

Should genetic testing become a standard component of clinical trials in order to advance evidence-based cardiology practice?

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Medical therapy currently assumes that all individuals with a given condition should be treated in the same way, guided by empirical evidence from clinical trials. This ignores interindividual differences, which may predict variable therapeutic efficacy or adverse drug reactions. Considerable evidence suggests that genetic makeup determines much of this variability, and major efforts are being made to harness genetics to predict drug responses. Pharmacogenetics can improve the power and informativeness of clinical trials and increase the value of new or existing drugs. However, the precise characteristics of the underlying genetic variants will strongly impact the ease with which pharmacogenetic studies can deliver these high expectations, to the extent that it is not yet efficient to systematically include genetic testing in clinical trials.

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THE PROBLEM

Both individual clinical practice and the cumulative experience reported in the literature indicate that conventional pharmacological therapy is a very imperfect science. The proportion of patients in whom the desired goal is achieved is variable, but usually surprisingly low, while adverse effects are a persistent problem. These include both the predictable problems of under- or overachieving the intended pharmacological intervention and unpredictable but stereotyped, “idiosyncratic” adverse drug reactions (ADRs). Monitoring drug levels, or a target response, can be instituted for a subset of drugs where the therapeutic window is narrow. However, in the vast majority of circumstances it is not possible to predict likely responders or those at risk of ADRs and so therapy is conducted on a “trial and error” basis.

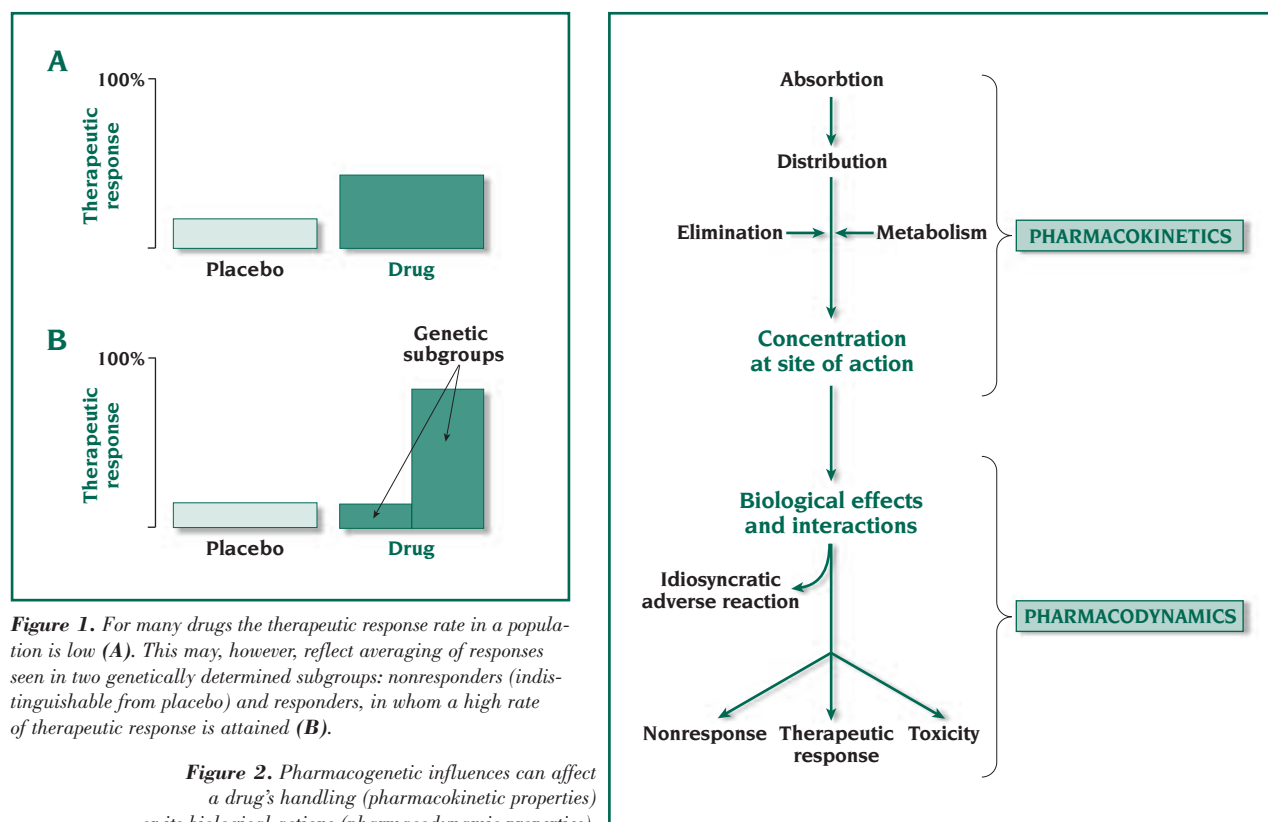
The disadvantages of this approach are obvious. At the very least, treatment is inefficient with substantial wastage of resources (both drugs and consultation time) and compliance is low. Even with extensive regulatory mechanisms in place, ADRs constitute a top ten cause of mortality.¹ Because serious unpredictable ADRs tend to be rare, they are often only documented late in the course of development of new therapies, or in postmarketing surveillance. Many otherwise good agents are therefore lost and the cost of bringing a drug to market drastically increased.

