Multigenic Control of Drug Response and Regulatory Decision-Making in Pharmacogenomics: The Need for an Upper-Bound Estimate of Genetic Contributions

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Abstract: Nature or nurture? To what extent genetics play a role in drug efficacy and safety? These questions are not new. They are however gaining increasing prominence with the implementation of pharmacogenomics in various facets of medicine ranging from therapeutics, drug development and regulatory science to research funding decisions. For predisposition to common complex diseases, twin and family studies have been the mainstay for estimating genetic components of the attendant risk. On the other hand, the rapid pace of drug development in the pharmaceutical industry and the need for faster regulatory decisions call for an approach of higher throughput to identify the compounds for which heritability is likely to play a significant role in their pharmacokinetics and/or pharmacodynamics. A second predicament related to multifactorial nature of drug effects is that one typically observes a considerable overlap in the distribution of drug response phenotypes among subpopulations identified by each pharmacogenomic biomarker. This is in sharp contrast to monogenic pharmacological traits wherein it is feasible to partition the patient populations into discrete subgroups by analysis of a single gene. Hence, as pharmacogenomic investigations progress from monogenic to increasingly multigenic or multifactorial drug response phenotypes, the regulatory decision-makers are faced with a dilemma: How can a reviewer or a clinician determine if a given separation of a drug response profile by a pharmacogenomic biomarker is worthwhile for clinical implementation? The present manuscript makes an attempt to address these broad and emerging issues in pharmacogenomics and regulatory science. We propose that a comparison of inter- versus intra-subject variability in drug response under minimal environmental exposure may provide an upper-bound estimate of heritability of drug efficacy and safety. It is also argued that seemingly modest changes in population averages may underestimate the dramatic impact of a genetic biomarker at the tails of a population. To this end, a conceptual framework for graded risk assessment among subpopulations with overlapping quantitative phenotypes is presented. We conclude with a broader discussion of the evolution of genetic biomarkers from monogenic to multigenic traits in pharmacology, the associated ethical, social and therapeutic policy corollaries and the challenges lying ahead.

Key Words: Pharmacogenomics, biomarkers, genetic components, twin study, drug regulation, repeated drug administration study, RDA, risk assessment, bioethics, therapeutic policy.

INTRODUCTION

The role of genetic factors for patient-to-patient and between-population differences in drug efficacy and safety is a central focus for pharmacogenomics. The thrust of pharmacogenomics in clinical medicine has been fueled by sequencing the human genome in 2001 and more recently, with the launch of the international haplotype mapping project aimed at determining the patterns of closely linked single nucleotide polymorphisms (SNPs) on each individual chromosome [Lander *et al*. 2003; Venter *et al*. 2003; International HapMap Consortium, 2003; Reidenberg, 2003]. The high throughput genomic technologies that spun off from these important research initiatives are increasingly utilized as key research tools, in effect, industrializing the scale of pharmacogenomic research in both academia and pharmaceutical companies [Aklillu *et al*. 2002; Kurth, 2003; Manasco and Arledge, 2003; McLeod, 2004]. On the other hand, an important implicit assumption underlying clinical pharmacogenomic studies is that the *targeted pharmacological trait is subject to appreciable genetic control*. Human geneticists dealing with common complex diseases are keenly aware of this fundamental assumption in genetic research. Hence, it is considered prudent to present convincing *a priori* evidence of the size of the genetic component for a trait under investigation such as breast cancer, diabetes or schizophrenia [Malhotra, 2002].

Typically, genetic components or heritability estimates are obtained using the twin method where pairs of monozygotic (MZ) and dizygotic (DZ) twins are evaluated

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for concordance in rates of disease prevalence. A higher concordance of the disease phenotype among monozygotic over dizygotic twins is taken as an evidence of heritability. Although twin studies are indeed very useful to address the heritability of common complex diseases, they have limited utility in the context of pharmacological responses to drugs and other xenobiotics [Malhotra *et al*. 2004]. Some of these limitations include difficulties in the recruitment of twins, obtaining clinical outcome data in both twins (since the twin pairs may not suffer from the same disease at the same time) as well as the financial cost of twin investigations. It should be acknowledged that family study methods can be used to estimate heritability – but usually of disease-related traits. As in twin studies, applications of family studies in pharmacogenomics meet however with similar limitations. The heritability estimates obtained by family studies tend to be lower than those derived by the twin method [Falconer, 1989; Jedrusik *et al*. 2003; Martin *et al*. 2004]. Moreover, the increase in throughput of genomic technology and the number of putative drug targets also demands an increase in the speed by which heritability estimates are obtained for numerous new molecular entities (NMEs) under clinical development in the pharmaceutical industry.

A second predicament related to the multifactorial nature of drug effects is that pharmacogenomic biomarkers may often identify subpopulations with varying degrees of overlap in the distribution of drug response phenotypes [Kalow *et al*. 2001a]. This is in sharp contrast to monogenic pharmacological traits wherein it is feasible to partition the patient populations into discrete subgroups by the analysis of a single gene. As pharmacogenomic investigations progress from monogenic to polygenic or multifactorial clinical endpoints, the decision-makers are faced with a practical challenge: How do we interpret the clinical significance and therapeutic policy implications of a pharmacogenomic test? More to the point, where do the therapeutically relevant subpopulations reside in a population exposed to a medicinal compound?

The present manuscript makes an attempt to address these emerging challenges in pharmacogenomics and regulatory science. We discuss (1) the Repeated-Drug-Administration (RDA) method as an alternative means to obtain heritability estimates prior to high throughput molecular pharmacogenomic research, (2) the various phases of clinical drug development where the RDA strategy can be implemented to support regulatory decision making, while integrating the available genomics or proteomics-based biomarker data and, (3) a population-based approach to pharmaceutical risk assessment with multigenic pharmacogenomic biomarkers. Lastly, the traditional reliance on population averages in pharmaceutical risk assessment is evaluated with a view to its ethical, social and therapeutic policy corollaries.

1. A. GRADED APPROACH TO THE ESTIMATION OF HERITABILITY IN PHARMACOLOGY

1.1. Setting Priority Levels for Pharmacogenomic Research

The availability of high throughput genomic technologies coupled with decreasing assay costs is a welcome development applauded by many investigators [Evans and McLeod, 2003]. A byproduct of this progress is that pharmacogenomics is now being applied to almost any drug. This has led to an enormous proliferation in the number of genetic association studies in the clinic – a much needed process for translation of molecular genetic findings to rational treatment guidelines and health policy [Albers and Ozdemir, 2004]. On the other hand, the potential shortcomings of disease-genetics or pharmacogenomic association studies have also been noted [Rietschel, 2001; Lerer, 2002; van den Oord, 2002; Ryan, 2003; Terwilliger and Weiss, 2003]. These include valid concerns such as suboptimal study designs wherein genotype-phenotype correlations are performed without regard to the biology or the mechanism of the targeted pathophysiological system, inadequate characterization of covariates and confounding due to locus and allelic heterogeneity.

In an ideal world with unlimited time and resources, it could have been reasonable to conduct pharmacogenomic studies for all compounds in clinical use or under development, including those subject to modest genetic control. The increasing pressures on the pharmaceutical industry to develop compounds in a timely manner for unmet medical needs and the heightened expectations of the patients and the public from genomic technologies call for a more prudent and rational strategy to select compounds for pharmacogenomic research, instead of the current all-comers approach noted above. That is, a higher priority can be assigned to drugs where genetics is deemed to play a major role in clinical response of the patients. Arguably, different levels of “composite pharmacogenomic risk” may presumably be established ranging from high (>50% genetic contribution), moderate (20–50%) or low (<20%) priority for pharmacogenomic investigations. Interestingly, there is already precedence set by regulatory agencies favoring a graded risk assessment for pharmaceuticals. For instance, using the increments from baseline in plasma midazolam area under the curve (AUC) following oral administration, there is work in progress by the US Food and Drug Administration to classify NMEs as weak (<2-fold), moderate (2- to 5-fold) or strong (>5-fold increase) CYP3A4 inhibitors [CDER, 2003a]. This classification system may benefit the drug labels such that new compounds do not need to be simply lumped together as a “CYP3A4 inhibitor”. Instead, varying levels of risk for drug interactions can be presented on the label or the package inserts for a NME found to inhibit CYP3A4 in clinical and *in vitro* studies. Conceivably, the decisions to initiate a pharmacogenomic study can be rationalized using a similar triage procedure at various phases of clinical drug development and regulatory decision making. This particular issue is discussed in more detail in Section *4*.

