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Biomarkers in Migraine

Biomarkers in Migraine: Their Promise, Problems, and Practical Applications

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Biomarkers are physical signs or laboratory measurements that "occur in association with a pathological process and have putative diagnostic and/or prognostic utility." Biomarkers hold considerable promise for understanding and intervening in the disease process of migraine. They may permit recognition of individuals at risk of developing migraine, improve the timing, accuracy, and precision of migraine diagnosis, and serve as indicators of treatment response and disease progression. Furthermore, they hold great promise for research. At the same time, there are important limitations to the use of biomarkers in migraine, including problems with validity, reliability, accuracy, and precision. Legal, ethical, and cost considerations are also important. This review describes the potential uses and limitations of biomarkers in migraine diagnosis, treatment, and research.

Key words: biomarkers, migraine, FDA, pharmacogenomics, proteomics, metabolomics

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Biomarkers are objective physical attributes that can be used to characterize and differentiate the biology of diseases and syndromes. Thus, among many other possibilities, a migraine biomarker might be a blood, urine, muscle, nerve, skin, or cerebrospinal fluid test result, a gene or gene product, an x-ray, magnetic resonance or computed tomographic imaging finding, or a characteristic pattern of electrical measurements generated on an electrocardiogram, electroencephalogram, or nerve conduction study. In short, a biomarker might be anything that can be detected, measured, and described in terms of physical qualities such as height, weight, depth, voltage, luminescence, resistance, viscosity, width, length, volume, or area. Although many definitions for the term biomarker have been proposed, for the purposes of this article and this special section of the journal, we adhere to the following:

> "A biomarker is a physical sign or laboratory measurement that occurs in association with a pathological process and has putative diagnostic and/or prognostic utility."[1]

Biomarkers have seen wide application in a number of medical fields. For example, variant alleles of the apolipoprotein E gene can predict an increased risk of Alzheimer's disease, thyroid stimulating hormone levels are used both to diagnose thyroid disorders and monitor treatment response, and forced expiratory volume measurements are followed as a surrogate endpoint in asthmatics.[2-4] Almost invariably, a combination of biomarkers will be more useful than any single marker: the classic triad of elevated blood pressure, proteinuria, and hyperreflexia in a pregnant woman is strongly associated with pre-eclampsia, whereas each element of the triad occurring alone can be associated with a wide variety of other diagnostic possibilities.[5]

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The migraine field is notable for its paucity of clinically useful biomarkers. In fact, current diagnostic criteria for migraine require the absence of any headache-related physical or neurological abnormality, thus defining migraine by the lack of any biomarker.[6] In view of expanding knowledge, this is unlikely to remain tenable. This article reviews general issues regarding the use of biomarkers as they relate specifically to migraine. These include types of biomarkers, and their potential use for migraine diagnosis, prognosis, and prediction and monitoring of treatment response. This article also will consider the application of biomarkers in migraine research, and discuss practical, legal, and ethical issues arising from their use.

Types and Categories of Biomarkers.—Biomarkers can be divided into a number of different categories or types. For example, biomarkers are often broadly divided into "biomarkers of exposure" or "biomarkers of disease." Another way of grouping biomarkers is to divide them into categories based on what the marker actually measures, such as a gene, an imaging pattern, a physical measurement, or proteins or protein patterns in various body fluids.

Biomarkers of Exposure and Disease.— Biomarkers of exposure are often referred to as "antecedent biomarkers" because they measure factors that are present before the disease, in this case migraine, develops. Antecedent biomarkers increase or decrease the risk of migraine, although they may not be either necessary or sufficient to cause it, and furthermore may not be directly involved in migraine causation.

In contrast, biomarkers of disease are measurements or surrogate markers that provide an indication of the presence or progress of migraine. They can be used for screening, for case finding and diagnosis, or for prediction and tracking of disease course or treatment response. Biomarkers of disease will be especially important for the future of migraine treatment, because it is now clear that migraine is not a single disorder but rather a cluster of several important headache subtypes, each with a different natural history and each requiring different treatment. (The various forms of familial hemiplegic migraine, for example, are some of these subtypes.) One important question that has not been investigated is whether migraine traits, even in the absence of headache expression, might contribute to the risk for associated problems, such as depression or stroke.

In any case, biomarkers of exposure would help identify persons at elevated risk of developing migraine. If it were possible to accurately determine who is at risk of severe, prolonged disability from migraine, it might make sense to treat those persons sooner rather than later once the disease develops, or even to institute pre-emptive measures before symptoms develop. Not enough is known yet about the natural history of migraine to be certain, but analogies with other diseases suggest that there may be a "window of opportunity" during which treatment will be most effective.[7] Currently, relatively few patients with migraine receive treatments known to be helpful for the disorder, and in those who do, the threshold for initiating treatment is quite high. For some patients, this may be too late.[8]

From a public health point of view, successful migraine pre-emptive strategies, or strategies that delayed its onset, could have large societal benefits, but the benefits to any one treated person might be small or nonexistent, and any treatment harms would have to be correspondingly insignificant. No combination of biomarkers will ever be perfect in determining risk: some people flagged as "susceptible" ultimately will not develop migraine even if untreated. Relatively complex, lengthy trials would be necessary to define gradations of risk, to identify the optimal type, timing, and duration of pre-emptive treatment, and to evaluate financial and nonfinancial harm to benefit ratios. Ultimately it may make sense to treat only those at high risk of developing very severe or disabling forms of the disorder, but we are a long way from being able to identify those individuals.

A high standard of proof is necessary to advocate anticipatory treatment of otherwise healthy, asymptomatic people, particularly for a disorder that, like migraine, is not life threatening.[9] Even more certainty about the harm to benefit ratio of an intervention would be necessary in the case of migraine, since the target population for pre-emptive treatment efforts largely would be comprised of young women of childbearing age.

