however, defined crosses can be made, so detecting modifiers is relatively easy. Moreover, because the repertoire of genes in humans is extraordinarily similar to that in other mammals and because the chromosomal arrangement of these genes has been strongly conserved during mammalian evolution, the identity and location of candidate modifier genes in humans can be reliably predicted from studies in model organisms.

Modifier genes are now leading to pioneering discoveries about the genetics and biology of hearing. In a study in this issue of the Journal, Schultz et al.1 show that five members of a family with autosomal recessive sensorineural hearing loss were homozygous for a missense mutation at a conserved site in CDH23, the gene that encodes cadherin 23, which mediates calcium-dependent cell-to-cell interactions. Mutations in this gene have previously been shown to cause a similar form of hearing loss in waltzer mice.2 The authors also note that three of the five affected persons had severe-to-profound hearing loss, whereas the other two had only high-frequency sensorineural hearing loss. This variability suggested the action of a modifier gene. Again, studies in mice pointed the way. Variants of the Atp2b2 gene, which encodes a calcium pump in the plasma membrane, modulate the severity of hearing loss in waltzer mice. Schultz et al. report that heterozygosity for a missense mutation in ATP2B2, the human homologue of Atp2b2, was associated with the severity of hearing loss in these five persons. During the study, they speculated that variation in the activity of the calcium pump influences cellular interactions that depend on calcium availability. They then tested whether this ATP2B2 variant modifies other forms of hearing loss and found that it affected hearing loss resulting from other genetic causes. Interestingly, heterozygosity for the ATP2B2 variant was not sufficient to cause hearing loss — an observation that is typical of most modifier genes: in the absence of their target gene variants, they generally do not have detectable effects on the trait. These discoveries about the genetic basis of sensorineural hearing loss in humans would have been much more difficult had there not been corresponding discoveries in mice to guide the way. This cross-talk between studies in humans and those in model organisms is probably essential for rapid progress.

Modifier genes complicate the genetics of sim-
ple traits and simplify the genetics of complex traits. Genetic variants differ in the magnitude of their effects (a phenomenon called “effect size”). Effect sizes range from simple mendelian traits, in which differences at a single gene account for all the phenotypic variation in a trait, to the other extreme, where a large number of genes with individually small effects control phenotypic variability. Modifier genes reduce the effects attributable to the mendelian variant that is the target of modification and increase those attributable to the modifier gene. As the number of modifier genes and the cumulative magnitude of their influence increase, the effect size of a single gene diminishes and genetic complexity increases. But just as they reduce the effects of genes associated with simple traits, modifier genes increase the influence of genes with subtle effects. In mouse models of diseases such as testicular cancer, modifier genes have been used to magnify genetic effects, rendering them amenable to analysis.

The considerable polymorphism of modifier genes is puzzling. If modifier effects are beneficial, why are they polymorphic, rather than fixed, such that all the members of a population might have the advantage of their beneficial effects? Presumably, the myriad ways that modifiers interact with many genetic variants mean that no single variant is universally beneficial. Instead, their polymorphism probably reflects the ongoing response of organisms to diverse genetic and environmental perturbations during the lifetime of an individual and the lifetime of the species. In other words, polymorphic challenges require polymorphic responses.

Modifier genes are evidence of the adaptive response of organisms to genetic and environmental perturbations. Every organism carries new and inherited mutations that adversely affect viability and fertility. They also encounter adverse environments that require adaptive responses. Organisms therefore face selective pressure to mitigate these deleterious effects. During evolution, individual organisms with new genetic variants that reduce or suppress detrimental effects will have selective advantages over those that do not have these modifier effects. With time, complex networks of functional interactions evolve to buffer individuals from both deleterious mutations and environmental perturbations. These networks provide genetic buffering and homeostasis, both of which are the focus of research on the systems biology of health and disease.

Ongoing discussions about the safety of several widely used drugs reflect the fact that developing effective and safe treatments for disease is profoundly difficult. Modifier genes represent a potentially powerful alternative. Their occurrence in healthy persons demonstrates their apparent safety; their ability to mute or suppress disease is evidence of their efficacy. Perhaps, once their mechanisms of action are understood, their benefits can be harnessed to prevent or treat disease. Perhaps the background noise of modifier genes can begin to silence the adverse effects of genes that cause disease.

From the Department of Genetics and the Center for Computational Genomics and Systems Biology, Case Western Reserve University School of Medicine, Cleveland.


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Malaria is a disease with far-reaching effects: 40 percent of the world’s population is at risk for malarial infection, approximately 300 million to 400 million cases occur annually, and each year, 1 million to 2 million people — predominantly young children — die of malaria. And yet there are no vaccines for parasitic diseases that affect humans, let alone a vaccine for malaria.

Sporozoites, the infectious stage of the malaria parasite *Plasmodium*, are small, slender, unicellular organisms that mature in the salivary glands of anopheline mosquitoes. Exposure to radiation-attenuated sporozoites, however, can elicit a cellular and antibody-mediated immune response sufficient to prevent malarial infection in humans (referred to as sterile immunity — i.e., total absence of the blood-stage parasites responsible for clinical disease). Volunteers immunized with radiation-attenuated sporozoites of *Plasmodium falciparum* and *P. vivax*, delivered by the bites of mosquitoes, were protected against infection when challenged with viable sporozoites. But there are logistical and technical obstacles to using sporozoites as vaccines, such as the need to attenuate infectivity by means of irradiation. Mueller and colleagues recently showed that genetically modified sporozoites induce complete immunity in a mouse model of malaria, thus overcoming these obstacles.

Having previously identified plasmodium antigens unique to the parasites’ preerythrocytic stage, the authors engineered *P. berghei* parasites lacking the *uis3* (up-regulated in infective sporozoites) gene. All mice immunized with multiple doses of *uis3*– sporozoites were fully protected against malaria when challenged with an intravenous injection of wild-type sporozoites or by the bites of infected mosquitoes. Just as important was the finding that the *uis3*– sporozoites elicited immunity when injected subcutaneously — a route acceptable for the delivery of vaccines in humans. Moreover, attenuated, whole-organism vaccines do not require exogenous adjuvants. The limited repertoire of adjuvants acceptable for human use impedes the development of “subunit” vaccines, in which single proteins or domains of proteins are the vaccinating agent.

The ability to elicit sterile immunity to a eukaryotic parasite such as *Plasmodium* is remarkable, given the complex life cycle of the parasite and the unique journey that sporozoites must take before reaching the liver, their initial site of replication in the host. While probing the skin in search of a blood meal, plasmodium-infected mosquitoes introduce saliva containing a small number of sporozoites into the subcutaneous tissue. The parasites leave the bite site, penetrate a capillary, and travel through the bloodstream to the liver.