1.2. Why Do We Need an Upper-bound Estimate of Heritability? - Genetic Components are not Physical Constants

An important consideration in pharmacogenomics is the recognition that the role of genetics in drug response depends on the environment (temporal, geographic or therapeutic) in which drugs are being administered. Thus, the

genetic component of a pharmacological trait is not a physical constant; its magnitude is subject to fluctuations based on the relative significance of environmental factors and gene-environment interactions at the time of a pharmaceutical intervention. A drug-related outcome (e.g. efficacy/safety) that appears to be under strong genetic control in a certain therapeutic setting may be entirely controlled by environmental factors in another context. The optimal design of pharmacogenomic studies may therefore require the identification of environmental conditions under which genetic factors make the largest contribution to drug response. Consider, for instance, the *CYP2D6* genetic polymorphisms that segregate the human population into extensive and poor metabolizer categories [Alvan *et al*. 2001]. In the presence of a potent inhibitor, virtually the entire population of patients may appear as poor metabolizers of CYP2D6 substrates, with no apparent role for genetics to explain variability in pharmacokinetics. The genetic components of drug response may also differ in various stages of a chronic disease or infection as exemplified by alterations of the inherited acetylation capacity (NAT2) in early versus late stage Acquired Immunodeficiency Syndrome (AIDS) [O'Neil *et al*. 1997]. Similar observations were made in patients with advanced metastatic cancer [Williams *et al*. 2000]. The availability of a high throughput method to obtain heritability estimates would inform the clinicians and decision makers on the types of environmental conditions that would enhance the predictive utility of a pharmacogenomic test. That is, monitoring the changes in the size of genetic components can also help to dissect the mechanistic underpinnings of environmental components for a pharmacological trait.

A key concept in rational project management is the estimation of best- and worst-case scenarios to guide critical decisions prior to resource allocations [Albers and Ozdemir, 2004]. Keeping in mind the variable nature of genetic components explained above, an upper-bound estimate for heritability of drug response obtained under minimal environmental exposures, would provide a best-case interpretation of the relative significance of genetic factors. In the event an upper-bound heritability measure points towards only a minimal or low genetic component (e.g. <20%), it would be reasonable to postulate that the clinical utility of a pharmacogenomic test will likely diminish further upon exposure to environmental modifiers of drug response [Senn, 2001]. In such cases, research efforts can be refocused on drugs subject to moderate (>20%) or strong genetic control (>50%), thereby avoiding unnecessary investments on projects that may not lead to clinically meaningful biomarkers or genetic tests in the clinic.

The foundations of genetic basis of variability in drug effects have been firmly established since the first inception and detailed account of the idea more than 40 years ago [Kalow 1962, 2002a]. The interest in routine application of pharmacogenomics in the pharmaceutical industry, the regulatory agencies or clinical practice is more recent. A case in point is the issuance of a draft guidance document in November 2003 by the US Food and Drug Administration (FDA) to encourage regulatory pharmacogenomics data submission by drug developers [CDER, 2003b]. These developments also placed pharmacogenomics on the public agenda, raising a vast amount of expectation as well as public scrutiny [Hedgecoe and Martin, 2003; Williams-Jones and Corrigan, 2003]. Experts in regulatory science have recently made the observation that the key question is not whether pharmacogenetic- or pharmacogenomic-guided drug therapy will happen, but rather, *when* and *how* this transformation will take place [Lesko and Woodcock, 2002]. However, as it often happens in many new or rapidly evolving fields of research, the long term and broad acceptance by the medical and the scientific community will rely on the ratio of success stories with compounds developed by pharmacogenomic testing (numerator) *versus* the full complement of compounds subjected to pharmacogenomic investigations (denominator). It is also interesting to point out that pharmacogenomic data differ inherently from other types of biomarkers. For example, measurements of plasma drug concentration (a frequently used biomarker) and pharmacokinetics apply virtually to all small molecule drugs, but not all drugs are subject to genetic regulation of their disposition or pharmacodynamics. Hence, setting some level of pharmacogenomic research priority by fast-tracking compounds that are under strong genetic control can only benefit the field; this would also contribute towards a healthy management of expectations on the part of the consumers of pharmacogenomic tests in the future. Otherwise, any new scientific paradigm or technology applied indiscriminately across all available drugs without adequate research justification has the potential to do disservice in the long run to the attendant field of scientific inquiry.

2. METHODS FOR ESTIMATION OF HERITABILITY

2.1. Twin Studies

The current practice for presenting evidence on heritability of drug response reflects a blend of information from three different sources:

- Evidence for metabolism or drug transport by a known genetically polymorphic pathway;
- Presence of genetic variation in putative drug targets or biological pathways upstream or downstream the presumed mode of drug action;
- Role of inheritance for the target disease (genetic networks underlying diseases may serve, in some cases, as targets for drug interventions).

Most drug response phenotypes are influenced by both environmental and genetic factors. The approaches noted above therefore lack a quantitative perspective. They do not adequately inform the decision-makers regarding the percentage of variance in clinical endpoints that is attributable to genetic factors alone. For many drugs, a number of genetic polymorphisms have been reported to influence their pharmacokinetic or pharmacodynamic attributes. In such cases, it is difficult however to gauge the quantitative significance of a singular or a set of candidate genes in relation to the *composite genetic component* in pharmacological traits. The latter piece of information becomes essential when a decision has to be made as to

whether further investigations are warranted to identify additional molecular genetic factors that may contribute to the variability in drug response [Ozdemir *et al*. 2000].

In 1876, Galton argued that twins could provide "a means of distinguishing between the effects of tendencies received at birth, and those that were imposed by the circumstances of their lives; in other words, between nature and nurture". In essence, twin studies compare the within-pair variances in pharmacological phenomena in MZ and DZ twins [Endrenyi *et al*. 1976; Vesell, 1992]. A smaller variance in MZ compared to that in DZ twins points towards the importance of genetic factors for the regulation of the pharmacological parameter under investigation. However, twin studies do not explain the mode of inheritance, the chromosomal location or the number of genes contributing to heritability of a pharmacological trait. Importantly, the validity of twin studies requires that the following assumptions are met:

- MZ twins share 100% of their genes,
- DZ twins, on average, share 50% of their genes,
- MZ and DZ twins are under similar environmental influences.

If and when these criteria are met, the effect of the environment essentially cancels out and thus, twin studies provide a valuable estimate of the overall effect of different genotypes (i.e. MZ *versus* DZ) on pharmacological traits.

As noted earlier, there may however be practical limitations to the application of twin studies to drug response phenotypes, particularly in a high-throughput setting of industrial and clinical drug development constrained by other competing objectives and project timelines [Manasco and Arledge, 2002; Sheiner, 1991]. This oftentimes leads to the execution of the molecular genetic work before a heritability estimate is available for the putative pharmacogenomic phenotype. In other cases, essential molecular pharmacogenomic work may be delayed owing to lack of data on the size of genetic components responsible for variability in drug response. A further additional layer of complexity is posed by the ever changing nature of the size of genetic components that depend on the type of drug subjected to pharmacogenomic analysis or the route and time of drug administration [Ohlman *et al*. 1993; Ozdemir *et al*. 2000; Leabman and Giacomini, 2003]. As in the case of common complex diseases, the role of heredity can be more prominent for certain clinical endpoints (endophenotypes) for the same drug. For instance, genetic variation in the promoter region of the dopamine D2 receptor gene (*DRD2*) was associated with anxiolytic and antidepressive effects during treatment with dopamine antagonists in patients with schizophrenia [Suzuki *et al*. 2001]. By contrast, improvement in other clinical endpoints such as positive and negative symptoms of the psychotic illness was apparently not influenced by *DRD2* promotor variation in this study [Suzuki *et al*. 2001]. An adequate understanding of the specific clinical endpoints subject to strong genetic regulation and the scope of interacting environmental factors is thus crucial for the rational design of clinical pharmacogenomic studies. These theoretical considerations could be put into practice with the availability of a high throughput method to estimate the genetic components in pharmacological traits. This may benefit not only the heritability estimates in pharmacology but also enhance our knowledge of the role of environment on individuals' genetic make-up. Through changes in the magnitude of genetic components under different experimental settings, one may indirectly infer the pertinent gene-environment interactions for a given drug: consider, for instance, the changes in NAT2 genotype-phenotype correlations during the clinical progression of the HIV infection [O'Neil *et al*. 1997]. It would be anticipated that the genetic components of interindividual variability in NAT2 phenotype might be markedly smaller in a sample of patients with differing severity of AIDS compared to healthy subjects.

2.2. The RDA Method – an Alternative to Studies in Twins

For most small-molecule drugs (<500 daltons) with protein targets (e.g. receptors or enzymes), drug effects represent *dynamic* and reversible biological phenomena that decay over time. This is in sharp contrast with classical *static* phenotypes of interest to human geneticists, for example, stature or eye color. Twin studies are essential to study the heritability of such permanent physical characteristics of an individual or the disease phenomena which can not be experimentally induced or reproduced. Although it is not always explicitly stated, the independent variable in twin investigations is the degree of similarity in the genetic make up of two groups of individuals: the MZ (nearly 100% identical in the genome) and DZ twins (50% sharing of the genetic make up on average). If genetic factors are truly significant for a clinical phenotype (dependent variable), it follows that the phenotype of interest should be reproducible to a larger extent in pairs of MZ than DZ twins. It is worthwhile to note that the twin studies provide a genome-wide estimate of heritability of a phenotype in relation to the role of environment. Keeping in mind the description of the independent and dependent variables in twin studies, it is tempting to question whether the heritability estimates could be obtained instead by comparing the variance of a *dynamic* dependent variable (e.g. drug response phenotype) in the same person assessed repeatedly over time (akin to the MZ twin) against the variance among individuals who are unrelated to each other [Kalow *et al*. 1998, 1999a].