This careful attitude probably should extend also to nondrug interventions, even seemingly harmless "lifestyle" recommendations about diet or exercise. One expert, speaking about cancer prevention efforts, has pointed out that, "The lessons learned from chemoprevention trials include the realization that 'everything comes with its own baggage. . .that's certainly true when it comes to any medication or even seemingly innocuous things, such as vitamins'. And to ensure that all adverse effects of chemopreventive agents are detected, randomized controlled trials need to be carefully monitored and of sufficient duration. . .It's essential to develop agents that target known molecular pathways and which are nontoxic. . .we're talking about treating healthy populations that will have to take these agents probably for many years."[10]

Categorical Biomarkers.—*Genetic Biomarkers*.—Genetic markers are one class of antecedent biomarker. Genetic polymorphisms are differences in deoxyribonucleic acid (DNA) sequences that result in variant phenotypes—for example, blond or brown hair—and are common enough (population prevalence greater than 1%) that they must be the result of natural selection. Polymorphisms are also referred to as "variant alleles." Genetic polymorphisms can result from a number of different gene alterations, including a change of a single nucleotide in a DNA sequence, in which case they are known as single nucleotide polymorphisms (SNPs). SNPs are the most common type of polymorphism.[11] Polymorphisms are distinguished from deterministic genes, which cause simple genetic, or Mendelian, forms of disease. As is the case with many chronic diseases, most forms of migraine are likely to be genetically complex, with many different genes contributing to risk. It seems likely, though, that all of these variations, whether they increase or decrease vulnerability to migraine, will act through just a few final common pathways. The different genes that are associated with familial hemiplegic migraine, for example, all appear to influence neuronal cell membrane stability, although the mechanisms and phenotypes differ.[12] This illustrates how identification of genes involved in migraine vulnerability should also help generate and confirm hypotheses about migraine pathophysiology and increase the pace of future biomarker discovery. Researchers can evaluate putative migraine markers to see how they modify the disease risk associated with known biomarkers. We may discover, for instance, that a particular genotype denotes an individual at special risk of developing migraine only when exposed to certain environmental factors or stresses.

So far, the few genetic markers identified for migraine have not had a significant impact on classification. As knowledge of migraine genetics increases over the next few decades, though, changes in the headache classification system are likely to be necessary as it becomes obvious that phenotypically defined forms of migraine are heterogeneous. An alternative opinion, expressed in the current version of the International Classification of Headache Disorders (ICHD), is that "the genetics of migraine may simply prove to be so complex that, in daily practice and perhaps to some extent in research, we shall continue with clinically defined diagnoses."[6] In any case, while there may be a few uncommon types of monogenic migraine, it seems unlikely that there will ever be a single genetic test that will yield a yes or no answer about common forms of migraine.

Some genetic markers for migraine will turn out to be intermediate biomarkers, which can be characterized as direct or indirect steps in the causal pathway of migraine that are related to its development but are not the only determinant.[13] For example, a particular genetic polymorphism may cause migraine only in the presence of another factor, such as a toxic or infectious exposure. Such a polymorphism would be strongly related to migraine and in its causal pathway, but not its only determinant.

Identification of genes associated with migraine is best viewed as a necessary first step in the development of clinically useful biomarkers. The correlation between genetic profiles and migraine will be far from perfect. Environmental factors are likely to account for most of this phenotypic variability, but there are also genetic and epigenetic factors that will play a role as well. With regard to genetic factors, it is likely that in addition to susceptibility genes, modifier genes for migraine eventually will be identified. One author has commented that, "Susceptibility genes and modifier genes are 2 biologic phenomena that few clinicians

should ignore in the genome era…Modifier genes are distinct from susceptibility genes, in that they are genetic variants that affect the clinical manifestation of disease (as opposed to liability)."[14]

An understanding of epigenetic changes also will be important. Epigenetic changes are those in which the genes themselves are not changed, but their expression or activity is altered.[15] Epigenetic changes are one way in which psychological experiences or trauma, even remote events, particularly if they occur during important developmental stages, might influence the occurrence or activity of migraine. There are a number of ways in which epigenetic changes can occur. One common mechanism is methylation of receptors. One recent study found that infant rats receiving maternal licking and grooming were less stress responsive in adulthood.[16] The authors speculated that these long-term consequences were caused by permanent changes in glucocorticoid receptors in the hippocampus that dampened the brain's stress response system. The proposed explanation was that during this key period of development, licking, and grooming decrease methylation of a promoter region of the gluococorticoid receptor, which then silences specific genes.

Gene expression also is regulated by microribonucleic acids (microRNAs). These are proteins that control gene expression by degrading or repressing target messenger RNAs after transcription has taken place.[17] MicroRNAs can regulate large numbers of target genes, and can be activated in various ways. MicroRNA profiles currently are used to classify some types of cancer.[18]

A significant barrier to traditional genetic research has been the extremely large studies required to identify the very small effects of susceptibility and modifier genes. Migraine and other headache disorders have been and always will be in direct competition with many other diseases for research resources. There are encouraging signs that technological and political advances will reduce genetic research costs to a more feasible level. One author has commented that, "The era of genome-wide association studies that can efficiently evaluate most common genetic variants across the genome with several hundred thousand markers is upon us, owing to the nearing completion of a very dense genetic map, known as the International HapMap Project, and new high-throughput genotyping technologies."[19] Already the cost of some types of genetic studies has dropped significantly, due to new technology. The director of the National Human Genome Research Institute recently suggested that "for $3 million, scientists can identify 300,000 markers in 1000 people with a particular disease and 1000 healthy controls…enough statistical power to find a gene that raises the risk of that disease by at least 30%."[11] Furthermore, genetic data from existing government-funded studies, such as the Framingham Heart Study, will be available free of charge to other scientists.