**Figure 1 (facing page). Life Cycle of Plasmodium in the Liver and Blood.**

Malaria sporozoites enter a liver lobule through either the portal vein or the hepatic artery and glide along the sinusoidal cell layer by binding to extracellular-matrix proteoglycans secreted by stellate cells. Liver sinusoids are lined by endothelial cells containing fenestrations that allow substances destined for removal and detoxification by the liver access to hepatocytes. Interspersed in the layer of sinusoidal endothelia are Kupffer’s cells, resident macrophages of the liver, which are responsible for the clearance of foreign particles from the blood. Sporozoites use Kupffer’s cells to gain access to hepatocytes (Panel A). The sporozoites then migrate through several hepatocytes before settling into one and undergoing further development (Panels B and C). After gradually growing larger than their host cell during the liver stage of infection (Panels D and E), the parasites differentiate into thousands of merozoites (Panel F), which are released into the circulating blood (Panel G), where they rapidly infect red cells (Panel H). Maturation of the blood-stage schizonts, resulting in the periodic amplification and release of merozoites from red cells, leads to clinical malaria. Using a mouse model, Mueller et al. recently showed that the *uis3* gene, which is expressed by sporozoites and early liver stages, is critical to the development and maturation of the parasite in the liver. Unable to differentiate into merozoites (red bar between Panels B and C), *uis3*– parasites act as a vaccine and elicit immunity. Similarly, radiation-attenuated sporozoites also fail to produce merozoites and generate sterile immunity.
Clinical Implications of Basic Research

Sporozoites traverse Kupffer's cells and migrate through several hepatocytes before developing into developing forms.

Liver stage

Immunization with UIS3- sporozoites and radiation-attenuated sporozoites

Blood stage

Schizonts

Merozoites released into the circulation

Rapid infection of red cells followed by periodic amplification and release of merozoites leads to malaria.
sinusoid, sporozoites are thought to recognize extracellular-matrix proteoglycans that protrude into the sinusoidal lumen and bind sporozoite surface proteins, including one called the circumsporozoite protein. The parasites then glide along the sinusoidal cell layer until they encounter a Kupffer’s cell (a type of macrophage specific to the liver), which they invade, and thus gain access to hepatocytes (Fig. 1). Once inside the liver tissue, sporozoites pass through several hepatocytes, fatally wounding them in the process before finally settling down in a single hepatocyte to mature and then differentiate into thousands of merozoites, which are capable of infecting red cells.

Radiation-attenuated sporozoites and uis3− sporozoites are able to infect the liver but cannot undergo nuclear division. Consequently, maturation and differentiation into the merozoite stage are blocked, and the infection fails to proceed to clinical malaria. Studies of hosts immunized with radiation-attenuated sporozoites have shown that antibodies against a circumsporozoite protein are elicited. After the host has been exposed to the bite of an infected mosquito, these antibodies inhibit the motility of sporozoites, immobilizing the parasites in the skin, blocking liver infection, and enhancing opsonization and phagocytosis of the sporozoites. If sporozoites evade such antibodies, however, they can invade hepatocytes, where humoral immunity is no longer effective, but the circumsporozoite protein and other newly expressed liver-stage antigens provide targets for protective CD4+ and CD8+ T-cell–mediated immunity.

Mueller et al. exploited the P. berghei genome to construct a genetically modified parasite that undergoes normal sporozoite development but abortive hepatic-stage development.3 In so doing, they provide a means of delivering preerythrocytic parasite antigens in their native configuration. Although this particular example of reverse genetics has advanced hopes that an attenuated-sporozoite vaccine may soon be developed, important challenges remain. Critical questions regarding the longevity and mechanism of immunity induced by genetically attenuated uis3− parasites must be answered. Other challenges include the preparation of sterile parasites free of mosquito debris (currently, sporozoites are obtained by dissecting the salivary glands of infected mosquitoes), the development of efficient cryopreservation protocols, and the route of immunization. In the meantime, study of the uis3− parasites may help to identify antigens expressed in the early stages of liver infection that are critical to the development of cell-mediated immunity — thereby speeding the design of subunit vaccines, which are more amenable to mass production.

From the Department of Medical and Molecular Parasitology, New York University School of Medicine, New York.


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C-Reactive Protein Levels and Outcomes after Statin Therapy

TO THE EDITOR: Ridker and colleagues (Jan. 6 issue)\textsuperscript{1} suggest that statin therapy be targeted to achieve a C-reactive protein (CRP) level of less than 2.0 mg per liter in patients with a recent acute coronary syndrome. Having examined the data presented in Table 1 of the article, we question this conclusion. After adjustment for known cardiac risk factors, the relative risk of recurrent coronary events was appreciably increased only in patients whose CRP level was in the highest quartile (>4.2 mg per liter). Patients whose CRP level was in the second or third quartile (0.9 to 4.2 mg per liter) had only moderate increases in relative risk, and after adjustment for other risk factors, those increases were not statistically significant. Although the authors found that event rates differed between patients with a CRP level of 2.0 mg per liter or greater and those with a level below 2.0 mg per liter, this difference was probably driven by data from patients with CRP levels in the highest quartile. Lowering CRP levels that are in the highest quartile may be beneficial. However, randomized clinical trials are needed to address this question. Until such trials have been conducted, we believe that it is premature to recommend aggressive use of statins to reduce CRP levels.

Beth Cohen, M.D.
David Singh, M.D.
University of California, San Francisco
San Francisco, CA 94143


TO THE EDITOR: The finding that high levels of CRP are associated with a poorer outcome than low CRP levels after an acute coronary syndrome is probably due to inadequate statistical adjustment for important confounding variables. According to data from Kinjo et al.,\textsuperscript{1} high CRP levels measured 25 days after an acute coronary syndrome were associated with poorer long-term outcomes than were low CRP levels, as well as with important prognostic factors that Ridker et al. did not consider in their “fully adjusted model.” These critical covariates include classic prognostic factors after an acute coronary syndrome, such as higher Killip class, a higher peak level of creatine kinase, and the use or nonuse of revascularization during the index hospitalization.\textsuperscript{1} These are true confounding variables — associated with both exposure (i.e., CRP levels) and outcome.\textsuperscript{2} Harb et al. demonstrated that the association between a high CRP level and poor outcomes after myocardial infarction was attenuated and became nonsignificant after adjustment for similar confounders, including ejection fraction.\textsuperscript{3} Thus, the weak CRP association observed (the lower level of the confidence interval for the relative risk in the highest CRP quartile was only 1.1 in the “fully-adjusted model”) probably represents nothing more than residual confounding due to important prog-

nistic factors associated with CRP in patients with an acute coronary syndrome.

Philip Greenland, M.D.
Donald M. Lloyd-Jones, M.D.
Northwestern University Feinberg School of Medicine
Chicago, IL 60611

Arthur J. Moss, M.D.
University of Rochester School of Medicine and Dentistry
Rochester, NY 14642

TO THE EDITOR: Ridker et al. demonstrated that patients with acute coronary syndromes in whom low CRP levels are achieved after statin therapy have a decreased risk of recurrent myocardial infarction or death, regardless of the levels of low-density lipoprotein (LDL) cholesterol attained. The observed benefit was largely attributed to the antiinflammatory effects of statins, as assessed by the inflammatory biomarker CRP, thus adding to the lipid-independent, pleiotropic properties of these agents. This interpretation notwithstanding, recent work provides evidence of a direct inhibitory effect of statins on CRP biosynthesis by human hepatocytes.1 Statins also up-regulate the production of nitric oxide synthase,2 which is expressed in hepatocytes; boosting hepatic nitric oxide production might, in turn, suppress CRP synthesis.3

The nature of the CRP reduction observed after statin therapy merits some scrutiny in light of these new findings. A complementary analytical approach would be to measure other hepatic positive acute-phase reactants (e.g., ferritin) and negative acute-phase reactants (e.g., albumin), as well as more proximal inflammatory biomarkers (e.g., interleukin-6) to help decipher the direct and indirect modulatory effect of statins on the hepatic synthesis of CRP.