In a given individual, within-subject variance (SD_w^2) is determined by environmental factors and measurement errors ($SD_w^2 = SD_{environment}^2 + SD_{measurement\ error}^2$). Notably, the second term ($SD_{measurement\ error}^2$) includes not only measurement error but also biological variation, random and nonrandom (e.g. circadian). On the other hand, between subject variance (SD_b^2) can be formulated as ($SD_b^2 = SD_{environment}^2 + SD_{genetic}^2 + SD_{measurement\ error}^2$). As originally proposed and demonstrated by Kalow *et al*. [1998], the genetic component (r_{GC}) of variability in a time-dependent pharmacokinetic or pharmacodynamic occurrence can be described with the following equation:

$$r_{GC} = \text{Genetic component} = (SD_b^2 - SD_w^2) / SD_b^2 \quad (1)$$

r_{GC} values approaching 1.0 point to overwhelming genetic control, whereas those close to zero suggest that

environmental factors dominate. In essence, any dynamic biological process exhibiting time-dependent decay and negligible carry-over effects between repeat observations should be amenable to RDA studies to dissect the genetic contribution to inter-individual variability in the corresponding biological phenotype [Ozdemir *et al*. 2000].

Table **1** illustrates the criteria to determine whether a pharmacological intervention and the targeted pathophysiological process are amenable to investigation by the RDA method. For pharmacological traits, general considerations for studies with a repeated-measures design would be applicable, such as the need for minimal or no carry-over in drug effects between repeat drug administrations. The latter is particularly important when the RDA method is applied to obtain heritability of pharmacodynamic endpoints. Insofar as the targeted disease process is concerned, it is essential that the underlying pathophysiological mechanism remains unchanged between repeated drug treatments.

2.3. Statistical Significance and Sample Size in RDA Studies.

We recommend that the between- and within-subject variances are obtained from the interindividual and residual mean squares, respectively, based on a one-way analysis of variance (ANOVA) (Endrenyi *et al*. 1976; Rosner, 1990). Attrition of the subjects between repeated drug administration periods may lead to an uneven number of repeat observations among subjects in an RDA study. The calculation of variances using ANOVA is advantageous because it allows the inclusion of data from all subjects and repeat study periods.

The formula for r_{GC} can be reorganized as

$$r_{GC} = 1 - (1/F), \text{ where } F = SD_b^2/SD_w^2 \quad (2)$$

Let n = total number of subjects in a RDA study,

k_i = number of drug administrations in the ith subject.

The ratio SD_b^2/SD_w^2 follows the *F*-distribution with (n-1) (numerator) and $\sum_{i=1}^{n}(k_i-1)$ (denominator) degrees of freedom (Rice, 1995). In order to test the statistical significance of the r_{GC} point estimate, the *null hypothesis* (H_o) can be expressed as $\{SD_b^2 = SD_w^2\}$ in which case the r_{GC} attains a value of zero, thus, indicating lack of heritability. A comparison of the experimentally obtained value of the SD_b^2/SD_w^2 ratio with the tabulated F value at the degrees of freedom noted above, with type I error level set at 0.05, allows statistical analysis of the significance of r_{GC}. The difference between SD_b^2 and SD_w^2 can be declared significantly larger than 0.00 when the empirically derived F value is higher than the tabulated F value, conferring statistical significance to the r_{GC} point estimate.

The lower (3) and upper (4) 95% confidence limits for the estimated F can be calculated by the formulas (Freund and Walpole 1980):

$$F_{observed} / F_{0.025,\, b.d.f,\, w.d.f} \quad (3)$$

$$F_{observed} \times F_{0.025,\, w.d.f,\, b.d.f.}, \quad (4)$$

where b.d.f and w.d.f are the degrees of freedom for the between- and within-person variances in the ANOVA, respectively, and $F_{0.025}$ is the tabulated F-statistic at the 2.5% significance level with the indicated degrees of freedom. Accordingly, the upper and lower-bound 95% confidence limits for the r_{GC} point estimates can be calculated by entering the F values obtained in (2) and (3) into formula (1).

The 95% confidence limits of the r_{GC} depend on three factors: (i) the number of subjects, (ii) the observed F value (SD_b^2/SD_w^2), and (iii) the number of repeat drug administrations. We have earlier proposed that the lower-bound 95% confidence limit of the r_{GC} could provide

Table 1. Considerations for Application of the RDA Method to Obtain *a Priori* Heritability Estimates in Clinical Pharmacogenomic Studies.

	Criteria
	Pharmacological endpoint:
▪	May decay and return back to the baseline (pre-drug) value over time
•	Molecular pharmacodynamics is comprised of a reversible interaction between the drug and its target
•	No carry-over in pharmacological effects between the repeated drug administration periods
	Target disease for the pharmaceutical intervention:
•	Clinical course of the disease permits drug holidays to allow for repeated drug administration
•	Preventative pharmaceutical interventions for chronic diseases (e.g. antilipidemic and antihypertensive agents) more suitable than acute diseases that may require uninterrupted and emergency drug administration
•	Stable disease pathophysiology between repeat drug administrations
•	Studies in healthy volunteers require a suitable surrogate endpoint or, alternatively, a known validated or probable valid biomarker* to serve as a repeat measure

Pharmacological effect refers to a pharmacokinetic or pharmacodynamic endpoint.

*Definitions per recent draft guidelines for regulatory pharmacogenomics data submission [CDER, 2003b].

guidance for estimation of the required sample size in a RDA study [Kalow *et al*. 1999b]. That is, for a given lower-bound 95% confidence limit, observed F value (SD_b^2/SD_w^2) (or, equivalently, the r_{GC} point estimate) and the number of repeat observations planned for a RDA study, the corresponding number of subjects can be calculated [Kalow *et al*. 1999b]. Accordingly, Table **2** presents the sample sizes for the required number of subjects with two, three or four repeated measures and various lower-bound 95% confidence limits of r_{GC} ranging from 0.50 to 0.80. As an example, to attain a lower-bound 95% confidence limit of 0.50 for the heritability of drug response in a study designed to accommodate repeated drug administration on four occasions and an anticipated F value of 5.0 (r_{GC} = 0.80), a sample of 10 subjects would be sufficient (Table **2**).

2.4. The Uniform Environment Assumption and Other Caveats for RDA and Twin Studies – the Asymmetry Between Pharmacogenomics and Disease Genetics

Twin studies, historically, have met with criticism on several fronts. First, it is usually questioned whether and to what extent the principal assumptions of twin studies, notably the equal environmental contribution to traits investigated in MZ and DZ twins, can be met with reasonable confidence. This is an important consideration because environmental differences within/between MZ and DZ twin pairs may confound the heritability estimates. Such concerns also apply to RDA studies regarding the uniformity of environmental influences on within- and between-subject variances. To this end, it is worthwhile to bear in mind that most chronic human diseases initiate and progress over a considerable period of time before clinical findings or disease-related traits are apparent. Thus, human genetics dealing with common complex diseases remains, for the most part, an observational science wherein the molecular genetic findings are correlated with a disease process that is not experimentally controlled or reproducible for obvious ethical and practical reasons. This means that the environment and the degree of its impact on the genetic component of disease predisposition are often difficult to estimate with adequate certainty or even incalculable. For all the parallels between the genetics of common complex diseases and pharmacological phenotypes, the very difference between the *observational* nature of study designs in disease genetics and the ability to *experimentally* produce a drug response phenotype in a setting carefully controlled for environmental factors is perhaps one of the most salient tenets of pharmacology that offers a notable advantage to control for environmental confounding in pharmacogenomic investigations. Thus, the concerns for equal environmental influences can be answered with a fair degree of confidence in applications of twin or RDA studies to pharmacological traits. On the other hand, estimation of heritability for drug effects is subject to other shortcomings, in particular, the low throughput of twin studies to dissect the genetic components for various clinical endpoints or types of medications. It is especially in this context that the simplicity and practicality of RDA studies may offer a marked advantage over twin studies to set priority levels for clinical pharmacogenomic research in time-constrained pharmaceutical industry or regulatory settings (see also Section *4*. for additional considerations).

A second concern over twin studies focuses on the ability to extrapolate the heritability estimates obtained in a well-controlled experimental pharmacology setting to patients in the clinic or other populations where environmental influences may take precedence over genetics [Vesell, 1992]. As a general rule, however, any heritability estimate is relevant only for the index population in which it was studied. The genetic components may also differ, for example, depending on the time interval between RDAs, the dose and route of drug administration. It is important to recognize that pharmacogenomic or human genetic studies follow a "two-tier" approach; twin or the RDA analysis represents only the first step towards assessment of the role of genetic factors for variability in pharmacology, to be complemented by subsequent molecular investigations. A high genetic component (r_{GC} >0.50) as measured by the RDA method suggests that further molecular genetic explorations are justified. Conversely, a low genetic component (r_{GC} <0.20) obtained under basal minimal environmental exposure would indicate that genetics is unlikely to play a major role for the corresponding pharmaceutical trait. Table **3** illustrates the genetic components or heritability of variability in drug metabolism, as measured by the RDA or the twin/family method, respectively. Although there is a

Table 2. Number of Subjects Required to Observe a 95% Lower Confidence Limit (0.80 to 0.50) for Three Different Point Estimates of r_{GC} (0.90, 0.80 or 0.70).