THE PROMISED SOLUTION: PHARMACOGENETICS

In keeping with all other biological traits, individual variability in drug response will inevitably share both genetic and environment components. In some instances, the heritability of variable drug response is known to be high, leading to the notion that a population might be partitioned into responders and non-responders by their genetic makeup (*Figure 1*). Further, because a drug's action is mediated by a relatively circumscribed number of target and signaling proteins, it is reasonable to hope that the number of genes determining a particular response may be lower, and the size of individual genetic effects may

SELECTED ABBREVIATIONS AND ACRONYMS

ADR	adverse drug reaction
CETP	cholesterol ester transfer protein
MDR	multidrug resistance
SNP	single nucleotide polymorphism



be larger (and hence more tractable), than in the common complex trait diseases. Given this, the obvious clinical need, and the advances arising from the Human Genome Project, major investments are being made toward unraveling such genetic factors, and the field of pharmacogenetics is burgeoning. Thus, the question posed for this article now raises real issues, whereas, even 5 to 10 years ago, it would have made little sense.

Pharmacokinetic and pharmacodynamic variation

Many instances of interindividual variation in drug handling have long been known to be genetic, for example, those attributable to variants of the cytochrome P450-metabolizing enzymes. Such pharmacokinetic variation can be attributed to genetic variants impacting on absorption, distribution, metabolism, and

excretion with marked effects on the bioavailability of a given agent (Figure 2). Systematic analysis of such genetic variants therefore holds the promise of tailoring individual dosage regimens to achieve the desired levels at the target organ. For example, the cytochrome P450 CYP2C9 enzyme metabolizes many clinically important drugs, including warfarin, the angiotensin II receptor antagonists, and fluvastatin. Uncommon variants in CYP2C9 (affecting ≈ 1 in 250) reduce its activity and markedly influence dose requirements²; life-threatening bleeding episodes have been reported in poor metabolizers of CYP2C9 exposed to warfarin.³

Variation that will affect a drug's action and interactions, that is, that will alter its pharmacodynamic profile (Figure 2), is currently less well understood at the DNA level, but the genetic influence is likely to be

just as pervasive. Variation within the target molecule, for example, G-protein-coupled receptors or second messenger systems, will be of significance, but perhaps more important will be the genetic heterogeneity underlying the etiology of most common conditions. A genetic classification of disease may predict biological, hence therapeutic, responses. For example, only in a subset of asthma patients do elevated leukotrienes contribute to the disease; accordingly, stratification by genotype in the 5-lipoxygenase gene predicts response to antiasthma treatment with a specific leukotriene inhibitor.⁴ By combining genetic sources of both pharmacokinetic and pharmacodynamic variation, it is reasonable to hope that a genetic classification may allow prediction of responders, nonresponders, and adverse responders to relevant classes of drugs (Figure 3, page 38).

GENETIC APPROACHES

As with analysis of inherited disease, many of the early precedents relate to single gene influences on phenotype. These are likely to provide informative examples, but, as with disease, probably account for a minority of pharmacogenetic variation. Examples include major effect vari-

ants in key enzymes in metabolism (see above) and in drug transport, for example, genetic variants in the multidrug resistance (MDR)-1 gene, important for handling a number of chemotherapy agents and also digoxin.⁵ Inherited mutations underlying susceptibility to malignant hyperthermia illustrate a monogenic pharmacodynamic adverse re-

sponse.⁶ Single gene examples of disease-causing mutations that define the genetic etiology of a heterogeneous condition, and hence predict response to treatment, include the different genetic etiologies of long QT syndrome.⁷ Subtle variants in the same genes illustrate the way in which rare genetic variants can underlie idiosyncratic ADRs; for example, sporadic mutations, as well as relatively prevalent single nucleotide polymorphisms, in potassium channel subunits, have been shown to underlie antibiotic-induced cardiac arrhythmias.^{8,9} For identification of both single gene effects and the presumably more prevalent oligogenic influences on drug response, a choice of genetic strategies exists.