Proteomic and Metabolomic Biomarkers.—Not just migraine genes, but the proteins they code for, and their metabolites, will turn out to be important in migraine expression. Pathophysiologic models of migraine that are based on well-studied systems, such as the serotonergic or dopaminergic systems, probably oversimplify matters. These models will have to be reevaluated once genes involved in migraine, and their products, have been identified. However, it is not that simple, either, since a great deal depends upon how and when these gene products are expressed. Estimates are that, at any particular time, only 15% of genes are expressed.[20] Not simply proteins, but protein and metabolite patterns that are associated with various phases of acute migraine attacks or with specific headache disorders should be sought. Proteomics, measurement of the complete set of proteins from a genome, and metabolomics, measurement of the complete set of metabolites in human tissues and fluids, therefore hold much promise as a way to understand the contributions of the various genes involved in migraine. These patterns can be sought in body tissues or fluids such as blood, urine, or cerebrospinal fluid, with a goal of identifying what has been termed a "disease fingerprint" or "disease signature" for migraine.

New techniques allow isolation of typical protein or metabolite patterns from small fluid samples, and can provide very high diagnostic sensitivity and specificity.[21] A complex computer software program (The ProteinChip® Biomarker System) already is available that provides a biomarker-based

classification scheme for prostate cancer, and one can imagine something similar being developed for migraine. The ProteinChip® program allows users to control test parameters depending upon "the clinical question being addressed. For example, confirmatory tests require high sensitivity, while screening tests require high specificity." In general, combinations or "batteries" of biomarker tests improve sensitivity and specificity.[22] Proteomic or metabolomic profiles might help identify not only a particular type of headache disorder, but also the stage or phase of the illness or of an individual attack.[23]

Physical and Physiologic Biomarkers.—Electroencephalogram and electrocardiogram findings, structural brain changes, and reproducible tests of cerebellar function are just a few of the physical and physiological measurements that might serve as biomarkers of migraine, although in most cases they are reflections of migraine and not its cause. Many of these biomarkers have the advantage of being relatively easy to measure and detect, and in fact possess many of the qualities of the "ideal" biomarker in that they are easy to obtain and there is a large window of opportunity for collection or measurement. Physiological differences between migraine patients and controls may make possible model-based methods for the prediction of migraine or its treatment response that are easily applicable in the clinical setting with a minimum of special testing or equipment.[24]

Imaging Patterns.—Functional neuroimaging techniques visualize patterns of regional brain activity. Characteristic patterns of brain activity may serve as biomarkers for migraine and other headache disorders, in the same way that a particular pattern of electrical activity can identify various types of seizure disorders. Activation patterns may be helpful in predicting response to treatment, in addition to identifying the type or stage of a disorder. Using radioligands, it even may be possible to image receptors or cell pathways that are involved in migraine or its treatment. These functional changes may mark either the migraine state (that is, are detectable during an attack—useful for diagnosis), or the migraine trait (present interictally—useful for screening).[25] In migraine, these pathological patterns of activity are especially valuable as biomarkers because there are often no structural lesions.

Table.—Potential Applications of Biomarkers in Migraine Research and Treatment

Type of Biomarker	Application
Biomarkers of exposure	Identify those at risk of developing migraine
	Identify those at risk of migraine complications
Biomarkers of disease	Permit diagnosis at all stages of the illness
	Differentiate subtypes of migraine
	Identify prognostic factors and predict treatment response
	Monitor migraine progression and complications

II. Potential Uses of Migraine Biomarkers.— Several important roles can be envisioned for migraine biomarkers, and are summarized in the Table. These include (1) diagnostic uses; (2) research and clinical trial applications; and (3) treatment uses.

Diagnostic Use of Migraine Biomarkers.—Migraine is homogeneous enough in its presentation to have been recognized as a distinct syndrome since antiquity. No single feature permits a definite diagnosis, but certain features in combination have diagnostic reliability that obviates the need for further testing. Despite this, not all migraine is the same: scratch the surface, and it proves to be frustratingly heterogeneous. Not only does the clinical presentation of migraine vary dramatically from one patient to the next, it also can vary within an individual patient from one headache to the next, over the lifespan, depending upon the type or timing of treatment, or even depending upon the specific triggering event.

Furthermore, migraine attacks and migraine patients that are indistinguishable on the basis of clinical features often respond differently to treatment, have different triggers, and display a different natural history. There even may be subclinical forms of the disorder. Diagnostic criteria for migraine emphasize specificity over sensitivity, which enhances their research value but reduces their clinical worth. The criteria are cumbersome and relatively subjective. The lack of

easily applied, objective tests that reliably confirm a clinical suspicion of migraine may be one explanation for the overuse of other tests that at least rule out alternative diagnoses. For these reasons, validated diagnostic biomarkers of disease for migraine would undoubtedly find wide application and lead to improvement of current severe deficiencies in migraine diagnosis.

Biomarkers may make precise, early diagnosis of migraine possible, which will provide an opportunity for intervention in earlier forms of the disease. Many of the cautions discussed previously with regard to preventive treatment of susceptible individuals also apply to individuals who are determined to have early stages of migraine, except that since in this case we are dealing with those who actually have manifested biological evidence of the illness itself (not simply biological evidence of risk) the standards required to accept some treatment risk can be relaxed accordingly. A strong argument can be made that aggressive interventions or lifestyle changes are defensible in the case of those with a strong family history of severe, progressive migraine.