Bertrand L. Jaber, M.D.
Nicolaos E. Madias, M.D.
Caritas St. Elizabeth’s Medical Center
Boston, MA 02135
bertrand_jaber@cchcs.org


THE AUTHORS REPLY: Drs. Jaber and Madias correctly note that multiple mechanisms have been proposed for the statin-lowering effect of CRP, and they seek information regarding proximal inflammatory markers. In this regard, our data and those of others have indicated that statins lower CRP levels but do not consistently reduce levels of interleukin-6.1

Drs. Cohen and Singh interpret our data to suggest that only patients with the highest CRP levels benefited from statin therapy. However, as shown in Table 1 of our article, we observed a linear relationship between inflammation and hard clinical outcomes across the full spectrum of CRP levels—a finding compatible with data from studies of primary prevention.2 Furthermore, as shown in Table 2, additional benefits were observed among patients who not only had LDL cholesterol levels below 70 mg per liter but who also had CRP levels that were reduced to less than 1 mg per liter.

Dr. Greenland and colleagues speculate that the strong relationship in our data between CRP levels measured at 30 days and subsequent vascular events might be due to residual confounding by the Killip class, peak creatine kinase level, and use or nonuse of early revascularization. In our study, we reported a relative risk of 1.8 for patients with a CRP level in the top quartile in a multivariate analysis adjusted for sex, smoking status, the presence or absence of diabetes, the presence or absence of a history of hypertension, body-mass index, achieved LDL cholesterol level, and assignment to antibiotic therapy or placebo (95 percent confidence interval, 1.2 to 2.7; P=0.003). After additional adjustment for the Killip class, peak level of creatine kinase, and use or nonuse of early revascularization, the relative risk was 1.9 (95 percent confidence interval, 1.3 to 2.9; P=0.004).

We cannot agree with Dr. Greenland and colleagues that the data reported by Kinjo et al. support a role of residual confounding, when that study

showed a statistically significant, ninefold increase in the risk of death from cardiovascular causes among patients in stable condition after a myocardial infarction who had elevated levels of CRP, even after adjustment for usual risk factors and for use or nonuse of revascularization, Killip class, creatinine kinase level, infarct location, and use or nonuse of adjunctive medical therapies. Moreover, in their discussion of the THROMBO study, in which CRP levels were a univariate but not multivariate predictor of recurrent events, Dr. Greenland and colleagues do not point out that in the same data set, the LDL cholesterol level was not predictive at all, even in univariate analyses. Thus, if it is concluded on the basis of the THROMBO study that CRP levels after myocardial infarction have little clinical utility, then it must also be concluded that there is no utility in measuring LDL cholesterol, a position at odds with all current recommendations and one we do not accept.

Paul M Ridker, M.D.
Christopher P. Cannon, M.D.
Eugene Braunwald, M.D.
Brigham and Women’s Hospital
Boston, MA 02115
pridker@partners.org


Molecular Prediction of Recurrence of Breast Cancer

TO THE EDITOR: Paik et al. (Dec. 30 issue) imply that their multigene assay provides more objective and reproducible information than an assigned tumor grade. Any RNA-based gene-expression profile of neoplasms is heavily dependent on tumor cellularity. Moreover, different areas of the tumor may be expected to show different gene-expression patterns. Therefore, selection of the area to be measured becomes a critical issue. Many breast-cancer specimens may be represented in two or more paraffin blocks, and it is unknown how the recurrence score in the current study would have been affected by the choice of block. The authors studied within-patient variability in only two cases. It was not stated how these two cases were chosen and what their mean recurrence scores were. The only number provided is the standard deviation of 2.2 recurrence-score units, and it is not clear whether this represents the average of the two cases. The reported standard deviation may be acceptable if the recurrence score was high but may not be acceptable if the latter was low. A much larger body of data on intratumoral variability in the recurrence score should be made available.

Joseph Geradts, M.D.
Roswell Park Cancer Institute
Buffalo, NY 14263
joseph.geradts@roswellpark.org


TO THE EDITOR: Paik et al. report that their multigene assay “provided significant information beyond tumor grade.” However, they beg the question of possible bias in how they designed the comparison of their assay results to tumor grade by taking the unusual step of stating the institutional affiliations of the pathologists who determined tumor grade, rather than documenting the pathologists’ expertise. Shak, a coauthor of the article, has asserted that the choice of pathologists is irrelevant, since he and his colleagues intended to compare their assay results to tumor grade as it might be determined in the “community,” but this seems a disingenuous way to allow bias. Since they advocate submission of tissue to their reference laboratory at Genomic Health, the proper test is to compare...
their assay results to tumor grade determined by pathologists with special expertise in breast disease (i.e., those in pathology reference laboratories). To allow readers to evaluate for themselves whether bias existed in the comparison of the multigene assay results to tumor grade, the authors should document the three pathologists’ experience in breast pathology at the inception of the study.

William H. Goodson III, M.D.
California Pacific Medical Center Research Institute
San Francisco, CA 94115


TO THE EDITOR: The article by Paik et al. raises two concerns. First, the practical implications of the test results are unclear. If tamoxifen will be given regardless of the recurrence score, the additional cost of testing would be an enormous burden to health care systems. Second, the 21-gene panel has no prognostic value for patients who do not receive tamoxifen.1 Since Paik et al. show that the assay had prognostic value only in tamoxifen-treated patients, it is unclear whether tamoxifen lowered the risk of recurrence for patients in the low-risk group, or whether there was a bias against high-risk patients in the tamoxifen-treated group.

Christopher G. Tang, B.A.
Albert Y. Lin, M.D.
Santa Clara Valley Medical Center
San Jose, CA 95128
albert.lin@hhs.co.santa-clara.ca.us


TO THE EDITOR: In their editorial, Bast and Hortobagyi1 suggest that the biomarker test described by Paik et al. allows precise prediction of risk and could spare patients with breast cancer the toxicity of chemotherapy. However, in my experience, the test has often been used for the opposite purpose. Many patients with breast cancer are determined to take aggressive treatment even for a minuscule reduction in the risk of recurrence.2-4 Women with a small tumor (<1 cm in diameter), who generally do not receive adjuvant chemotherapy, are now undergoing this test to find a justification for chemotherapy. Others, who are already undergoing chemotherapy, receive a high-risk score and on that basis are being treated more intensively than previously planned.

It is important to address the cost of this method, which is about $3,500 per test. According to Paik et al., 22 percent of women with breast cancer will have an intermediate score, which does not help with treatment decisions. Therefore, it appears that, in reality, this test may put additional pressure on women to get more treatment and may result in an increase in the cost of health care.

Tawee Tanvetyanon, M.D.
Loyola University Chicago Stritch School of Medicine
Maywood, IL 60153
ttanve@lumc.edu


THE AUTHORS REPLY: We agree with Dr. Geradts that selection of tissue for RNA analysis and variability between blocks are important considerations. Of the 668 cases in the published study, macrodissection based on prespecified criteria was performed to exclude normal tissue in 130 cases (19.5 percent). In addition to the reported variability between blocks in two patients, we have additional data in 36 patients. In 16 cases with macrodissection in the National Surgical Adjuvant Breast and Bowel Project (NSABP) trial B-20, the RNA concentration was 8.9 times as high in the enriched tumor region as in the normal region. The median difference between the whole section and the region enriched in tumor tissue in these macrodissected cases was 3.2 recurrence-score units. In 20 additional patients, we estimated the variability in recurrence scores among 60 blocks without macrodissection. Even with a varying degree of relative tumor volume, the standard deviation in the recurrence score between blocks from the same patient was 3.0 recurrence-score units. For 16 of the 20 patients, the standard deviation between blocks was less than 2.5 recurrence-score units. The variability in measurement of the recurrence score is less than the
known large variability among readers in the subjective assessment of tumor grade.\textsuperscript{1} When the recurrence score is used as a continuous function, consideration of variability should have less effect on the interpretation of the result.