Candidate gene studies

Candidate gene studies are limited in that they can only test known genes that are already plausibly implicated in the underlying process.

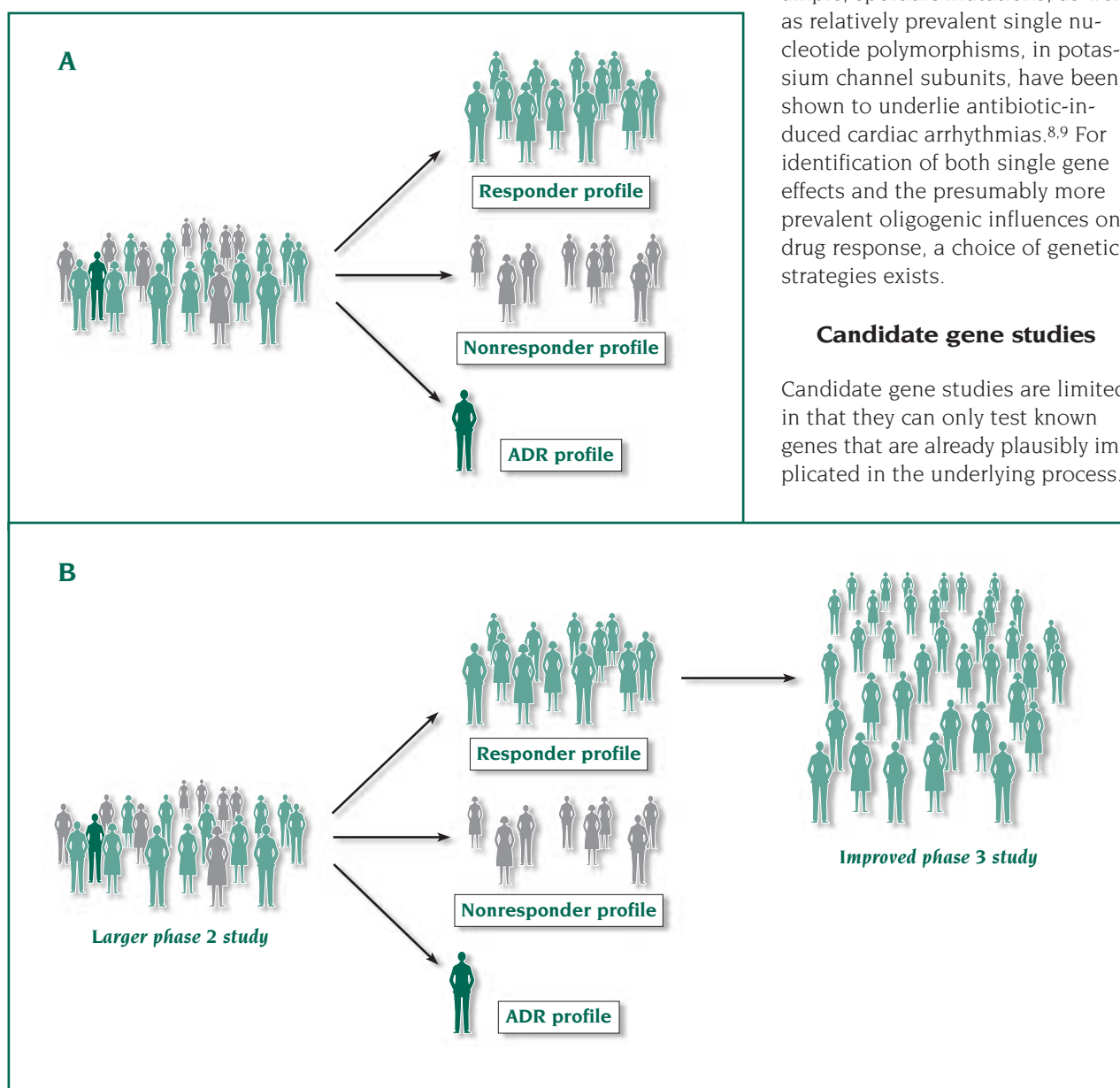


Figure 3. Detailed genetic analysis (either candidate gene or genome-wide single nucleotide polymorphism [SNP] typing) of extended early clinical trials aims to partition the population into responders, nonresponders, and those at risk of adverse drug reactions (ADRs) (A). If genotyping can identify responders, selected entry into subsequent phase 3 trials has the potential to improve power to show efficacy. Thereafter, the drug could be targeted to those it would benefit with reduction in risk of ADRs (B).