However, we are not very advanced in our understanding of the natural history of migraine. One persuasive argument against aggressive early treatment is that the natural history of migraine in most individuals is "benign," with eventual regression of headache frequency and severity.[26] This change in phenotype, though, does not necessarily reflect a reduction in risk from other aspects of the disorder, and may not mitigate its previous or future harms.[7] We do not know, for instance, if regression of headaches also implies regression of stroke risk or a reduction in risk for some of the structural correlates of migraine that are only now being identified. Judging treatment needs or regression of a complex neurologic illness on the presence or absence of a single clinical symptom, headache, may turn out to be unwise, particularly as we learn more about the pathophysiology of migraine. Additionally, headache regression is not universal: some migraineurs are severely affected well into old age, which serves as a reminder that there may be factors associated with initiation of migraine and its transformation or remission.[27] Combinations of biomarkers will add to the complexity of diagnosis, but eventually should help sort out which of several possible disease evolution patterns is likely to apply in a particular patient. Biomarkers of susceptibility, in particular, are very far from being a clinical reality. Potential markers must first be identified on the basis of retrospective studies in subjects who already have migraine, and then be tested in studies of migraine-free subjects to assess their ability to detect migraine vulnerability and onset in healthy individuals.

Research and Clinical Trial Applications.—The potential research benefits of migraine biomarkers are enormous. Migraine biomarkers would help identify homogeneous research populations, would improve research into migraine pathogenesis, and would allow identification of risk factors for migraine as well as factors related to treatment response, in addition to serving as trial endpoints. Biomarkers already are widely used in treatment research for other diseases, notably cancer. For example, a gene polymorphism that predicts benefit from trastuzumab has been identified, and use of this drug is limited to patients with appropriate markers.[28] Since cancer therapy generally is quite toxic, biomarkers help limit treatment side effects to those most likely to obtain some compensatory benefit. The same should prove true in migraine treatment.

The United States (US) Food and Drug Administration (FDA) has recognized that the use of biomarkers in clinical trials raises many issues. In March 2005 the agency issued nonbinding recommendations for the pharmaceutical industry regarding pharmacogenomic submissions (not including proteomic or metabolomic techniques). These guidelines principally focus on pharmacogenetic tests related to drug metabolism, some of which the document describes as having "well-accepted mechanistic and clinical significance." Others, though, "are not well enough established scientifically to be appropriate for regulatory decison making."[29]

The FDA guidance document distinguishes between two types of pharmacogenomic markers. Valid biomarkers are those "which are measured in a test system with well-established performance characteristics" and for which "there is an established scientific framework or body of evidence that elucidates the physiologic, pharmacologic, toxicologic, or clinical significance of the test results." The example provided in the document is that of variations in the CYP2D6

enzyme, which are already reflected in some drug labels. Observational or exploratory markers are those which "alone, are insufficient for making regulatory decisions."[29]

The FDA subdivides the category of valid biomarkers into "known valid biomarkers," defined as those "that have been accepted in the broad scientific community" and "probable valid biomarkers," defined as those "that appear to have predictive value for clinical outcomes, but may not yet be widely accepted or independently verified by other investigators or institutions." The document further comments that a clinical trial may generate information "sufficient to establish a significant association between a pharmacogenetic test result and clinical outcomes" and that when this happens "the test result represents a probable valid biomarker." Although the FDA does not require submission of exploratory pharmacogenomic data, it strongly encourages voluntary submission of such data "to support scientific contentions related to dosing and dosing schedule, safety, or effectiveness. . .to stratify patients. . .or to identify patients at higher risk for an adverse event. . ."[29]

The FDA guidance document offers suggestions for sponsors who want to incorporate pharmacogenomic data into a drug label, in which case the test must be integrated into the clinical trial with the intention that it will appear in the label for (1) informational purposes, for example to provide information on dose adjustment based on drug metabolism genotype; or (2) to guide treatment, for example used to choose a dose, identify patients at risk of side effects, or identify likely responders. In the latter case the FDA recommends "codevelopment of the drug and the pharmacogenomic tests. . .and submission of complete information on the test/drug combination. . ."[29]

Homogeneous Trial Populations.—The research benefits of identifying homogeneous populations are enormous, and this probably will be one of the most important and earliest uses of migraine biomarkers. It is difficult to distinguish the different types and etiologies of migraine on the basis of purely subjective descriptions, no matter how detailed or thorough they may be. Until quite recently, disease endpoints for migraine have been defined mainly in conceptual terms, and attempts to operationalize the definition in the form of structured diagnostic criteria have been laudable but flawed. Currently, trial subjects in most migraine studies are selected on the basis of an ICHD migraine diagnosis, and trial results then are assumed to be generalizable to most or all patients with migraine. ICHD criteria, though, distinguish poorly or not at all among potentially important subgroups of migraineurs whose disease biology and treatment responses may differ. These subgroups include migraineurs with early, middle, or late stages of migraine, those with white matter lesions, and those who have varying degrees of migraine frequency, severity, or comorbidity. In fact, some of those subgroups have been excluded systematically from clinical trials.[30]

Biomarkers of disease should help clarify whether such practices are defensible, and increase confidence in clinical applications of tested treatments to migraineurs whose characteristics match those of the trial population. This promises to reduce significantly the number of people whose time, money, and patience is wasted trying therapies that are likely to be ineffective.