We disagree with Dr. Tanvetyanon’s opinion that there is no value in an intermediate recurrence score. For patients with an intermediate recurrence score, the test indicates that they are not at the highest or at the lowest risk of distant recurrence. Moreover, the likelihood of recurrence for a tumor with a recurrence score of 19 is different from that for a tumor with a recurrence score of 30, although both are classified as “intermediate” risk. Like any new test, the recurrence score will most likely undergo further optimization over time and become integrated with contributions from clinicopathologic features.

With regard to the comments of Mr. Tang and Dr. Lin, we reiterate that the practical application of the recurrence score is to permit more informed decision making concerning the addition of adjuvant chemotherapy in patients with node-negative, estrogen-receptor–positive breast cancer. An individual tumor can have a low recurrence score and a low risk of distant recurrence because the tumor is less aggressive, responsive to tamoxifen, or both. It is likely that the genes evaluated in the calculation of the recurrence score capture both the prognosis and the responsiveness to tamoxifen.

Finally, we would like to point out that we failed to acknowledge grant support from the National Cancer Institute for the study: 5-U10-CA69651 was awarded to the NSABP Biostatistical Center and grant U10-CA12027-34 to the NSABP Operations Center.

Soonmyung Paik, M.D.
Norman Wolmark, M.D.
National Surgical Adjuvant Breast and Bowel Project
Pittsburgh, PA 15212
spaik.nejm@nsabp.org

Steven Shak, M.D.
Genomic Health
Redwood City, CA 94063


Attention Deficit–Hyperactivity Disorder

\textbf{TO THE EDITOR:} Rappley’s review of attention deficit–hyperactivity disorder (Jan. 13 issue)\textsuperscript{1} omits important diagnoses that can mimic attention-deficit disorders — sleep disorders and convulsive disorders. Increased daytime behavioral problems occur with sleep disorders, and vice versa.\textsuperscript{2-5} Daytime inattention and fidgetiness often result from sleep-disordered breathing (including obstructive sleep apnea and upper airway resistance syndrome) and periodic limb movements of sleep, which may occur in isolation or with the restless legs syndrome. These conditions result in hypoxemia and sleep fragmentation. Narcolepsy can also make a child appear inattentive when he or she has very brief naps. Children with primary generalized absence epilepsy (petit mal seizures) may also appear inattentive because of untreated seizures. Physicians must include detailed questions concerning sleep disorders and epilepsy in the evaluation of children with attentional, academic, and learning problems.

Jay E. Selman, M.D.
Columbia University College of Physicians and Surgeons
New York, NY 10032
jay_selman@yahoo.com

**Prophylaxis against Rabies**

**TO THE EDITOR:** As noted by Rupprecht and Gibbons (Dec. 16 issue), delay before postexposure prophylaxis against rabies is initiated may result in treatment failure and death. The incubation period for rabies in dogs could be much longer than 10 days. Furthermore, in previous studies, the rabies virus was isolated from the saliva and cerebrospinal fluid of many dogs before they had any signs of rabies, and up to 18 percent of the infected dogs died without having shown any signs of illness beforehand. Therefore, management by 10-day observation in many areas where rabies is prevalent might put patients’ lives at risk. According to World Health Organization (WHO) recommendations, all patients with a category 3 exposure (i.e., a transdermal bite or contamination of the mucous membranes with saliva) should receive immune globulin and vaccine immediately, and treatment should be stopped only if the animal remains healthy throughout the 10-day period of observation or is euthanized and found to be negative for rabies by appropriate laboratory tests.

Sathit Kurathong, M.D.
Bangkok Metropolitan Administration Medical College
Bangkok 10300, Thailand
skurathong@hotmail.com


**TO THE EDITOR:** In their review, Rupprecht and Gibbons recognize that rabies immune globulins are in short supply and note that “multisite intradermal vaccination is another possible strategy to accelerate the immune response.” However, the risks associated with not using immune globulins in cases of severe rabies exposure should be emphasized. A similar statement, since withdrawn, from the Thai Health Ministry led to one tragic death. The eight-site method was used without immune globulin in a Thai child with facial dog bites, and the child died of rabies 15 days later. Recent studies have shown that the multisite accelerated method will result in increased antibody titers by day 14.
but not in significantly earlier ones.  This topic was discussed at a 2004 WHO meeting, at which the need for immune globulin in the optimal treatment of severe rabies exposure was reaffirmed.

Henry Wilde, M.D.
Thai Red Cross Society
Bangkok 10330, Thailand

Thiravat Hemachudha, M.D.
Chulalongkorn University
Bangkok 10330, Thailand


TO THE EDITOR: In their review of rabies prophylaxis, Rupprecht and Gibbons mention that multiple intradermal vaccination is an alternative strategy for accelerating the immune response. Because of the high cost of rabies vaccine, the intradermal route is often used in tropical countries. However, in these countries, chloroquine is used regularly. In our view, it is important to mention that weekly oral chloroquine prophylaxis against malaria has been associated with an impaired antibody response to intradermal rabies vaccination. If chloroquine is being used concurrently, intramuscular injections are preferable. If the intradermal route is chosen, chloroquine should not be used.

Erwin Van den Enden, M.D.
Institute of Tropical Medicine
2000 Antwerp, Belgium


THE AUTHORS REPLY: In response to Dr. Kurathong: As we discuss in our review, postexposure prophylaxis against rabies should begin when rabies is considered seriously. In developed countries, where rabies is controlled among domestic animals, suspect dogs are observed and postexposure prophylaxis administered only when there is a significant suspicion of rabies.

It is important to differentiate between the incubation period (i.e., the time between exposure and onset) and the period when transmission is likely. Incubation periods are quite variable, lasting from days to years (average duration, approximately one to three months). In contrast, animals excrete rabies virus for only a few days before obvious illness. The 10-day observation period refers to the suspect biting dog. Postexposure prophylaxis is unnecessary unless the animals sickens, after which euthanasia and laboratory diagnosis should be performed. No documented human cases have occurred when the dog remains normal during this period. If there is an increased prevalence of rabies, as there is in developing countries, postexposure prophylaxis begins after a bite and is discontinued if the animal remains normal or test results are negative.

The comments of Drs. Wilde and Hemachudha and of Dr. Van den Enden highlight potential difficulties in postexposure prophylaxis in countries where rabies is endemic. Rabies vaccines are potent, but many factors affect immunity, including genetic characteristics, nutrition, concomitant diseases, and concomitant use of other drugs. Serologic analyses of vaccine recipients, such as Peace Corps volunteers, showed substantial differences in responses after vaccination. Antimalarial drugs, such as chloroquine, may interfere with optimal responses after primary vaccination with intradermal vaccine. If antimalarial drugs are administered, vaccination should ideally be given by the intramuscular route. Nevertheless, there have been no documented instances of failure of postexposure prophylaxis as a result of the use of antimalarial drugs. Unfortunately, malaria is endemic in most regions where canine rabies persists: the costs of intramuscular vaccination make it impractical for routine use in these regions, as opposed to occasional use in travelers. The high costs and lack of availability of rabies immune globulin, which we agree is routinely indicated for high-risk exposures, also remain a problem in these areas.