However, this limitation may be less of a handicap in investigating drug responses as the downstream pathways are often quite well mapped out. Thus, DNA can be typed for variants affecting proteins thought likely to be involved in drug handling, the target itself, and downstream signaling. A prominent, but as yet unsubstantiated, study of this type is the proposed role of cholesteryl ester transfer protein (CETP) polymorphisms in the response to "statins."¹⁰ In parallel, the candidate net can be thrown much wider by harnessing the emerging genomic and proteomic tools for identification of novel candidate genes.¹¹ For example, DNA microarray experiments for expression profiling (comparing normal and pharmacologically manipulated systems) can prioritize genes for screening for DNA variants. Such pharmacogenomic approaches can also provide new data for mechanistic studies and perhaps even new markers for early signs of drug toxicity.

Genetic analysis of variants in candidate genes is conducted by association study, comparing the incidence of a given variant in cases and controls—in this instance, for example, responders versus nonresponders or those with ADR versus those without. In principle, this is a simple study design lending itself easily to collection of the necessary data within the framework of a randomized control trial. In practice, different properties of the underlying genetic polymorphisms (particularly the allele frequency and mode of gene action) have a dramatic impact on the trial sample size needed.¹² In addition, experiences with candidate gene analysis in disease association studies indicate the importance of taking other complexities into consideration. These include analyses of multiple variants in a gene to gain information on

haplotype structure, consideration of gene-gene and gene-environment interactions, and awareness of the confounding effects of population admixture.

Systematic approaches to defining pharmacogenetic variants

The power of genetics is at its greatest when genome-wide analyses can be performed without the constraints of prior hypotheses. The conventional approach to genome-wide analyses has been to use linkage analysis in families, as has been widely successful in single gene disorders and has had some notable recent successes in complex traits.^{13,14} However, data on drug responses will not often be available in related individuals, and so linkage analysis is generally not an option. Systematic approaches to genome-wide association have been much discussed, and may be now coming of age.¹⁵ Once the consensus sequence of the Human Genome Project was complete, focus shifted to defining the extent of variation in individuals. An extremely large set of single nucleotide polymorphisms (SNPs) is being assembled by public and private efforts, particularly the SNP consortium, with a view to constructing genome-wide high-density SNP maps. Major uncertainty exists, however, as to the extent of linkage disequilibrium across the human genome, and particularly as to whether conserved "blocks" exist in a way that will much reduce the number of SNPs that need to be typed to provide genome-wide coverage.¹⁶ In the meanwhile, there is a premium on intragenic polymorphisms and, particularly, those polymorphisms likely to exert a direct functional effect. The first genome-wide SNP association study to detect a disease-susceptibility allele has just been published, like-

ly heralding a new era of very large-scale association analyses. This study, which implicates a cytokine gene in the etiology of coronary artery disease, involved typing of over 60 000 SNPs in 100 cases and 500 controls as a preliminary experiment, prior to reevaluating the most promising 600 SNPs in more than 2000 cases and controls.¹⁷

Optimism exists in some quarters that it will be possible to use "abbreviated SNP maps" to identify categories of drug response by complex pattern recognition rather than by going on to formally define a particular susceptibility variant (or set of variants).^{18,19} Thus, the hope would be that an SNP profile could be established for patients displaying either adverse events or a therapeutic response. If such a profile was sufficient to capture genome-wide susceptibility information, and yet was relatively simple to perform, then it could be used in individual preprescription analyses.¹⁸

Even if the optimism is well placed, SNP genotyping capacity and prices will need to continue to improve at least as steeply as they have done over the past few years. One potential solution to the cost of SNP typing, which is being explored in complex trait genetic analyses as well as in pharmacogenetic studies, is the pooling of DNA samples among cases and among controls (as distortions in the frequency of particular variant between cases and controls could, in theory, be detectable by typing just the two pools).