Lower limit†	Two repeat measurements*			Three repeat measurements*			Four repeat measurements*		
	0.90	0.80	0.70	0.90	0.80	0.70	0.90	0.80	0.70
0.80	33	--	--	22	--	--	18	--	--
0.70	14	95	--	9	66	--	7	57	--
0.60	9	33	187	5	22	133	4	18	116
0.50	7	19	60	4	12	41	3	10	35

†95% lower confidence limit of r_{GC}. *Number of repeat measurements in each subject. Reproduced with permission from Kalow *et al*. 1999b.

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Table 3. Comparison of Genetic Components and Heritability Estimates of Selected Pharmacological Traits, as Measured by the RDA or, the Twin/Family Study Methods, Respectively.

Enzyme or model drug	RDA analysis of published data	Index catalytic reaction or parameter	r_{GC}	Twin or family study	Index catalytic reaction or parameter	Heritability
CYP1A2	Denaro et al. 1996	Caffeine metabolic ratio* (p.o.)	0.69	Rasmussen et al. 2002	Caffeine metabolic ratio* (p.o.)	0.72
		Caffeine plasma clearance (l/h/kg)	0.82			--
CYP2D6	Ereshefsky et al. 1996	Dextromethorphan O-demethylation (p.o.)	0.97	Steiner et al. 1985	Debrisoquin 4-hydroxylation (p.o.)	0.79
CYP3A4#	Lobo et al. 1986	Nifedipine plasma $AUC_{0-\infty}$ (p.o.)	0.82	Penno et al. 1981	Antipyrine 4-hydroxylation (p.o.)	0.88
	Kashuba et al. 1998	Midazolam plasma clearance (i.v.)	0.96			--
Ethanol disposition	Kopun and Propping, 1977	Absorption rate (mg/ml x 30 min) (p.o.)	0.60	Kopun and Propping, 1977	Absorption rate (mg/ml x 30 min) (p.o.)	0.57
	Kopun and Propping, 1977	Rate of metabolism (mg/kg x h) (p.o.)	0.57	Kopun and Propping, 1977	Rate of metabolism (mg/kg x h) (p.o.)	0.41
	Kopun and Propping, 1977	Elimination rate (mg/ml x h) (p.o.)	0.71	Kopun and Propping, 1977	Elimination rate (mg/ml x h) (p.o.)	0.46

*Caffeine metabolic ratio = (AAMU + 1U + 1X) / (17U). # RDA analyses with various substrates collectively suggest that 60% to 90% of person-to-person variability in CYP3A4 activity may be due to genetic factors [Ozdemir *et al*. 2000]. (i.v.): intravenous administration; (p.o.): oral administration. Caffeine metabolites in urine: AAMU, 5-acetylamino-6-amino-3-methyluracil, a stabilized conversion product of the acetylated metabolite (AFMU); 1U, 1-methylurate; 1X, 1-methylxanthine; 17U, 1,7-dimethylurate.

need for additional comparative evaluations, the general agreement between the two methods is encouraging, and warrants further explorations in pharmacogenomics (see Sections *3.1* to *3.4* for specific examples).

Within-subject variability in pharmacology has been known for a long time [Wagner, 1973; Bruguerolle, 1998]. These individual variations have oftentimes been considered as nuisance or noise by clinical pharmacologists or the pharmaceutical industry; this may reflect a missed opportunity to evaluate the genetic components of pharmacological variability. The RDA analysis offers the possibility to develop further insights into the biological meaning of intra-individual standard deviations in pharmacology. To this end, there is an increasing interest in characterization of within-subject variances in clinical trials and during therapeutic drug monitoring which should facilitate the RDA analysis in the near future [Karlsson and Sheiner, 1993; Kashuba and Nafziger, 1998; Leabman and Giacomini, 2003].

3. STRATEGIES AND CONTEXT FOR APPLICATION OF THE RDA METHOD IN PHARMACOGENOMICS

3.1. Estimation of Heritability for Novel Pharmacological Phenotypes Hitherto Unexplored for Pharmacogenomic Variabilities

The distribution of pharmacological traits is often interpreted with a view to the significance of underlying genetic factors. It is generally agreed that monogenic traits are characterized by bimodal distributions. The reverse rule however does not always hold. That is, bimodality, alone, can not be taken as a definitive evidence of monogenic control. Many environmental factors such as diet and drug interactions may lead to bimodal pharmacokinetics or drug effects [Kalow, 2001b]. Another notable misconception is the assumption that a unimodal distribution points toward a lack of genetic control in pharmacokinetics or pharmacodynamics. This in part stems from the evolution of the field of pharmacogenetics concerning primarily monogenic traits, to pharmacogenomics dealing with multigenic outcomes in pharmacology. For many of the complex multifactorial traits, it is therefore essential to establish a baseline heritability estimate before the subsequent molecular genetic work can be justified and planned.

Insofar as the genetic variability in drug disposition is concerned, pharmacogenetic studies have long focused on drug metabolism and more recently, on drug transport [Innocenti and Ratain, 2002; Weinshilboum, 2003]. By contrast, virtually no information is available on the heritability of renal elimination, the other major contributor to drug disposition *in vivo* [Leabman and Giacomini, 2003]. Since the early 1990s, there has been a growing interest in the evaluation of inter-individual variability in drug metabolism *in vitro* [Tucker *et al*. 2001]. However, these *in vitro* studies do not account for renal clearance *in vivo*. In effect, this contributes to some of the existing difficulties of extrapolating data obtained *in vitro* to the clinically relevant situation *in vivo*. An adequate knowledge of heritability and the precise molecular genetic factors that may influence person-to-person differences in renal clearance may facilitate not only pharmacogenomic-guided personalized medicine efforts but also the scope of drug disposition studies *in vitro*.

Recently, Leabman and Giacomini [2003] applied the RDA method to evaluate the heritability of renal drug disposition in humans. They used published data on between- and within-subject variance of renal clearance for compounds known to be eliminated by renal mechanisms. The r_{GC} values of drugs that undergo transporter-mediated secretion (ampicillin, amoxicillin and metformin) ranged from 0.64 to 0.94 ($p<0.05$), lending evidence for genetic control of renal secretion. On the other hand, compounds subject to passive secretion/reabsorption or glomerular filtration (digoxin, iohexol and terodiline) displayed genetic components considerably less than 0.50 (r_{GC} = 0.12–0.37) ($p>0.05$). Among the latter compounds, iohexol is primarily cleared by glomerular filtration; studies in monozygotic and dizygotic twins indicate that glomerular filtration rate is not subject to appreciable genetic control [Leabman and Giacomini, 2003; Hunter *et al.* 2002; Grim *et al.* 1979]. Terodiline is handled by passive reabsorption in the kidney. The low r_{GC} value (0.37) for renal clearance of terodiline is consistent with a multitude of environmental factors (e.g. urinary pH) that may cause temporal fluctuations in passive reabsorption in a given individual. For digoxin, the genetically variable efflux transporter MDR1 contributes to its renal secretion. However, the environmental influences on passive reabsorption of digoxin appear to supercede the potential role of genetics on active secretion in the kidney (r_{GC} = 0.12, $p>0.05$) [Leabman and Giacomini, 2003]. Collectively, these analyses demonstrate the value of the RDA approach to obtain baseline heritability estimates in biological systems or clinical endpoints hitherto unexplored for pharmacogenomic variability.

3.2. Residual Genetic Variability After the Initial Discovery of Molecular Genetic Variants

CYP3A4 is the most abundant form of Phase I drug metabolizing enzyme in the human liver and contributes to first-pass and systemic metabolism of numerous drugs with diverse chemical structures. Although there is up to 60-fold interindividual variation in CYP3A4 expression, the precise molecular genetic basis of individual differences in CYP3A4 catalytic function remains elusive [Ozdemir *et al.* 2000]. More than 30 single nucleotide polymorphisms (SNPs) have been identified within *CYP3A4* but the majority of these SNPs either occur at low frequency (<5%) in human populations or have a minimal impact on enzyme function [Lambda *et al.* 2002a, 2002b]. On the other hand, a twin study using antipyrine 4-hydroxylation *in vivo* found indication for high heritability of CYP3A4 activity (H_2 = 0.88) [Penno *et al.* 1981]. Consistent with this observation, RDA analysis using published data suggests that 60% to 90% of person-to-person variability in CYP3A4 function can be attributed to genetic factors [Gharaibeh *et al.* 1998; Kashuba *et al.* 1998; Ozdemir *et al.* 2000]. Following the initial discovery of molecular genetic variants in a candidate gene, the dissection of genetic components by the twin method or the RDA analysis thus provides the investigators with a decision-tool as to whether further molecular genetic investigations are worthwhile or not. As for *CYP3A4*, the prevalence of SNPs with demonstrated functional consequences (e.g. consider the *CYP3A4*2* allele with a frequency of 2.7% in whites and absent in Chinese and Blacks) is too low in comparison with the genetic component estimates discussed above (up to 90%) [Lamba *et al.* 2002b; Sata *et al.* 2000]. Among the CYP3A enzymes, the CYP3A4 is quantitatively the most important one, while CYP3A5 has been suggested to play a role in certain cases [Kuehl *et al.* 2001]. These two isozymes share to a great extent substrates, inhibitors as well as inducers. Hitherto there are no specific substrates that could be used to phenotype for the two enzymes separately. If there was, one could probably show that there is a nonunimodal distribution of the CYP3A5 activity, with a minor part of Caucasians expressing this enzyme. However, a major part of black Africans has functional CYP3A5 genes. Further investigations are thus warranted to search for the molecular genetic variants responsible for variations in CYP3A enzymes.