Clearly, elimination of canine rabies is the ultimate solution to problematic postexposure prophylaxis. Scarcely all biologic agents for use in humans are not a practical approach to the ef-
Case 38-2004: A Large Tumor of the Skull

TO THE EDITOR: In Case 38–2004 (Dec. 16 issue), Richardson et al. provide an excellent discussion of the treatment options available for patients with multiple myeloma. In my opinion, the diagnostic workup for the patient is missing two important tests that should be done routinely, especially in the setting of oligosecretory and extramedullary myeloma. The first is functional imaging with whole-body positron-emission tomography (PET) scanning. In this patient, a PET scan would have helped identify occult sites of plasmacytoma, thus providing an accurate assessment of his tumor burden. The second test is the measurement of monoclonal free light chains in the serum, which is an easily available measure of tumor activity, even in oligosecretory settings. It would have been helpful to know the level of measurable free light chains in this patient before and after radiation treatment and during relapse. Measurement of free light chains is quite sensitive and specific for monitoring the response and for detecting an early relapse. An accurate assessment of the tumor burden early in the course of disease could improve patient outcomes by prompting the implementation of definitive therapy, rather than palliation of symptoms.

Ashraf Z. Badros, M.B., Ch.B.
University of Maryland
Baltimore, MD 21201
abadros@umm.edu


THE AUTHORS REPLY: Dr. Badros suggests that whole-body PET scanning and measurement of free light chains in the serum should be done routinely. We agree that in the setting of nonsecretory myeloma, PET scanning and measurement of free light chains can be helpful in establishing the extent of disease, particularly if protocol participation or intensive therapy is planned. However, the routine use of these tests is not supported by the clinical practice guidelines of the National Comprehensive Cancer Centers Network.

We agree that studies have suggested that measurement of free light chains is sensitive and specific in monitoring the response and detection of early relapse, and if used in conjunction with PET scanning, provides an additional tool for the assessment of tumor burden. This patient chose a more conservative approach to therapy, and therefore, because more intensive treatment was deferred, the use of these additional tests would have been of less value in this particular setting.

Paul G. Richardson, M.D.
Dana–Farber Cancer Institute
Boston, MA 02115
Ara Kassarjian, M.D.
Wen Jing, M.D.
Massachusetts General Hospital
Boston, MA 02114

TO THE EDITOR: In reply to the letter from Grass (Jan. 13 issue),1 we would like to clarify that www.clinicaltrials.gov was expanded in October 2004 to allow for the registration of any clinical trial that has been approved by a human subjects review board (or the equivalent) and that conforms to the regulations of the appropriate national or international health authority. We encourage investigators and sponsors of clinical trials that meet these criteria to register their trials at this Web site. In addition, current U.S. law mandates the registration of trials that are designed to evaluate the efficacy of drugs for the treatment of serious or life-threatening diseases and that are intended to support a new drug application to the Food and Drug Administration.2 For more information about registration of clinical trials, see http://prsinfo.clinicaltrials.gov.

Deborah A. Zarin, M.D.
ClinicalTrials.gov
Bethesda, MD 20894
dzarin@mail.nih.gov


Distribution of C-Reactive Protein Values in the United States

TO THE EDITOR: Recent studies suggesting that C-reactive protein (CRP) may be an important risk marker for cardiovascular disease have stimulated the demand for testing. Nevertheless, many physicians may be unfamiliar with high-sensitivity CRP measurements. In fact, no description of the distribution of CRP values for the entire adult population (and relevant subgroups) in the United States is readily available to clinicians. We therefore sought to summarize the distribution of CRP for all U.S. adults by age, sex, and race or ethnic background.

We analyzed components of the most recent data released from the National Health and Nutrition Examination Survey (NHANES), 1999 through 2002, which includes a sample of 21,004 people.1 NHANES is conducted by the National Center for Health Statistics, Centers for Disease Control and Prevention, to assess the health and nutritional status of the civilian, noninstitutionalized population of the United States. The complex sampling design, data-collection methods, and weighting approach are described elsewhere.2 We report on the 8874 adults (20 years of age or older) who underwent high-sensitivity CRP testing.

All analyses incorporated four-year sampling weights (WTMEC4YR) to account for the differential probability of selection among subjects and for nonresponse, as well as design-effects variables — the stratum variable (SDMVSTRA) and the variable for the primary sampling unit (SDMVPSU) — in order to account for the survey’s complex, multistage sampling strategy. All analyses used the SVY series of commands in the Stata program, version 8.2.

The levels of CRP range from 0.1 to 296.0 mg per liter, with a distribution highly skewed to the right (mean, 4.3; median, 2.1) (Fig. 1 and Table 1). The levels of CRP are higher among women than among men (median, 2.7 mg per liter vs. 1.6 mg per liter) and increase with age (median, 1.4 mg per liter among those 20 to 29 years of age vs. 2.7 mg per liter among those 80 years of age or older), but they vary less across categories of race or ethnic background. Recently, researchers have begun to use a CRP level of 2 mg per liter or greater as the threshold for defining high cardiovascular risk.3,4 With use of this threshold, 52 percent of the adult population in the United States would be considered at high risk. The proportion of people with a level of 2 mg per liter or higher is substantial at all ages (e.g., 41 percent among those 20 to 29 years of age vs. 62 percent among those 80 years of age or older).

These data document that levels of CRP vary with sex and age. Half of U.S. adults have levels

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Table 1. Distribution of C-Reactive Protein Levels in the U.S. Population Overall and According to Sex, Age, and Race or Ethnic Group.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CRP Category</th>
<th>CRP Level</th>
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<tbody>
<tr>
<td></td>
<td>0–1.9 mg/liter</td>
<td>2.0–2.9 mg/liter</td>
</tr>
<tr>
<td>All adults</td>
<td>48</td>
<td>14</td>
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<tr>
<td>Sex</td>
<td>56</td>
<td>14</td>
</tr>
<tr>
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<td>13</td>
</tr>
<tr>
<td>Female</td>
<td>59</td>
<td>12</td>
</tr>
<tr>
<td>Age (yr)</td>
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<td>13</td>
</tr>
<tr>
<td>20–29</td>
<td>50</td>
<td>13</td>
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<tr>
<td>30–39</td>
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<td>50–59</td>
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<tr>
<td>70–79</td>
<td>51</td>
<td>13</td>
</tr>
<tr>
<td>Race or ethnic group†</td>
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<td>White</td>
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<td>Black</td>
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<td>14</td>
</tr>
<tr>
<td>Hispanic‡</td>
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<td>13</td>
</tr>
<tr>
<td>Other</td>
<td>44</td>
<td>12</td>
</tr>
</tbody>
</table>

* Percentages may not total 100 because of rounding. CRP denotes C-reactive protein, and IQR interquartile range (i.e., 25th to 75th percentile).
† Race or ethnic group was self-reported.
‡ “Hispanic” includes the National Health and Nutrition Examination Survey categories “Mexican Americans” and “Other Hispanics.”


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Altered Mental Status after a Marathon

TO THE EDITOR: Hyperthermia has long been recognized as a cause of altered mental status after a marathon, but in recent years exercise-associated hyponatremia has become increasingly common. In 2003, there was an unexpected increase in the number of London Marathon runners who presented to St. Thomas’ Hospital, situated near the finish line of the race, among them 17 runners with altered mental status (Glasgow Coma Scale scores, 11 to 13). Six of these 17 runners received a diagnosis of hyperthermia (temperature, >37.7°C), and 11 a diagnosis of exercise-associated hyponatremia (serum sodium level, <135 mmol per liter). There were distinct differences between these two groups in their characteristics at presentation (Table 1).