"Proof of principle" studies

In the meantime, a compromise position will likely be large-scale and systematic analyses of SNPs across each of a large number of candidate genes, thus broadening the scope and allowing the testing of more

than the most obvious genes while still falling short of a truly genome-wide analysis. Those studies that are currently offered as “proof of principle” fit this category. For example, a severe hypersensitivity reaction occurs in approximately 5% of individuals treated with the antiretroviral abacavir. As this side effect had overt immunological features, 114 polymorphisms in 12 candidate gene families were selected for an association analysis, allowing the identification of risk-associated variants in the tumor necrosis factor- α (TNF- α)/HLA B57 locus.²⁰ In a treatment cohort of 200 individuals, the presence of three human leukocyte antigen (HLA) alleles had a positive predictive value for hypersensitivity of 100% and a negative predictive value of 97%.²¹

Limitations

The feasibility of defining clinically useful pharmacogenetic variants will depend on parameters that will vary for each gene/drug interaction. As with all susceptibility alleles, the power of a genetic study and subsequent utility of a genetic marker will critically depend on the size of the genetic effect. Model organism genetics tells us that weak effect variants will far outnumber the occasional major effect variants. The overall contribution to the disease or response will reflect both the size of the effect and the population prevalence of the susceptibility allele. Thus, functionally significant variants in the various *CYP* genes tend to be present at between 2% to 10% of the population, an appropriate frequency to be optimistic about their utility. Variants that are much rarer will be less tractable and less effective in cost terms (unless the adverse effect is exceptionally severe). It is also likely that genotyping will only be the investigation of choice where there is not another

simple functional assay that gives a readout reflecting the cumulative impact of a number of genes. For example, a competing strategy to predict an individual's pharmacokinetic profile is the administration of a small panel of tracer substances followed by their analysis in blood and urine to give a detailed picture of the full complement of metabolic and excretion pathways variation.

HOW CAN PHARMACOGENETICS BE USED TO IMPROVE DRUG DEVELOPMENT, TRIALS, AND SURVEILLANCE?

If it is shown to be feasible to define pharmacogenetic variants with important predictive accuracy, then, in the near future, we should expect to see changes to the way in which drugs are evaluated in clinical trials.

More information from phase 2 studies

If possible, the most attractive option would be to perform comprehensive genetic analyses in phase 2 studies, even if this required an enlargement of such studies, in order to yield substantial benefits in the more costly phase 3 studies. Perhaps most plausibly this will first be achieved for ADR pharmacogenetics. DNA from individuals with ADRs would be compared with others enrolled in the trial by either the candidate gene or systematic approach. In pragmatic terms, this would most easily be achieved by archiving blood samples (for example, a stored blood spot much as is used for the Guthrie test) on all enrolled subjects as this would be cheaper and easier to orchestrate than retrospective collection of informative samples. Clearly, however, there are additional ethical considerations (discussed below). Once predictors of an adverse outcome are identi-

fied, genotyping could be performed at entry into phase 3 studies with considerable reduction in risk and with increased likelihood of ultimately gaining regulatory approval.

More efficient phase 3 studies

Much harder to achieve, but of significant promise, would be to use genetic analysis of larger phase 2 trials to define responder and non-responder profiles. If feasible this would allow smaller and more economical phase 3 trials restricted to a potentially susceptible population. Exclusion of predicted nonresponders would save such individuals from fruitless participation and would flag (at a much earlier stage) the need for additional therapies. Such genotype criteria have already entered the drug development process in a few instances. A phase 3 trial of desipramine required CYP2D6 genotyping to exclude the 7% to 10% of “poor metabolizers” who could be predicted to not benefit²²; this removed the need for continuous plasma concentration monitoring. An analogous approach, for example in primary prevention trials, is to use DNA typing for documented susceptibility variants to enrich the study for those most at risk; this would increase the event rate and the power of the study to resolve differences in treatment efficacy at an early stage.

Can genotyping improve trial power?

Under some circumstances, genotyping could reduce the trial size needed to show efficacy of a given agent. A large proportion of nonresponders can mask a good response in a subset (*see Figure 1*) such that genetic stratification could yield a more significant result. By modeling single gene effects, Cardon et al



have shown that the impact on sample size is critically dependent on allele frequency (best when the variant is common) and on mode of action (dominant effects needing smaller sample sizes than codominant or recessive).¹² Importantly, this means that sample size can be either much smaller, or much larger, than that needed in a nongenetic trial, and so attempts to gain power by genotyping in a large (eg, phase 3) study would only make sense where the details of the genetic factors contributing to outcome are known.