3.3. Biomarker Bridging Studies: Population-to-Population Variability in Heritability

Three years ago, the Human Genome Project provided the blueprint for some 30,000 genes in the human genome [Lander *et al.* 2001; Venter *et al.* 2001]. The International Haplotype Map Project, officially launched in October 2002, will soon identify the common SNPs and their patterns (haplotypes) on individual chromosomes in various human populations from Africa, Asia and Europe [International HapMap Consortium, 2003]. It is anticipated that the HapMap initiative will characterize 600,000 SNPs evenly spaced across the genome at a density of one SNP every 5 kb. Ultimately, the goal is to utilize the haplotype map of the human genome as a foundation for future clinical association studies of human diseases as well as drug efficacy and safety [Athanasiou *et al.* 2002; Judson *et al*. 2003]. In comparison to investigations with specific candidate genes, genome-wide association studies offer much promise in pharmacology because it may be possible to discover unprecedented genes predisposing to disease or drug toxicity and treatment failure [Wong *et al*. 2004; Wong and Licinio, 2004]. An important caveat in genome-wide inquiries is that they exploit the principle of linkage disequilibrium (LD), the co-occurrence of alleles at different genetic loci at a frequency greater or lesser than what would be expected due to random association alone. Consequently, the genetic loci that are reportedly associated with specific drug effects may not necessarily correspond to the causal genetic variants.

It could be argued by some investigators that causality may not matter in genome-wide association studies insofar as the identified surrogate genetic markers predict the clinical response to a given pharmaceutical intervention. This assertion may hold up only if generalizations are avoided and genetic testing to personalize drug therapy is limited to the index population where the pharmacogenomic association was initially observed. On the other hand, the degree of LD varies markedly in different regions of the genome as well among different populations [Patil *et al.* 2001; Reich *et al.* 2001; Goldstein, 2003; Hoehe, 2003]. Unless the causal genetic variants are ascertained, this means that the informativeness of SNP or haplotype markers in genome-wide association studies will be fraught with uncertainty when clinical predictions are extended to other populations beyond the immediate study sample. Moreover,

due to the multigenic nature of most human diseases and pharmacological traits, pharmacogenomic biomarkers can be population-specific; divergent sets of genes may influence the clinical phenotypes in different populations. Hence, without a clear knowledge of the causal genetic markers, an impasse can be quickly reached when pharmacogenomic testing is attempted at point of care in the clinic where patients with diverse genetic backgrounds seek medical care.

The concept of "bridging studies" has been traditionally applied to pharmacokinetics and drug exposure evaluations when regulatory drug approval is sought in various countries or populations across the world. It seems reasonable to postulate that biomarker-bridging studies are now also timely and pertinent, particularly in the context of genome-wide clinical pharmacogenomic studies. Although the causal genetic biomarkers should undoubtedly be identified eventually, estimation of heritability of a clinical endpoint in a new population may facilitate decisions concerning the biomarker-bridging studies. As an example of forward applications, let us assume that a genome-wide association study identified a genetic locus apparently predictive of drug toxicity for a compound that previously failed in clinical development. It is claimed by the sponsors of this hypothetical study that the finding is likely to have global applications to develop a diagnostic kit to aid prescription decisions in the clinic – although the precise causal genetic variants were unclear at the time of the initial association with drug toxicity. The latter claim may fail if variation in the causative gene plays a smaller role in a new sample of patients drawn from a different population. It is in this very context that genetic component analysis with the RDA method may guide the identification of populations demonstrating high heritability of drug response or toxicity wherein biomarker-bridging studies need to be conducted with higher priority.

3.4. Identification of Gene-Environment Interactions

As noted in Sections *1.2* and *2.1*, the size of genetic components in pharmacological variability is not a fixed value but can be influenced by various gene-environment interactions. The recognition of such environmental influences has paramount importance for the optimal design of clinical pharmacogenomic studies [Ozdemir and Lerer, 2005]. To this end, an interesting example can be found in earlier studies of cyclosporine disposition. Ohlman *et al.* [1993] investigated the within-subject and circadian variation of oral cyclosporine terminal elimination half-life in renal transplant recipients on repeated occasions at steady-state. They found that cyclosporine pharmacokinetics displayed large within-subject variability as well as circadian variation. An RDA analysis of their results reveals that the genetic component of variability in cyclosporine disposition exhibited time-dependency and that genetic contributions are more apparent at night ($r_{GC} = 0.68$) ($p < 0.05$) than during the day-time ($r_{GC} = 0.20$) ($p > 0.05$). This observation is indeed consistent with general pharmacokinetic principles; the clearance of intermediate (e.g. cyclosporine) to high extraction drugs can be influenced by variations in hepatic blood flow [Wilkinson and Shand, 1975]. The hepatic blood flow is likely to exhibit a larger variability in an individual subject during the day-time than the night-time, owing to, for example, variations in scheduling of meals, exercise and physical activity. Collectively, these observations suggest that genetic factors may play a more dominant role in cyclosporine disposition at night-time in patients with renal transplantation, while environmental effects become prominent during the day-time. By extension, pharmacogenetic tests may be more important for such drugs if they are intended to display pharmacodynamic effects mainly at night time. Fig. (**1**) illustrates the influence of various environmental (e.g. time of drug administration) and pharma-

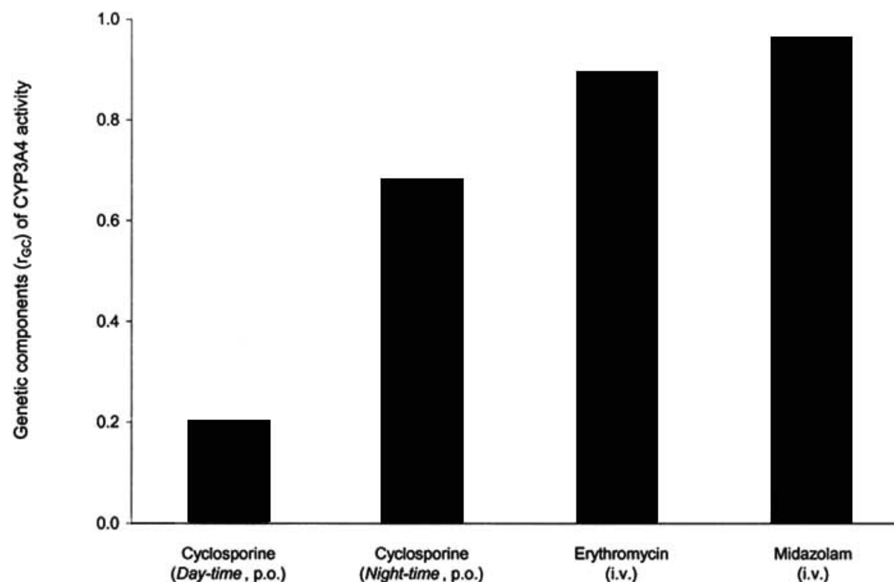

Fig. (1). Variability in genetic components (r_{GC}) of CYP3A4 activity depending on the time (day-time or night-time *cyclosporine* administration in patients) and the substrate (*erythromycin* or *midazolam* – both administered intravenously in healthy volunteers) [Ohlman *et al*. 1993; Kashuba *et al*. 1998; Gharaibeh *et al*. 1998].

cological factors (e.g. type of drug) that can all modify the size of genetic components [Ohlman *et al.* 1993; Gharaibeh *et al.* 1998; Kashuba *et al.* 1998]. The awareness of such caveats would clearly benefit how future pharmacogenomic studies are conceived and designed. It is worthwhile to stress that the RDA method, or twin studies, can identify the size of genetic components but the results do not necessarily elucidate the identity or the number of candidate genes responsible for genetically-determined variability in drug effects.