All six of the runners with hyperthermia presented with collapse and confusion 185 to 385 minutes after the race had begun; five of them had not completed the marathon. Fourteen runners received a diagnosis of exercise-associated hyponatremia. Eleven of these runners were confused, and one had seizures. All of them had completed the marathon. Their serum sodium levels ranged from 116 to 133 mmol per liter. The first runner in this group pre-
sented 398 minutes after the race had begun, after the last runner with hyperthermia had arrived. There was a substantial delay between completion of the marathon and presentation at the hospital among the runners with hyponatremia (mean, 243 minutes; range, 132 to 391). None of these runners could recall having completed the event, but a collateral history, available for some, described them as having been lucid when they finished the race and then becoming confused later.

The exact pathophysiology of exercise-associated hyponatremia is still debated, but it is thought to be due to excessive ingestion of hypotonic fluids in the setting of neurohumoral changes that reduce free water excretion.\textsuperscript{1-3} It is usually assumed that the fluid ingestion occurs during the exertion, and most previous reports have described the development of symptoms during or at the end of an event, although late presentations (including presentation after a marathon) have been reported.\textsuperscript{3} We postulate that delayed presentation is due to absorption of hypotonic fluid after the completion of an event, often leading to delayed symptoms and presentation to a hospital not primarily designated to receive event participants. After an educational campaign warning runners of the dangers of excessive drinking, there was only one reported case of exercise-associated hyponatremia after the 2004 London Marathon.

Adrian M. Goudie, F.A.C.E.M.
Swan District Hospital
Perth 6056, Australia
adrian.goudie@health.wa.gov.au

Dan S. Tunstall-Pedoe, D.Phil, F.R.C.P.
Homerton Hospital
London E9 6SR, United Kingdom

Mary Kerins, F.F.A.E.M.
King’s College Hospital
London SE5 9RS, United Kingdom


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BOOK REVIEWS

**The Great Betrayal: Fraud in Science**


Irrespective of the difficulty in determining its incidence, misconduct in research — fraud, falsification, and plagiarism — has a corrosive effect on the scientific enterprise. It violates the norms of scientific integrity, leads researchers down spurious paths, and, in the case of clinical research, uses false data to endorse treatments. Misconduct erodes trust among researchers and the public’s confidence in and support of research. Moreover, good-faith whistle-blowers can suffer devastating personal and professional consequences, and institutions must bear the burden of the human and financial costs of investigating allegations of misconduct.

In *The Great Betrayal*, Horace Freeland Judson discusses these and other aspects of misconduct in research and the resulting effects on the scientific enterprise. He chronicles and analyzes a range of recent and historically distant cases in various scientific fields and countries. In this book, Judson displays his talents as a science writer and contemporary historian who is skilled in interviewing and observing and collecting and using primary source documents. His analysis buttresses the view that incidents of misconduct in research are not idiosyncratic, isolated events. Rather, he argues convincingly, they involve patterns that arise from “a culture of fraud,” which is not confined to science but exists within — and is attributable to certain features of — many other professions.

I found Judson’s treatment of two topics particularly informative and insightful. The first is peer review of grant applications and manuscripts. The fairness of the present methods of peer review is under attack, and the susceptibility of the present system to corruption (Judson’s word) vigorously debated. The second topic is the importance of authorship in the social system of science. With support from actual case histories, Judson examines why the theft of intellectual property, including the many varieties of plagiarism, is such a serious misdeed. He also discusses the failure to give authors credit when it is due and the awarding of “gift” or “ghost” authorship.

In the book’s epilogue, Judson presents his reasons for believing that the sciences, in accordance with inexorable “Malthusian limits,” are at an “inevitable” stage of transition from growth to a “steady state.” His corollary thesis is that “the transition to the steady state is fraud’s deep context.” Although I am not fully persuaded by the case he makes for an approaching steady state — which, he is careful to point out, does not mean “the end of science” — his argument is thought-provoking. Nor am I convinced that a transitional phase is the “deep context” of fraud, given the long history of fraud in science and the varieties of fraud in many other professions. But if Judson is even partially correct, these matters have a significant bearing on misconduct in research, on the ways in which it is handled (or not) by institutions, and on the steps that could be taken to curtail misconduct and improve the responses to it.

*The Great Betrayal* merits a wide readership, especially among those who are concerned with the responsible conduct of research. Even experts on misconduct will find a wealth of material in this book. One can only hope that people who still obstinately insist that misconduct is not a serious problem because it is a rare event committed by a few “bad apples,” or because science is a self-correcting enterprise, will read this important book.

Judith P. Swazey, Ph.D.
Boston University Schools of Medicine and Public Health
Boston, MA 02118
jswazey@midmaine.com

**Hope or Hype: The Obsession with Medical Advances and the High Cost of False Promises**


Armed with support from the Robert Wood Johnson Foundation, Deyo and Patrick make a well-documented — if depressing — argument that doctors, scientists, and laypersons
alike are far too easily seduced by industry hype for merely new (as opposed to truly better) drugs and medical devices. Deyo and Patrick are appropriately tough on the Food and Drug Administration’s (FDA’s) drug approval process, in part because the agency’s mission does not include weighing one drug against another but, rather, merely approving a new drug if it works at all, even if it has no advantages over cheaper drugs already on the market. The authors are even tougher on the FDA’s process for approving medical devices, deftly hanging the agency by its own quotes, such as this gem: “New devices are less likely than drugs to have their safety established clinically before they are marketed.” And, of course, they note that it is not part of the FDA’s mission to regulate surgical procedures.

But the basic message from Deyo and Patrick, both professors at the University of Washington, is that we are all too ready to believe that new, expensive, or aggressive care must be better than older, cheaper, or milder treatments. It is a cultural thing, they argue, citing one study that showed that whereas 34 percent of Americans believe that modern medicine can cure almost anything, only 27 percent of Canadians and 11 percent of Germans do.

There is little that is new in this book for anyone who has followed the medical journals and the mainstream press over the past decade. But it is an excellent reference for the reader who wants details of the horror stories that have grabbed headlines: the rise and fall of the fenfluramine–phentermine diet pill (sometimes referred to as “fen–phen”); the high failure rate associated with some cardiac pacemakers; the widespread use of bone marrow transplantation for advanced breast cancer before studies finally showed that it was no more effective, and could be more dangerous, than standard chemotherapy; the appalling suppression or delayed publication of “negative” results in studies funded by drug makers. Citing example after example, Deyo and Patrick are at their most successful when they detail the degree to which the pharmaceutical industry, the most profitable industry in the United States, sometimes abuses its enormous power.

Happily, just when you are about to move on to something, anything, else, Deyo and Patrick come up with a comparatively upbeat ending, exploring some remedies for America’s ills. They like the idea, endorsed last September by a coalition of editors of medical journals, including this one, of a national registry for clinical trials in order to make it harder for the manufacturers of drugs and devices to suppress negative findings. They want to stop drug companies from claiming marketing expenses as tax deductions — a no-brainer, in my mind. And they want a better post-marketing surveillance system for drugs and devices. None of this will be easy. Fixing the mess, the authors conclude, will “require action by doctors, hospitals, the media, and the government.”