Biomarkers as Trial Endpoints.—In addition to aiding the selection of homogeneous migraine trial populations, biomarkers are likely to be used eventually as time-saving surrogate trial endpoints that predict desired final endpoints, or to serve as drug targets and intermediate or ultimate clinical endpoints in their own right. Many pharmaceutical companies have already been collecting and storing blood or other DNA samples from migraine subjects in clinical trials, with the intention of searching for such markers. One potential problem with the use of biomarkers as surrogate endpoints is that although they may increase the efficiency of a trial, shorter, smaller trials probably mean that some important longer-term harms (or advantages) of a treatment will be missed. This could exacerbate the already serious problem of unanticipated postmarketing drug effects. The ability to identify patients at risk of a particular serious side effect might lead manufacturers to exclude such patients from clinical trials of the drug, or conclude that such a drug was not worth the extra costs entailed by testing. One recent case, in which a new drug was approved along with a requirement that patients be tested for a biomarker

(in this case an enzyme that metabolizes the drug) before it could be prescribed, illustrates that such fears are not merely theoretical. This excerpt from a contemporary newspaper account describes the event:

> "Aczone, a new drug for acne, was approved by FDA in July, but a requirement is that patients first be tested for enzyme deficiency that puts them at risk of developing anemia from the drug. This illustrates a barrier. . .companies fear that if testing for such genetic markers is required, it will discourage doctors from prescribing a drug or limit its sales to a subset of patients. Upon learning of the requirement, Astelias, one of its developers, abandoned the drug. The other developer is continuing with development, hoping to have the requirement limited or lifted."[31]

A hopeful possibility is that biomarkers that predict side effects also might help resurrect useful drugs whose toxicity problems could be predicted or managed using biomarker technology. In the migraine field, for example, the highly effective but potentially toxic drug methysergide might return to clinical use if its rare but serious side effect of retroperitoneal fibrosis could be predicted or detected on the basis of biomarkers. Similarly, biomarkers that predict treatment benefit in small migraine subgroups might prevent drugs from being discarded or overlooked when they appear to have no benefit in larger, unselected patient populations. It has been suggested that biomarkers that identify subgroups that benefit from the controversial cancer drug gefitinib, which showed limited overall benefit in larger trials, might "save the drug from being pulled off the market."[32]

Treatment Applications.—Valid, reliable biomarkers would help define precise, rational migraine treatment regimens based on an understanding of migraine pathology in a particular patient. At present, migraine treatment is focused on symptoms rather than on concepts of underlying pathological processes, and titration and dose targets are based on clinical judgment. Available treatments take effect slowly, provide only partial relief for the majority of patients, and have important tolerability and, in some cases, safety drawbacks.[33] Treatment algorithms are difficult to apply to individual patients, and imprecise in distinguishing patients who require specific treatments or doses.

From a clinical perspective, it is easiest to imagine uses for biomarkers that accurately predict and reflect response to particular migraine treatments. Currently, it is necessary to choose and adjust migraine medication based on clinical symptoms and response. This is time consuming and relies heavily on the development of side effects as a crude means of determining the dose and type of treatment. Treatment based on biomarkers would instead match therapy to the individual biologic characteristics of a particular patient's migraine. Biomarkers might also help to plan and monitor treatment. If a biomarker normalizes or disappears, for example, it might signal that treatment is successful or even that it should end.

With regard to migraine, the availability of such tools is currently in the realm of wishful thinking, but in other areas of medicine, notably oncology, concerted effort has been devoted to applying biomarker technology to clinical decisions. An ambitious program has developed "an algorithm that incorporates the iterative nature of assay development into an evaluation process that includes developers and end users. . . the assay uses reverse transcription evaluation of a set of 16 genes that were shown to strongly associate with the risk of recurrence of breast cancer in women who presented with early stage disease . . . it provides information to aid the physician and the patient in making important clinical decisions, including the aggressiveness of the therapy that should be recommended."[34]

Biomarkers also may provide a true opportunity for stage-specific treatment. Headache clinicians have long suspected that treatment that is effective in patients who have just begun to have migraine may be ineffective or even harmful later.[7] Certainly this is true in many other illnesses, but at the present time we have no scientific way of staging migraine treatment. It seems likely that in some cases migraine treatments have been abandoned as useless when the real problem is "too little, too late." Treatment trials in which patients experience adverse effects without benefit have been termed "unmitigated failures," in contrast with those that provide benefit with few or no side effects, which have been labeled "unqualified successes."[35] One hope is that migraine biomarkers will eventually increase the likelihood that "unqualified success" will occur early in the course of migraine treatment,

eliminating the frustrating trial-and-error method that currently applies.

III. Technical, Ethical, and Legal Considerations.—It is usual to think of biomarker development in migraine in terms of its promise, with little attention to potential pitfalls or problems. There are important general limitations, however, to the use of biomarkers that will also apply to their application in migraine. Most of these limitations derive from the fact that biomarkers are signs and measurements and like all such entities are not infallible. Still other problems will arise because of ethical and legal considerations.

Technical Issues.—The major technical issues to be considered in applying biomarker technology to migraine include the need to establish the reliability and validity of a biomarker, to characterize its receiver operating characteristics, and to understand issues of causality and confounding. The interested reader can consult several excellent reviews on the subject of the technical aspects of biomarkers, but a complete discussion of them is beyond the scope of this article.[36-39]

Several things are worth brief mention, however. The first is that to be cost-effective and widely accepted, biomarkers for a nonfatal illness like migraine would have to be accurate, inexpensive, and easily obtained.[40] The second is that the presence of a biomarker never will stand alone as incontrovertible proof of migraine unless that biomarker has been shown to be "always and only" a sign of migraine. For example, white matter lesions or posterior infarcts are associated with the presence of migraine, and may be a manifestation of it, but also are found in other illnesses.[41] Confounding can occur when an intermediate biomarker is related to an unidentified factor that also is related to the exposure. This affects the validity of the association between the biomarker and migraine. Preliminary observations suggest that this type of confounding may best explain the relationship between patent foramen ovale (PFO) and migraine with aura. The presence of a PFO appears to be strongly associated with migraine with aura, for example, and even may have prognostic implications. However, PFO and migraine with aura may both be caused by a third, possibly genetic, factor that affects both cardiac structure and migraine susceptibility.[42]