4. A FRAMEWORK FOR THE INTEGRATION OF RDA ANALYSES IN REGULATORY DECISION-MAKING

The concept of using demographic, pharmacokinetic, and more recently, genomic data to predict the likelihood of drug efficacy and safety is central to personalized medicine and rational therapeutics [Reidenberg 1974, 1999; Dahl, 1977; Dahl, 2002; Kayaalp, 2002; Ozdemir *et al.* 2001]. On the other hand, it has been instructive to observe over the past three decades the critical role of regulatory decision-making in the widespread implementation of new technologies and therapeutic approaches on practices in the pharmaceutical industry and clinical medicine. For example, the theoretical foundations of population pharmacokinetic modeling and the statistical software capable of conducting such analyses have been available since 1970s [Sheiner, 1997]. Yet, the routine use of population pharmacokinetics in clinical drug development has been considerably facilitated in part through recent guidelines developed by regulatory agencies [CDER, 1999]. Therefore, it is conceivable that advances in regulatory science and policy will play a similar and significant role to drive the application of pharmacogenomics technology in medical therapeutics and rational clinical drug development.

Interestingly, a recent search of 2000 entries in the Physicians' Desk Reference (year 2003 electronic version) identified only 51 labels containing pharmacogenomic information [CDER, 2003a]. Moreover, the majority of the pharmacogenomic data in the labels were not presented in a form that could be readily applied to clinical practice [observations made by Dr. Lawrence Lesko, CDER, 2003a]. Although it is difficult to gauge the precise number of drug labels that ought to include data on host genetic make-up for rational dose titration or choice of medications, this example suggests that pharmacogenomic information is likely not adequately represented in current drug labels. Hence, there is a need to implement pharmacogenomics in the very early phases of clinical drug evaluation such that the generated information can inform the drug labels in a timely manner prior to New Drug Applications (NDAs) to regulatory agencies. We suggest that the advances made by recent draft regulatory guidelines on pharmacogenomic data submissions can be complemented by additional considerations on heritability of drug exposure and response. The regulatory review process could potentially benefit if the genetic components in pharmacokinetics, safety or efficacy were calculated and interpreted by reviewers of the NDA dossiers. For example, if strong evidence for heritability of certain endpoints is identified by the RDA analyses, this may then likely allow the regulatory agencies to request from drug developers molecular pharmacogenomic data to better forecast the risk/benefit ratio associated with the drug candidate. Conversely, unnecessary investments and focus on compounds under modest genetic control can presumably be avoided by the sponsors when a NME is found to display a low r_{GC} value (<0.20).

The discrepancies between the efficacy of NMEs in animal models and the clinic, coupled with lack of early and reliable predictors of drug toxicity have recently prompted a surge in the scope and nature of biomarkers strategies, ranging from gene-expression and proteomics to metabonomics approaches [Lesko and Atkinson, 2001; Downing, 2002]. It is hoped that these technical advances will provide, at least in selected cases, an early indication or proof-of-principle, for drug efficacy in healthy volunteers or patients in phase 2A clinical trials. Increasingly, it is thus becoming feasible to quantify drug activity and pharmacodynamics during phase 1 and other early stage clinical trials with the use of biomarkers. Although these early measures of pharmacological activity can not be uniformly extrapolated to changes in clinical endpoints in real-life patient populations at point of care, biomarkers nonetheless offer a unique opportunity to ascertain the molecular mechanisms of drug action. We therefore reason that early phase clinical biomarker data can be effectively utilized to obtain an estimate of genetic components in healthy volunteers and carefully selected patient populations under uniform and basal environmental influences. Conceivably, a small cohort of healthy volunteers or patients can be repeatedly tested in phase 1 and phase 2A clinical trials – provided that the targeted disease allows interruptions in pharmacotherapy and the other criteria presented in Table **1** are met. For instance, an antihypertensive or oral antidiabetic agent in clinical development can be re-evaluated on several occasions in volunteers or stable patients to calculate the r_{GC} values for pertinent biomarkers or surrogate endpoints. Early phase drug formulation and bioequivalence studies as well as drug-food interaction investigations may serve as additional and valuable sources of data for RDA analyses. It is worthwhile to note that End-of-Phase 2A (EOP2A) meetings between the regulatory agencies and the drug developers (sponsors) are becoming a mainstay before critical decisions are made to proceed to costly confirmatory large-scale phase 3 trials. Even though the focus of the EOP2A meetings is usually on proof-of-principle for efficacy and safety, it seems reasonable also to consider, at this stage, whether, and under what conditions, a molecular pharmacogenomics program should be developed in parallel to phase 3 trials. This is particularly important for pharmacogenomics as the relative significance of genetics may vary depending on the NME, clinical end point and the environment in which drugs are administered. Subsequently, a second estimate of r_{GC} can be calculated in phase 3 by repeat observations on a cohort who have participated in phase 2A studies. It is noteworthy that although the r_{GC} estimates in phase 1/2 studies with healthy volunteers or patients with mild disease may intuitively expected to be higher than patients with advanced disease, it is conceivable on theoretical grounds that in some cases the disease process itself may unmask genetic components, thereby allowing a higher r_{GC} in patients evaluated during phase 3 clinical drug development. A flowchart for the

integration of the RDA method in various stages of clinical drug development and key definitions of the pertinent terminology are presented in Fig. (**2**).

5. MULTIGENIC TRAITS AND PHARMACEUTICAL RISK ASSESSMENT

5.1. A Framework Based on Changes in Population Tails

A glance at leading journals in pharmacogenomics uniformly attests to the vast number of candidate gene studies reported in the literature over the past five years. With the progressive decline in the cost of genetic testing, technological difficulties in pharmacogenomics have been partially overcome and many investigators across the world are now able to pursue the correlative genotype-phenotype studies in the clinic. There exists however a large and lamentable gap between such statistical associations and their translation to clinical treatment guidelines and therapeutic policy. The skeptics of pharmacogenomics and its role in customized therapies note two potential shortcomings pertaining to clinical relevance of genetic biomarkers: (1) the overlap in quantitative drug response phenotypes between various genotypes in a candidate gene study and, (2) the ostensibly greater range of phenotypic

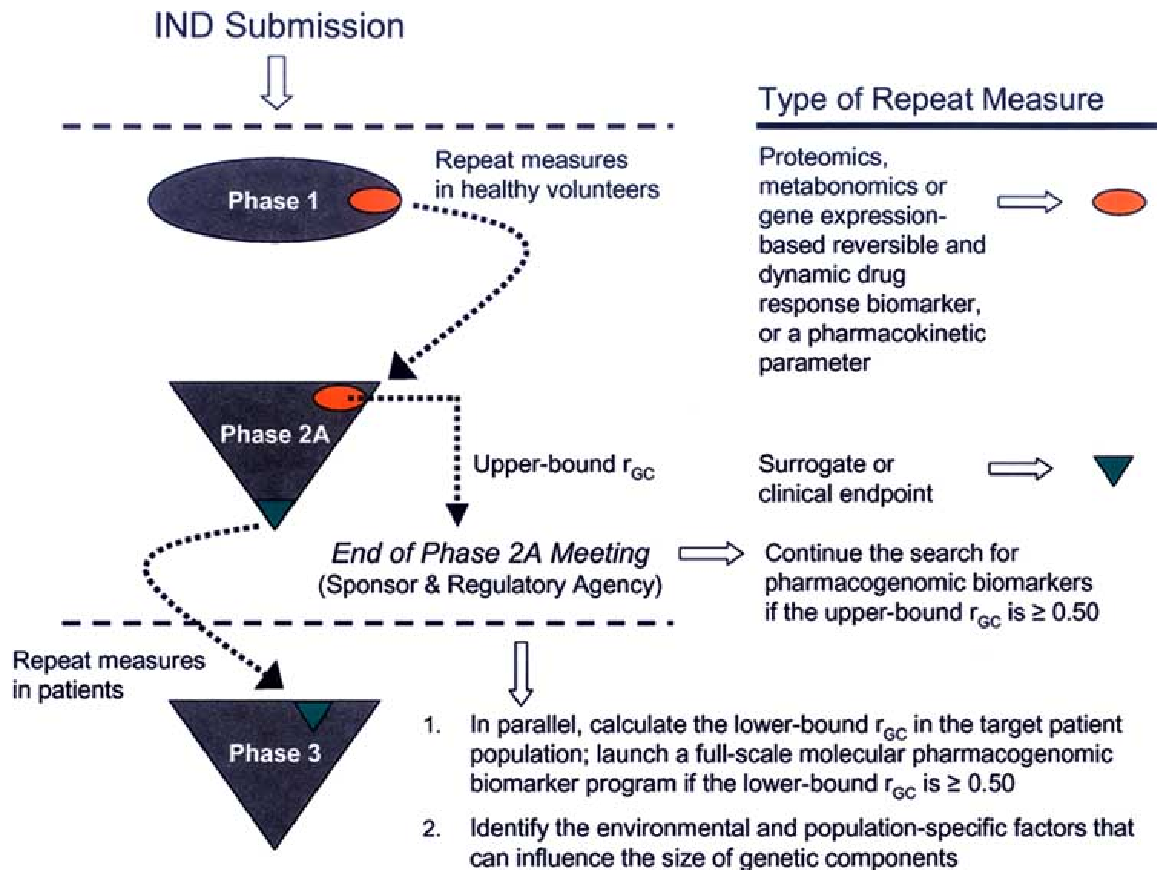

Fig. (2). A conceptual framework for application of RDA analyses in early and late phase clinical drug development to guide decisions to launch a molecular pharmacogenomic and diagnostic program parallel to the NDA critical path. Environmental factors (e.g. dose and formulation, route of drug administration) that may enhance the relative role of genetics in pharmacological variability can be inferred through their impact on the size of genetic components. *IND*: Investigational New Drug. *Metabonomics*: The quantitative study of cellular metabolites formed and degraded under genetic or (patho)physiological influences using a multivariate systems approach [Aardema and MacGregor, 2002; Nicholson *et al*. 2000].