Judy Foreman, Ed.M.
Suite 301, 4 Brattle St.
Cambridge, MA 02138
judyforeman@myhealthsense.com

**Double Standards in Medical Research in Developing Countries**

The controversy at the center of this book regarding the use of placebo in trials in developing countries came to light in 1997. The studies in question were evaluating the efficacy of zidovudine in the prevention of perinatal transmission of HIV, even though the drug had already been proved effective. The dispute filled hundreds of articles in medical journals and the lay press, and it led the public to question the existence of ethical guidelines in research and their application in developing countries.

As some contenders in the debate provocatively asked, were these studies in Africa or Asia not merely modernized versions of the Tuskegee experiments? Were we not condoning or even promoting double standards in medical research in developing countries? In her book, Ruth Macklin asks equally critical questions: Should clinical trials that would be considered unethical in the United States or Europe be conducted in developing countries? What standard of care should be secured for study participants during the trials? If the tested therapies prove more effective, acceptable, or cost-effective than the current “standard of care,” should they be made available to the study participants?
participants and others in their community or country and, if so, through which mechanisms? These very questions prompted the landmark revision of the Declaration of Helsinki in 2000 to reaffirm that a new medical method of prevention or treatment should be tested against the best current methods, a point reiterated in the footnotes of the 2004 revision.

As one of the world’s leading experts in medical ethics, Macklin has taken an active role in virtually all aspects of the controversy in an attempt to help, as she puts it, to overcome the difficulty of crafting ethical guidelines that are “usefully prescriptive without being hopelessly aspirational.” In this book, she visits the essential components of the debate, including ethical standards in research, justice, exploitation, informed consent, the affordability of drugs, human rights, and the harmonization of international guidelines. Her writing is accessible; chapters start with a debate surrounding case studies and offer an outline of existing guidelines and a presentation of opposing arguments. One unique feature is that Macklin, as an insider, can effectively depict the parties involved, their constituencies, and their role in the process of revising or confronting guidelines and institutional positions. She helps the reader understand why statements on the same topic from the World Medical Assembly, the Food and Drug Administration, the International Federation of Pharmaceutical Manufacturers and Associations, and the International Conference on Harmonization do not match.

Although it is fascinating to visualize the actors developing their arguments, this format is also one of the book’s drawbacks, since the conceptual bases of the debate are somewhat sidetracked. The reader then needs to fill in the gaps. Placebo-controlled studies, for example, are often referred to as easier, and therefore cheaper, to implement than are studies with active controls. Although this may often be the case, the basis for this statement is not obvious and deserves more attention. Moreover, these two kinds of trials do not answer the same types of questions. Another issue that deserves more attention is cultural and economic relativism. If the goal is to come up with guidelines that have practical applications, this issue needs to be faced squarely.

Yet Macklin succeeds in widening the scope of the debate, particularly in two important chapters on the critical issues of making drugs affordable and protecting human rights. She explores the subject of drug pricing and intellectual property, reminding the reader that the ethical basis of research goes far beyond protecting human subjects from harm or abuse. It also mandates the promotion of access to better health care and to the product of the knowledge acquired through research, especially in developing nations where the needs are immense. Macklin draws on the 1948 Universal Declaration of Human Rights and the 1966 International Convenant on Civil and Political Rights to remind the reader that embedded in these declarations are the right to the means of improving and protecting health and the right to benefit from the advancement of medical science.

Although this book will be essential reading for those conducting or participating in research, their views during and after the controversial studies are not examined. Citations are mostly from newspaper articles, guidelines, regulations, and commentaries in interviews but rarely from articles in medical journals. Macklin states that “most of the people who have given any thought to the matter have already formed an opinion and there would be little point in trying to convince them otherwise,” but I would argue that some investigators may have changed their opinions and may want to revisit the arguments, precisely because of the points raised in this book — how the studies’ results got translated into public health policy, for example. What seems to be lost here is the fact that much of the debate surrounding the zidovudine trials was not about whether study participants should have received placebo but about what the researchers had known, proven, believed, or hypothesized that allowed them to use placebo. For example, the question of whether an established, effective intervention exists or is likely to be available in the near future is not always straightforward and is certainly central to the debate.

I took immense pleasure in reading this book but also suffered from quite a bit of frustration at times. I believe this is the hallmark of an important book — the kind you loan to a friend for the pleasure of arguing about it later.

Marc Lallemant, M.D.
Institut de Recherche pour le Développement
75480 Paris, France
marc@phpt.org

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The essays in this book arose from a conference dedicated to the “History of Human Experimentation during the Twentieth Century,” held in 2001 at the University of Lübeck in Germany. Eight of the 23 contributors (including the editors) live in Germany; the remaining authors are from the Czech Republic, France, Israel, Japan, Russia, the United Kingdom, and the United States, which gives this book an international flavor.

Roelcke and Maio point out in their preface that “historical arguments are frequently used to propagate or attack” positions in current bioethics debates. And they rightly assert that when players in these debates use such arguments, the past is often oversimplified. They also point to the strangely ahistorical aspect of many discussions within bioethics, with the observation that “there seems to be a lack of awareness of the historical dimensions implicit in today’s value preferences.”

Roelcke ends the introduction by stating that the book can be understood as “an argument for the necessity to include systematic historical reflection in present day ethical debates on human subjects research.” He identifies diligent research as crucial to historical scholarship and bemoans the tendency among bioethicists to rely on “deficient, faulty, or misconceived secondary or tertiary [historical] literature.” He also calls on historical researchers to avoid interpreting the past largely (or exclusively, in the worst cases) by making comparisons with the present. In his introduction, Roelcke alludes to an additional advantage that arises from careful historical work — an appreciation for the contingency of change. This gain is stated with more force by Nadav Davidovitch in a valuable essay on changing perceptions of the placebo: “The advantage of the historical perspective is that it offers the possibility of a de-familiarization of the existing situation, by understanding that the state of affairs is not a ‘given,’ but is part of an ongoing struggle, with the possibility of negotiation and change.”

Some of the contributions to this book are excellent, with Davidovitch’s essay heading the list. Paul J. Edelson’s piece contrasting the contributions of two whistle-blowers, Henry K. Beecher (of the United States) and Maurice Pappworth (of the United Kingdom), is also very useful. Edelson convincingly builds his analysis around a juxtaposition of Beecher as “the quintessential ‘insider’” and Pappworth as the “very definite ‘outsider.’” Edelson, however, misses an interesting layer of complexity with respect to Beecher’s status as an insider. Henry Beecher was born Henry Unangst. Young Henry changed his surname to Beecher when he left Kansas for Harvard Medical School, correctly calculating that Boston would be more impressed with a moniker that matched the prominent abolitionist clan (to which he was distantly related through his maternal grandmother). Edelson concludes his essay with perhaps the most interesting general point in the book. He asserts (drawing on the work of Robert A. Nye) that we can properly understand the reaction of physicians to the regulation of human experimentation only by taking into account the “culture of honor” — the central concept from which professions (of medicine or anything else) arose.

Much of this book, however, fails to live up to the standards and expectations set out by the editors. Many of the essays are so poorly organized that the basic points are difficult to discern. Some of the authors fall into the unenlightening trap of presentism. One contributor tosses out the statement that “the process of establishing modern experimental medicine . . . was often, but not exclusively[,] based on unethical experimentation.” Simply to label experimentation of the past as “unethical” is to close off some of the most interesting lines of historical inquiry. And, given the editors’ call for careful historical scholarship, their choice of citation style is a frustrating mystery. The particular combination of footnotes and reference lists they use is awkward for published works and all but unworkable for archival sources.