Biomarkers need not be causally related to migraine, though, in order to be useful. Intermediate biomarkers might also be related to or caused by an environmental exposure that results in migraine. For example, elevated antibody titers against a particular virus might be a biomarker for migraine because they reflect the presence of an organism that causes migraine, not because they themselves are involved in its development. There has been relatively little scientific investigation of toxic, infectious, or other environmental influences on migraine. It is entirely plausible that such exposures might modify the risk of developing migraine if they interact with genetic susceptibilities, or might alter the course of migraine once it has developed. One author has pointed out that with chronic neurological diseases, an important practical difficulty with biomarkers of exposure is finding markers "that are stable over the long periods required for prospective studies of chronic neurological disease. . .banked serum or plasma may be of value in some instances. . ."[43]

Another important caveat is that the technical characteristics of a migraine biomarker that have been established in one situation or group may not apply over the entire natural history of the disorder, in all subgroups, or in all situations. Measurement error or imprecision will account for some of this variability, but so too will factors such as biological variability.

Lesko and Atkinson have identified the technical attributes of a useful biomarker as (1) clinical relevance; (2) sensitivity and specificity to treatment effects; (3) reliability; (4) practicality; and (5) simplicity.[1] The Antecedent Biomarkers Group, convened to examine the use of biomarkers in Alzheimer's disease, has described criteria for effective biomarkers in that disorder, many of which apply to migraine as well.[44] These include:

- Detection of a fundamental feature of neuropathology. . ."validated in neuropathologically confirmed cases"
- Diagnostic sensitivity and specificity of over 80%
- Reliable, reproducible, noninvasive, simple to perform, and inexpensive
- Established by

- Two independent studies with similar findings
- Published in a peer-reviewed journal.

Ethical Issues.— Ethical issues are likely to arise that are related to costs and the fair distribution of biomarker benefits and burdens to all segments of the migraine population. Despite the fact that biomarker development will produce efficiencies in clinical trials and improve the overall management of migraine, it is unlikely to reduce treatment expense. It may also decrease incentives for pharmaceutical companies to pursue drug therapies. Most of the costs associated with bringing a drug to market are fixed, and drugs that gain formal FDA indications for smaller and smaller biomarker-defined subgroups means less scope for companies to recoup their research investments unless they increase the price of such drugs.

Another inadvertent result of the more precise drug labeling made possible by biomarkers is that "off-label" prescribing of such drugs might increase, since it always will be possible that treatments tested in a subgroup of migraineurs with a particular biomarker profile also will be effective in patients with other profiles. On the other hand, there are powerful disincentives to "off-label" prescribing that are likely to intensify in the future.[45] It seems entirely possible that more exact characterization of the migraine population in treatment trials will be used as a basis for treatment limitations and denials by insurance and other third party payers. They might refuse to pay for the studied treatment in any patient whose biomarker profile is not identical to that of trial subjects, or might balk at the prospect of paying for biomarker testing.

Thus, biomarker development seems likely to open a new and largely unanticipated front in the struggle between those who seek to apply new technology for the benefit of patients, and those who have a vested interest in using it to maximize profits. A likely paradox is that pharmaceutical companies may not have sufficient financial incentives to conduct the trials necessary to characterize a drug's performance in patients with a potentially endless array and combination of biomarkers, while insurance companies would seek to limit care and costs by refusing to pay for treatments on the basis that such studies had not been done or were not convincing. This combination of perverse incentives could seriously slow the translation of achievements in biomarker research into improvements in migraine care, with patients and their doctors, as usual, caught in the middle.

Legal Issues.—Legal and political issues, some of them novel, will surely arise with the advent of migraine biomarker technology. A fear of malpractice suits could lead to premature clinical use of biomarkers, particularly those that might detect unusual forms of migraine or help avoid serious treatment side effects. This would be unfortunate if it prompted extensive use of a biomarker in clinical practice before its true utility and cost-benefit profile had been clearly established; such a situation did occur with the use of prostate specific antigen testing for prostate cancer, suggesting that this is a danger that will recur again with biomarkers in many areas of medicine.[46] As discussed previously, pharmaceutical companies might use biomarkers to exclude subjects at high risk of drug side effects from clinical trials, to avoid liability. While this practice might decrease the risk of harm to individual trial subjects, if carried to an extreme it also may mean that the harm to benefit balances of some treatments simply will be assumed, rather than proved.

Yet another legal aspect of biomarker development has to do with whether genes, gene sequences, gene products, or tests for these things can be patented. The prospect of paying a licensing fee in order to be able to do scientific or clinical research on a particular migraine gene or protein, or paying a royalty for the use of a test based on these things, is appalling to many. A 1980 US Supreme Court decision involving a genetically engineered bacterium cleared the way for patents on "anything under the sun that is made by man."[47] Over the next decade, thousands of patents on genes and DNA sequences were sought, in some cases by companies and researchers who had no understanding of what they sought to patent, but merely wanted to stake a claim. Patents have been issued for approximately 20% of the human genome.[48] In response to this flurry of biological patent activity, the US patent office tightened its scrutiny of biotechnology patents and now grants patents only in cases where "specific and substantial utility" and applicability

to research has been shown.[49] Several other recent developments have calmed fears that gene patents will impede research. For example, after a significant struggle, scientists have extracted an agreement that information derived from the Human Genome Project immediately will enter the public domain.[50] Courts outside the United States, notably those in Europe and Canada, generally have not ruled favorably on gene patents.[47,51] In a closely watched case with obvious implications for the medical use of biomarker technology, the US Supreme Court is set to rule this fall on the legality of a patent sought for "the simple correlation of an elevated level of the amino acid homocysteine with a deficiency of 2 B vitamins."[52]

CONCLUSION

No review article can conclude without a catalogue of research that remains to be done. In the headache field biomarker development has focused almost exclusively on migraine. There has been little work on tension-type headache, posttraumatic headache, or cluster headache, to list just a few other headache disorders that also deserve attention. The precedents and knowledge derived from the study of migraine biomarkers undoubtedly will provide vital direction when the time arrives to apply biomarker technology to other headache disorders.