Key Definitions

NIH Biomarkers Definitions Working Group [2001]

- Biological marker (biomarker): A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention.
- Surrogate endpoint: A biomarker that is intended to substitute for a clinical endpoint. A surrogate endpoint is expected to predict clinical benefit, harm, or lack of benefit or harm based on epidemiologic, therapeutic, pathophysiologic, or other scientific evidence.
- Clinical end point: A characteristic or variable that reflects how a patient feels, or functions, or how long a patient survives.

variation within each subpopulation compared to the difference in mean values between subpopulations defined by pharmacogenomic markers. These criticisms could in part make sense from a purely statistical and descriptive point of view, but they lack a clinical pharmacology perspective. As a general rule, population tails represent the individuals who are either most vulnerable or refractory to pharmaceutical interventions. Hence, insofar as the pharmaceutical risk assessment is concerned, it makes sense to evaluate the impact of changes in population means on the tails of a phenotypic distribution. Any change in the proportion of individuals who reside in population tails is more likely to translate into clinically meaningful events in the form of drug toxicity or resistance to drugs and environmental catastrophes [Kalow, 1992, 2001a]. This diversity in pharmacological effects by members of a population also has significance in how evolutionary forces may shape a population or species [Kalow, 2002b].

Fig. (**3**) shows two populations with an overlapping quantitative trait, catalytic function of a drug metabolizing enzyme. Each population is represented by a standardized normal distribution with a mean of 0.0 and a standard deviation of 1.0. It is assumed that drug toxicity is observed when the catalytic function decreases below a threshold value of X_o. Even though the mean enzyme functions are moderately different with considerable overlap in distributions, the proportion of patients with clinical drug toxicity is markedly higher in population B than population A (Fig. **3**). A graphical comparison of the relative changes in AUC at population tails between the two populations exemplifies this point further (Fig. **4**). Evidently, the degree of changes in population means underestimates the relative increase in the risk of drug toxicity in population B that has a slower catalytic activity. Importantly, the lower the toxicity threshold X_o (i.e. the rarer an adverse drug reaction or the wider a therapeutic window) is, the more it will be affected by changes in population mean values (Fig. **4**). Drugs with a narrow therapeutic window have traditionally received much attention in regards to pharmaceutical risk management. However, the data in Fig. **4** suggest that the concept of edge effect would be particularly relevant for drugs with a wider therapeutic window. Consider, for example, the case when the toxicity threshold X_o is positioned at the 10th (narrower therapeutic window) or the 1st (wider therapeutic window) percentile (Fig. **4**). Thus, for a given change in the population mean, the percentage elevation in toxicity risk would be higher for compounds with a wider therapeutic window (Table **4**).

The idea of using changes in population tails to gauge the impact of interethnic differences in quantitative phenotypes was earlier named as the "edge effect" [Kalow, 1992]. We herein reason that this concept also has implications for interpretation of the clinical significance of multigenic biomarkers and the development of therapeutic or regulatory policies towards rational implementation of future pharmacogenomic discoveries in the clinic. Thus, using the precedence discussed earlier on the classification of CYP3A4 inhibitors (Section *1.1*) and as an example of forward applications, subpopulations identified by a pharmacogenomic biomarker may presumably be catalogued into different risk categories by considerations of the degree of change in edge AUCs. Risk categories may reflect, for instance, a low (<2-fold), moderate (2 to 5-fold) or high/marked (>5-fold) increase in the edge AUC in the

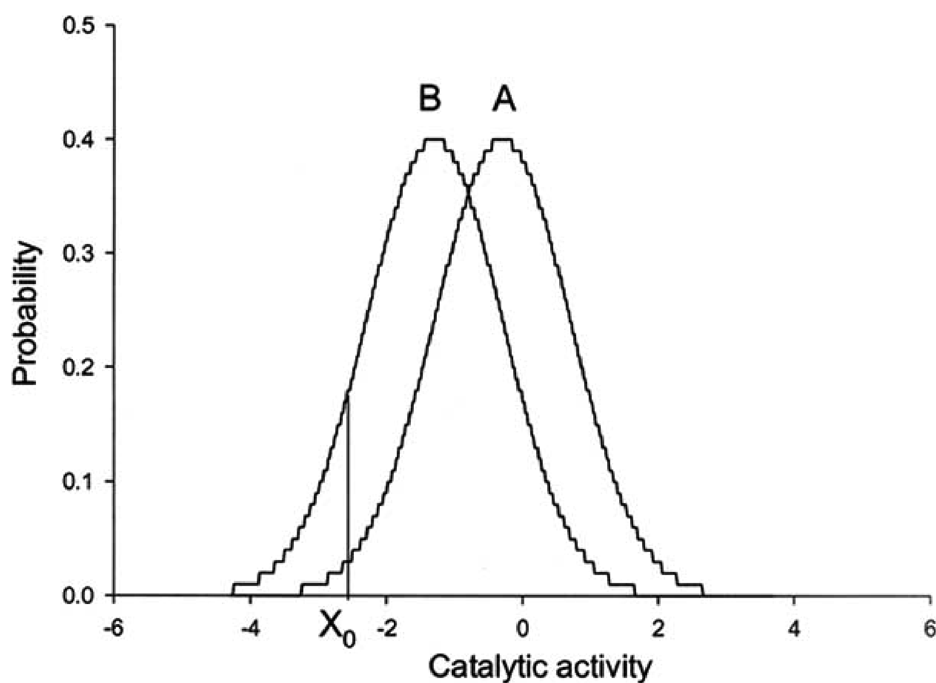

Fig. (3). Standardized normal distributions of catalytic activity of a hypothetical drug metabolism pathway in two populations (A: *fast*; B: *slow*). X_o reflects the threshold activity below which drug toxicity is anticipated. Reproduced with permission from Kalow *et al*. 2001a.

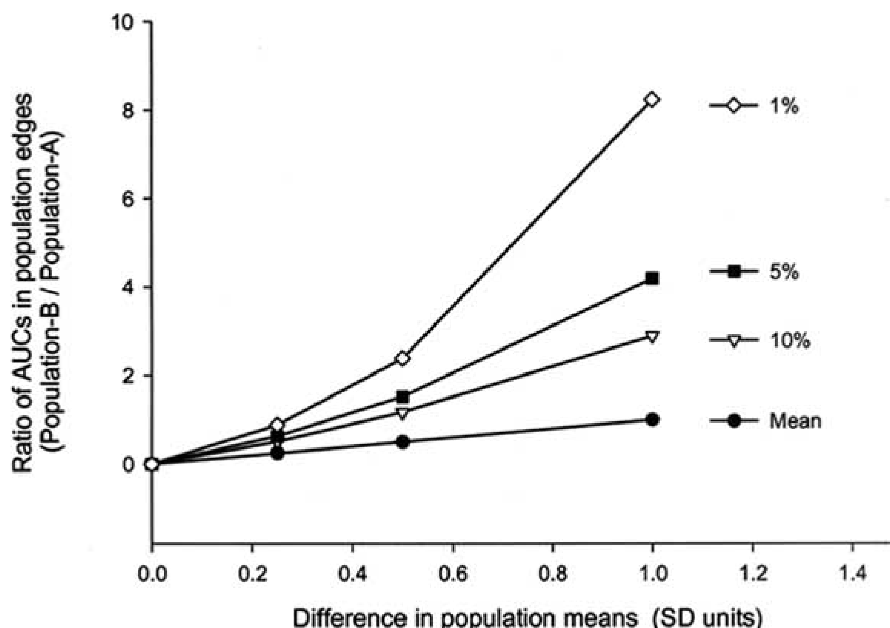

Fig. (4). Ratio of AUCs at two population edges (B/A) as a function of differences in population means when the toxicity threshold is positioned at the 10th, 5th, or the 1st percentile in the index population A. Note the *nonlinear* increase in the ratio of edge-AUCs (population B/population A) compared to the *linear* increase in the difference between population means.

variant population (e.g. Population B) relative to the index reference population (e.g. Population A) (Fig. **3**). Table **4** illustrates the data presented in Figure 4 in a numerical form and with the additional assignment of various risk levels to changes in edge AUCs. For example, note that a small 25% SD difference in mean catalytic activity between population A and population B may be reflected by an 89% increase in risk for toxicity when the X_o is set at 1% (Table **4**). The threshold values for assignment of the risk into different categories (low, moderate, high) shown in Table **4** may of course differ depending on the severity and the nature of clinical endpoint (e.g. drug toxicity) in the clinic. In contrast to monogenic pharmacogenetic traits with bimodal distributions, it is thus clear that risk assessment for multigenic and multifactorial pharmacological effects requires additional population-based considerations to complement decision-making on clinical relevance of pharmacogenomic biomarkers.