Surveillance

Analysis of DNA from the full phase 3 trial population—and indeed from postmarket surveillance—may, however, be needed to document the susceptibility to serious, but rare, ADRs. Similarly, if the power to resolve drug responses or resistance is not adequate in phase 2, or the research not complete (a realistic situation at present), DNA analysis may be needed from selected individuals from the phase 3 study (those informative for a given response). It appears to be the view of most pharmaceutical companies that this might most economically be performed by sample archiving at the point of enrolment rather than by retrospective collection.

Ethical and legal issues

Informed consent is often considered to be especially problematic in genetic studies. However, the main reason for this is the potential of genetic analysis to reveal data that have implications for individuals other than the study subject (ie, first-degree relatives) or that have the potential to yield predictive information about future disease liability. These concerns should be much less prominent in pharmaco-

genetic analyses as the familial impact is low and drug response variants will generally not relate to underlying disease risks. Where they do, for example, in classification of genetic subtypes of a heterogeneous disease, the problem is generally less in clinical trials because the individuals are already either known to be affected or at high risk (in the case of primary prevention trials). One issue that will need exploring and specific explanation during the consent process, is that pharmacogenetic testing has obvious commercial implications and data arising from a study (for example, an academic study) might later lead to exploitable intellectual property. Concerns have also been voiced about the partitioning of populations into those for whom a treatment may not be offered, but, so long as the genetic test is sound, these individuals would not have benefited from the proposed treatment anyway. One can anticipate a move toward reimbursement being restricted for efficacious therapies, or licensing being subject to pairing with a pre-prescription diagnostic test; appropriate regulation will only be feasible if the underlying genetic science can be clearly validated.

Other postulated benefits

One justification sometimes offered for routine collection of DNA on all participants in a study is the hope that such a DNA collection could contribute to research into the underlying disease etiology. While genetic analysis into the basis of disease susceptibility holds huge potential in the long term for identification of new targets and the derivation of rational therapies, the power of such studies is dependent upon the selection of extreme phenotypes. Thus, patients with typical age of onset, or severity, of disease (as are usually enrolled into clinical

trials) are often of insufficient genetic enrichment to be informative. Similarly, disease-gene identifying studies need to be designed with genetic issues paramount: in particular, this will involve close genetic matching of the control subjects and it may also require collection of affected family members (in order to perform genome-wide linkage studies). Further, a quite different consent process is needed. Thus, such approaches would not support a proposal for DNA collection to become a standard component of routine clinical trials.

ROUTINE GENETIC TESTING: IF NOT YET, WHEN?

At present, much of the research into the potential of pharmacogenetics is driven by commercial pressures and is conducted by the pharmaceutical industry.^{18,19} This is not to say that advances in the field will not be of sound benefit for evidence-based cardiology, but rather that commercial considerations may determine the timetable of when and how systematic genetic sampling becomes a standard component of clinical trials. Where it is anticipated that genetic testing could accelerate phase 3 studies, facilitate regulatory approval, or even salvage a drug that had had to be dropped due to an unacceptable ADR rate, we can expect to see imminent applications. Certain drug indications will have greater priority. Pharmacogenetic predictions will be most relevant where treatment is administered long term, where there is a narrow therapeutic window (and no easy alternative way to measure or predict levels), or where there are serious unpredictable idiosyncratic complications. In many other instances, where treatments are well tolerated with fewer serious complications, pharmacogenetics will

move more slowly even though it may yet have much to offer in terms of prioritizing treatments by likely efficacy. Because of the current advantages of relatively simple genetic systems and candidate genes, pharmacogenetic tests are likely to be introduced first in situations where the biology is already quite well understood. Such knowledge will also be necessary to determine whether genotyping could reduce trial size.

While we are not yet at the stage where systematic genetic testing can be considered a cost-effective component of clinical trials, we are already close to the time where systematic collection of such samples can be justified—to allow genotyping in informative subsets thereafter. The field is moving particularly fast and the indications are that pharmacogenetics (particularly in the field of adverse events) will be the first area of complex trait genetic analysis to impact directly on health care. While the advent of truly “personalized” medicine is still a long way off, the days of blind treatment identical for all are numbered.

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