The editors are to be commended for rendering all of the contributions into one language, and I am grateful they chose English. The copyediting challenges inherent in working with authors who are not writing in their native tongue are often considerable. In this case, the final results fall well short of any reasonable standard. The text has dozens of clunkers such as the following: “It was quite usual to use hospital patients for experiments.” And trite expression seems to have gone largely
unchallenged. For example, we are offered the following worn (not to mention inaccurate) metaphor that racism was the 2000-pound elephant sitting unremarked-upon in the center of the laboratory. (A ton is more like the weight of a water buffalo; a standard elephant weighs about 12,000 pounds.)

Jon M. Harkness, Ph.D.

University of Minnesota
Minneapolis, MN 55455
harkn008@umn.edu

NOTICES

Notices submitted for publication should contain a mailing address and telephone number of a contact person or department. We regret that we are unable to publish all notices received. Notices also appear on the Journal's Web site (www.nejm.org/meetings). The listings can be viewed in their entirety or searched by location, month, or key word.

LUNG SUMMIT 2005: INNOVATIONS IN PULMONARY & CRITICAL CARE MEDICINE

The summit will be held in Cleveland, April 21 and 22. It is presented by the Cleveland Clinic Department of Pulmonary, Allergy, and Critical Care Medicine.

Contact Daniel M. Laskowski, Cleveland Clinic, Center for Continuing Education, KK-31, 9500 Euclid Ave., Cleveland, OH 44195; or call (800) 232-2273, extension 43702; or see http://www.clevelandclinicmeded.com/registration.htm; or e-mail laskowd@ccf.org.

2ND INDO-U.S. WORKSHOP FOR PHYSICIANS AND SURGEONS

The workshop will be held in Chennai, India, April 22 and 23. It is sponsored by the Diabetes Research Centre. Chennai.

Contact Dr. Vijay Viswanathan, Diabetes Research Centre, 4 Main Rd., Royapuram, Chennai-600 013, India; or call (91) 44-25954913; or fax (91) 44-25954919; or e-mail dr.vijay@vsnl.com.

5TH EXTRAORDINARY INTERNATIONAL SYMPOSIUM ON RECENT ADVANCES IN OTITIS MEDIA

The symposium will be held in Amsterdam, April 24–27. Contact Congress Care, P.O. Box 440, ’s-Hertogenbosch 5201 AK, the Netherlands; or call (31) 73 6831238; or fax (31) 73 6901417; or see http://www.ommen2005.nl; or e-mail info@ommen2005.nl.

41ST ANNUAL ROBERT M. JERESATY, M.D., CARDIOVASCULAR SYMPOSIUM

The symposium will be held in Hartford, Conn., April 27 and 28. It is sponsored by the Heart Institute of Connecticut, Saint Francis Hospital and Medical Center.

Contact Hoffmann Heart Institute of Connecticut, Saint Francis Hospital and Medical Center, 114 Woodland St., Hartford, CT 06105-1299; or call (860) 714-5555; or fax (860) 714-5980.

HARRINGTON SPINE SYMPOSIUM

The symposium will be held in Kansas City, Kan., July 28–30. Contact Dr. Marc Asher, 3901 Rainbow Blvd., MS 3017, Kansas City, KS 66160; or call (913) 588-6174; or fax (913) 588-8796; or see http://www.harringtonsymposium.com.

OCCUPATIONAL SAFETY AND HEALTH EDUCATION AND RESEARCH CENTER

The following courses will be offered in Chapel Hill, N.C.: “Supervising Asbestos Abatement Projects” (Refresher Course, April 27); “Building Inspection and Management Planning for Asbestos” (Refresher Course, April 28); “Designing Asbestos Abatement Projects” (Refresher Course, April 29); “Certified Hazardous Material Manager (CHMM) Review” (June 6–9; Exam, June 10); “Sampling and Evaluating Airborne Asbestos Dust (NIOSH 582)” (June 20–24); and “Asbestos Operations and Maintenance” (July 11).

Contact Occupational Safety and Health Education and Research Center, University of North Carolina, 3300 Hwy. 54 West, Chapel Hill, NC 27516-8264; or call (919) 235-3320 (national) or (919) 2101 (North Carolina); or fax (919) 966-7579; or e-mail oshercww@sph.unc.edu; or see http://www.sph.unc.edu/osherc/.

INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

The “3rd Annual Meeting” will be held in San Francisco, June 23–25.

Contact International Society for Stem Cell Research, 60 Revere Dr., Suite 500, Northbrook, IL 60062; or call (847) 509-1944; or fax (847) 480-9282; or see http://www.issscr.org; or e-mail issscr@isscr.org.

MEDICAL SPANISH WORKSHOP

The workshop will be held in San Francisco, April 29–May 2 and in Las Vegas, May 3–16. It is sponsored by the University of Arizona, College of Medicine.

Contact Tamara Rios, Rios Associates, 3729 N. Bay Horse Loop, Tucson, AZ 85719; or call (520) 907-3318; or fax (520) 322-2441; or e-mail convesp@aol.com or tamra85719@aol.com; or see http://www.medsspanish.org.

JOHNS HOPKINS UNIVERSITY

The following courses will be offered in Baltimore, unless otherwise indicated: “50th Annual Topics in Clinical Medicine” (May 2–6); “The 55th Institute for Spirituality and Medicine — Spiritual Well-Being: The Individual, Community, and Health Care Institutions” (May 9–11); “The 3rd Annual Postgraduate Seminars in Dermatology” (June 18); and “Perioperative Management — In Its 21st Year” (Aspen, Colo., Aug. 15–18).

Contact Office of CME, Johns Hopkins University School of Medicine, Turner 20, 720 Rutland Ave., Baltimore, MD 21205-2195; or call (410) 955-2959; or e-mail cmenet@jhmi.edu; or fax (410) 955-0807; or see http://www.hopkinscme.org.

FALK FOUNDATION

The following symposia will be held: “Colitis: Etiologies and Therapeutic Strategies” (Birmingham, United Kingdom, May 6 and 7); “Diverticular Disease: Emerging Evidence in a Common Condition” (Munich, Germany, June 17 and 18); “Gastro-Conference Berlin 2005 (Part I): Highlights in Gastrointestinal Oncology” (Berlin, Oct. 1 and 2); and “Gastro-Conference Berlin 2005 (Part II): Disease Progression and Disease Prevention in Hepatology and Gastroenterology” (Berlin, Oct. 3 and 4).

Contact Falk Foundation e.V., Congress Division, Leinenweberstr. 5, P.O. Box 6529, D-79041 Freiburg, Germany; or call (49) 761-15 14 0; or fax (49) 761-15 14 359; or e-mail symposia@falkfoundation.de; or see http://www.falkfoundation.de.

UNIVERSITY OF COLORADO

The following courses will be offered: “2nd Rocky Mountain Metabolic Syndrome Symposium” (Denver, April 29); “41st Annual Internal Medicine Program” (Estes Park, Colo., July 17–22); “32nd Renal Disease and Electrolyte Disorders Course” (Aspen, Colo., July 25–29); and “Annual Psychiatry Conference” (Aspen, Colo., Aug. 3–5).

Contact Office of CME, University of Colorado School of Medicine, 4200 E. 9th Ave., #C295, Denver, CO 80262; or call (303) 372-9054; or fax (303) 372-9065; or e-mail cme.meetings@uchsc.edu; or see http://www.uchsc.edu/cme.