We are at a very early stage of migraine biomarker development, but nonetheless one where certain things are clear. One desirable consequence of biomarker identification will be the firm establishment of migraine as a legitimate, biologically based medical problem. This is heartening because the all too common view of migraine as a quasi-medical complaint plays a large part in its stigma and poor care. Other predictable and similarly desirable outcomes include refinements in migraine diagnosis, treatment, and research, as well as the appealing prospect of being able to identify migraine-susceptible individuals at a stage when simple interventions are highly effective.

All of this is very exciting, but these advances do not come without equally daunting costs. For each benefit there is a countervailing drawback, pitfall, or unintended consequence—sometimes several. One undesirable consequence of the biomarker revolution will be to focus attention on ever smaller groups of patients with migraine subtypes that were formerly indistinguishable by clinical methods, but that now can be precisely defined by biologic measurements. This may impede the study of broad, general patterns, and mechanisms that different forms of headache have in common, and limit appreciation for widely applicable, over-arching principles of causation or treatment. By restricting the size and diversity of trial populations, biomarkers also might reduce the chance for serendipitous or unexpected findings that historically have played a critical, hypothesis-generating role in medical research.

Yet another important and so far unexamined disadvantage of the biomarker revolution might be a further decline in respect for the contribution of clinical care and judgment in migraine management. It is an unfortunate fact of modern medicine that those who make basic science discoveries are venerated, while those who specialize in applying these discoveries at the level of the individual patient are held in low regard. Will the brave new world of migraine biomarkers add to this problem by relegating the doctor to the role of ordering tests, compiling results, and then applying expert-determined treatment algorithms in a manner calculated to produce the highest pay-for-performance returns? One hopes not. In fact, it seems likely that the best possible use of migraine biomarkers will require considerable clinical expertise and imagination. For one thing, biomarkers that predict treatment benefit may well assort independently of those that predict side effects or a host of other important factors, so that clinical trials never could be done to provide guidance on how to proceed in every individual circumstance. Biomarkers will augment, not supplant, the need for clinical judgment, which will be even more necessary to advise patients about complicated issues and to interpret and apply a potentially vast and confusing array of resulting information.

Conflict of Interest: None

REFERENCES

1. Lesko LJ, Atkinson AJ. Use of biomarkers and surrogate endpoints in drug development and regulatory decision making: Criteria, validation, strategies. *Ann Rev Pharmacol Toxicol*. 2001;41:347-366.

2. Mayeux R, Saunders AM, Shea S, et al. Utility of the apolipoprotein E genotype in the diagnosis of Alzheimer's disease. Alzheimer's disease centers consortium on apolipoprotein E and Alzheimer's disease. *N Engl J Med*. 1998;338:506-511.
3. Ladenson PW. Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer. *Clin Chem*. 1996;42:183-187.
4. Detels R, Tashkin DP, Simmons MS, et al. The UCLA population studies of chronic obstructive respiratory diseases. Agreement and disagreement of tests in identifying abnormal lung function. *Chest*. 1982;82:630-638.
5. Packer CS. Biochemical markers and physiological parameters as indices for identifying patients at risk of developing pre-eclampsia. *J Hypertens* 2005;23:45-46.
6. International Classification of Headache Disorders II. *Cephalalgia*. 2004;24(suppl 1):1-160.
7. Loder E, Biondi D. Disease modification in migraine: A concept that has come of age? *Headache*. 2003;43:135-143.
8. Loder E, Sheftell F. The quality of headache care in the United States: Review and analysis of recent data. *Headache*. 2005;45:939-946.
9. Rose G. Sick individuals and sick populations. *Int J Epidemiol*. 2001;30:427-432.
10. Hampton T. Clinical trials point to complexities of chemoprevention for cancer. *Med News Perspectives JAMA*. 2005;294:29-31.
11. Kaiser J. Genomic databases. NIH goes after whole genome in search of disease genes. *Science*. 2006;311:933.
12. Vanmolkot KR, Kors EE, Turk U, et al. Two de novo mutations in the Na, K-ATPase gene ATP1A2 associated with pure familial hemiplegic migraine. *Eur J Hum Genet*. 2006;14:555-560.
13. Kosmeder JW II, Pezzuto JM. Intermediate biomarkers. *Cancer Treat Res*. 2001;106:31-61.
14. Drumm ML, Konstan MW, Schluchter MD, et al. Genetic modifiers of lung disease in cystic fibrosis. *N Engl J Med*. 2005;353:1443-1453.
15. Rodenhiser D, Mann M. Epigenetics and human disease: Translating basic biology into clinical applications. *CMAJ*. 2006;174:341-348.
16. Weaver IC, Cervoni N, Champagne FA, et al. Epigenetic programming by maternal behavior. *Nat Neurosci*. 2004;7:847-854.
17. Sevignani C, Calin GA, Siracusa LD, Croce CM. Mammalian microRNAs: A small world for fine-tuning gene expression. *Mamm Genome*. 2006;17:189-202.
18. Lu J Getz G, Miska EA, et al. MicroRNA expression profiles classify human cancers. *Nature*. 2005;435:834-838.
19. Haston CK, Hudson TJ. Finding genetic modifiers of cystic fibrosis. *N Engl J Med*. 2005;353:1509-1511.
20. Green ED, Waterston RH. The human genome project: Prospects and implications for clinical medicine. *JAMA*. 1991;266:1966-1975.
21. Diamandis EP. Mass spectrometry as a diagnostic and a cancer biomarker discovery tool: Opportunities and potential limitations. *Mol Cell Proteomics*. 2004;3:367-378.
22. Bearer CF. Markers to detect drinking during pregnancy. *Alcohol Res Health*. 2001;25:210-218.
23. Fin OJ. Immune response as a biomarker for cancer detection and a lot more. *N Engl J Med*. 2005;353:1288-1290.
24. Klerman EB, Gershengorn HB, Duffy JF, Kronauer RE. Comparisons of the variability of three markers of the human circadian pacemaker. *J Biol Rhythms*. 2002;17:181-193.
25. Bramanti P, Grugno R, Vitetta A, Marino S, DiBella P, Nappi G. Ictal and interictal hypoactivation of the occipital cortex in migraine with aura. A neuroimaging and electrophysiological study. *Funct Neurol*. 2005;20:169-171.
26. Franceschi M, Colombo B, Rossi P, Canal N. Headache in a population-based elderly cohort. An ancillary study to the Italian Longitudinal Study of Aging (ILSA). *Headache*. 1997;37:79-82.
27. Mazzotta G, Ballai V, Alberti A, et al. Characteristics of migraine in an out-patient population over 60 years of age. *Cephalalgia*. 2003;23:953-960.
28. Plosker GL, Keam SJ. Trastuzumab: A review of its use in the management of HER2-positive metastatic and early-stage breast cancer. *Drugs*. 2006;66:449-475.
29. Guidance for Industry: Pharmacogenomic Data Submissions. US Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research, Center for Biologics Evaluation and Research, Center for Devices and Radiological Health. March 2005. Available at URL http://www.fda.gov/CBER/gdlns/pharmdtasub.pdf on April 26, 2006.
30. International Headache Society Clinical Trials

Subcommittee. Guidelines for controlled trials of drugs in migraine: Second edition. *Cephalalgia*. 2000;20:765-786.
31. Biomarker study can save Iressa. Reed Life Science News. Available on www.genpromag.com/-ShowPR~PUBCODE~018~ACCT~1800000100~ISSUE~0503~RELTYPE~RLSN~PRODCODE~00000000~PRODLETT~E.html. Accessed online April 12, 2006.
32. Pollack A. A special drug just for you, at the end of a long pipeline. New York Times, Tuesday, November 8, 2005.
33. Silberstein SD, Goadsby PJ. Migraine: Preventive treatment. *Cephalalgia*. 2002;22:491-512.
34. Taube SE, Jacobson JW, Lively TG. Cancer diagnostics: Decision criteria for marker utilization in the clinic. *Am J Pharmacogenomics*. 2005;5:357-364.
35. Mancini GB, Schulzer M. Reporting risks and benefits of therapy by use of the concepts of unqualified success and unmitigated failure: Applications to highly cited trials in cardiovascular medicine. *Circulation*. 1999;99:377-383.
36. Schulte PA, Perera FP. Validation. In: Schulte PA, Perera FP, eds. Molecular Epidemiology: Principles and Practices. San Diego: Academic Press; 1993:79-107.
37. Pepe MS, Thompson ML. Combining diagnostic test results to increase accuracy. *Biostatistics*. 2000;1:123-140.
38. Thompson ML, Zucchini W. On the statistical analysis of ROC curves. *Stat Med*. 1989;8:1277-1290.
39. Haker S, Wells WM, Warfield SK, et al. Combining classifiers using their receiver operating characteristics and maximum likelihood estimation. Lecture Notes in Computer Science 2005; Volume 3749: 506-514. Published proceedings of MICCAI 2005: 8th International Conference, Palm Springs, CA, October 26-29, 2005. Editors: James S. Duncan, Guido Gerig. Available on http://spl.harvard.edu:8000/pages/papers/haker/haker-miccai2005.pdf. Accessed online May 3, 2006.
40. Rubenstein JH. The cost-effectiveness of biomarkers for predicting the development of oesophageal adenocarcinoma. *Aliment Pharmacol Ther*. 2005;22:135-146.
41. Porter A, Gladstone JP, Dodick DW. Migraine and white matter hyperintensities. *Curr Pain Headache Rep*. 2005;9:289-293.
42. Dalla Volta G, Guindani M, Zavarise P, et al. Prevalence of patent foramen ovale in a large series of patients with nigraine with aura, migraine without aura and cluster headache, and relationship with clinical phenotype. *J Headache Pain*. 2005;6:328-330.
43. Mayeux R. Biomarkers: Potential uses and limitations. *NeuroRx*. 2004;1:182-188.
44. Antecedent biomarkers in Alzheimer's disease: Uses, limitations, and future directions for research. Available on http://www.alzforum.org/res/enab/workshops/bioma-rkers.asp. Accessed online April 13, 2005.
45. O'Reilly J, Dalal A. Off-label or out of bounds? Prescriber and marketer liability for unapproved uses of FDA-approved drugs. *Ann Health Law*. 2003;96:429-431.
46. Hirst G. Consent before testing men for prostate cancer—a challenge? *Aust Fam Physician*. 2005;34:889-891.
47. Stix G. Owning the stuff of life. *Sci Am*. 2006;294:76-83.
48. Jensen K, Murray F. Intellectual property landscape of the human genome. *Science*. 2005;310:239-240.
49. Grisham J. New rules for gene patents. *Nat Biotechnol*. 2000;18. Available on http://www.biotech-info.net/new_rules.html. Accessed online April 14, 2006.
50. Marris E. Free genome databases finally defeat Celera. *Nature*. 2005;435:6.
51. Matthijs G. The European opposition against the BRCA gene patents. *Fam Cancer* 2006;5:95-102.
52. Ruttimann J. US to rule on research patent. *Nature* 2006;440:587.