6. SOCIETAL, ETHICAL AND THERAPEUTIC POLICY CONSIDERATIONS

Pharmacogenomics has the valuable potential to increase the efficacy of therapies, and to reduce adverse events and the number of subjects needed for clinical trials. Further, targeting drug responses which, unlike complex diseases, have not been subject to millennia of evolutionary selection to counter their effects, may give way to more immediate clinical returns [Goldstein *et al*. 2003]. In concurrence with recommendations of the 11th International Conference of Drug Regulatory Authorities (ICDRA) on implications of regulatory decisions, these possibilities advance the mandate of regulatory agencies to promote and protect public health on the basis of efficacy, safety, quality and cost effectiveness [WHO, 2004]. A method that may provide a graded risk assessment for decision-making on the feasibility of targeting or optimizing drug responses through pharmacogenomics would be a valuable contribution to these ends.

The RDA method may begin to address, in this early stage of implementation of pharmacogenomics in the clinic, a number of significant social, ethical and regulatory policy concerns. These issues include and relate to regulatory oversight, confidentiality and privacy, informed consent, availability, access and clinician's changing roles and responsibilities [Graham, 2001; Buchanan *et al.* 2002]. Additionally, consent and responsibility extend beyond the individual to communities in pharmacogenetic and pharmacogenomic research. This, in turn, requires the recognition of heterogeneity in human relations and cultural identities, as in the human genome, and in discerning where it is appropriate to obtain community consent and consultation, community consultation alone, or not at all [Weijer and Miller, 2004]. Both national and international regulatory agencies such as the ICDRA will likely play important roles in shaping the therapeutic policies for implementation of pharmacogenomics in drug development and at point of care in the clinic. Estimation of the genetic components of variability in drug effects can help to identify individuals or communities who are most likely to benefit from pharmacogenomic research while preventing or minimizing the risks associated with such ethical concerns in populations wherein genetics may play a relatively minor role in their response to pharmaceutical interventions. Insofar as cultural identities may synthetically blend biological (e.g. genetic) and environmental substrates, and can be formed, for example, around disease groups, lifestyle

Table 4. A Population-based Conceptual Framework for Graded Risk Assessment Between Overlapping Subpopulations Identified by Multigenic Pharmacogenomic Biomarkers.

Decrease in mean catalytic activity expressed in SD units	Ratio of Subjects with Drug Toxicity in Population B versus Population A					
	$X_o < 10\%$	Risk Category	$X_o < 5\%$	Risk Category	$X_o < 1\%$	Risk Category
0.10	1.18	Low	1.22	Low	1.29	Low
0.25	1.51	Low	1.63	Low	1.89	Low
0.5	2.17	Moderate	2.52	Moderate	3.39	Moderate
1	3.89	Moderate	5.19	High	9.24	High

X_o represents the threshold catalytic activity below which clinical toxicity is experienced. Relative toxicity risk is calculated by the ratio of the number of individuals who display a catalytic activity below X_o in population B (slower activity) versus population A (faster activity). Risk categories were assigned based on the relative increase in AUC at the edges of population B versus population A. Low risk = <2-fold increase in edge AUC, moderate risk = 2 to 5-fold increase; high risk = >5-fold increase. Splus 4.5 (MathSoft, WA) was used to evaluate the normal and inverse normal cumulative distribution functions and the AUCs.

choices and perceived phenotypic or recently identified genetic characteristics, a method for the estimation of heritability in pharmacology that better accommodates these pluralities and diversities within human societies is to be welcomed.

A marked increase in population mobilities, global migrations and abrupt shifts in habitat and environment by human groups raises the stakes for effective methods for clinicians to ensure safe and efficacious treatments. Simple demographic or phenotypic classifications of patients or disease states do not meet the complexities of the global populations whom clinicians now encounter locally [Seyhan, 1992; Harding, 1998]. Yet these clinicians must decide upon whom to perform and how to interpret a pharmacogenetic test and whether to administer therapies. Further, mean values of phenotypic traits in populations may create cultural or pharmacological stereotypes and will likely prove to have less external validity in the real world than do population edges, which better reflect and incorporate the pluralistic diversities of clinical practices. While numerous social and technical factors affect genetic testing [Williams-Jones and Graham, 2003] as well as drug response, medical researchers and clinicians provide a first line source of hope to the patient but also hold a responsibility not to fill these sufferers with unrealistic speculatory promises and the geno-hype that may be advanced by occasional overly enthusiastic statements in the media on ramifications of preliminary research findings. In an era of heightened public expectations for genetic research and its promises, claims made by individual academic or commercial interest groups on discovery of genetic tests require adequate scrutiny and acceptance in the broader international community of scientists and ethicists before they are widely publicized [Webster *et al.* 2004]. As in any field of research endeavor, a cautious and accountable approach thus requires a strategy that is seen to have an efficient hierarchy of rational discriminators that can be used towards evidence based decision-making in pharmacogenomics. The identification by the RDA method of the size of genetic components and the attendant gene-environment interactions operational in pharmacological variability may contribute towards these goals.

Informed consent and privacy issues in pharmacogenomic research have long constituted one of the focal points of interest in bioethics. Analysis of the ethical implications of genetic research at a societal level, however, may require additional considerations [Hedgecoe, 2004; Williams-Jones and Burgess, 2004]. In this regard, a particular concern is the issue of *equity* [Nuffield Council on Bioethics, 2003]. It is anticipated that pharmacogenomics may create niche populations wherein drugs may work optimally with minimal risk for toxicity. This may also create subpopulations for whom effective drugs are not developed due to the small size of the particular patient group, or for a large but economically poor population. There is very little support for the view that the pharmaceutical marketplace will adequately address the needs of the pharmacogenetically-defined minorities without a comprehensive public policy response [Melzer *et al*. 2003]. The question remains as to whether financial or legislative mechanisms will be in place to ensure that such therapeutic orphan patient populations also have access to, and benefit from, genomics-based new medications. When drugs are developed for neglected populations or orphan therapeutic indications, there may be cause for concern over misrepresentation or excessive promotion of compounds introduced with a pharmacogenomic test [Quick *et al*. 2003]. To this end, a "ratio-based" scaled ethical analysis appears to be necessary for a balanced interpretation: that is, one should take into account not only the selected or singular examples of compounds developed by pharmacogenomic guidance (numerator), but also the entire complement of lead compounds (e.g. those with a high r_{GC}) that could have benefited from co-development with pharmacogenomic biomarkers (denominator).

While the RDA method calculates the genetic component of variability in pharmacology, its accounting of intra-individual variations may provide an avenue that recognize and bring us closer to establishing the environmental components which should not be overlooked in pharmacokinetics and pharmacodynamics. The age old debate on the contributions of genetics *versus* environment to (patho) physiological traits and more recently in pharmacology has resulted in a somewhat mutually exclusive perception of

these two factors. The ability to characterize the heritability of pharmacological traits in a high throughput manner may soon increase the general awareness of the "plasticity" of genetic components: that is, the relative significance of genetic factors can only be deciphered adequately within the proper context of the very environment in which drug effects are elicited. Looking further, it can be anticipated that this intimate interplay between genetics and the environment as well as the ever changing size of genetic components in pharmacology may eventually evolve into a dialectical and complementary synthesis of these two fundamental elements governing drug effects.

7. CONCLUSIONS AND FUTURE PERSPECTIVES

Pharmacogenetics started with studies of the effects of single gene variants on the fate and action of drugs. In the meantime, we have seen that most inter-individual differences in drug response are multifactorial: that is, they result from actions and interactions of many genes, in addition to environmental inputs. Modern genomic methods will discern many of these factors, thereby leading to and into pharmacogenomics.

Pharmacogenomics is a relatively recent arrival on the scientific scene, but it will remain with us as long as there are physicians who prescribe drugs for patients: variations in drug response will be ever present. The variations may demand efforts, either to assure that the intended therapeutic effects are achieved, or to avoid drug-induced toxicity to the patient [Service, 2004]. It is now clear that many of these additional efforts will not be successful without considering a patient's genes. However, obtaining this information will often require the use of genomic methods. Utilisation of this information will also demand the knowledge of drug-gene interactions: genes control drug responses, and drugs may affect gene function by changing gene expression.

To achieve progress in this area, an important stepping stone will be information on the extent to which a given drug-response variation has genetic, and to what extent environmental causes. This question, which means the assessment of heritability, is dealt with to a considerable extent in this paper: in pharmacology, twin studies can be complemented or substituted by RDA studies; that is, by administering a drug on two or more occasions to a group of subjects, and comparing mathematically the intra- and the inter-individual variations. Heritability data, however obtained, are not physical constants: they may vary between populations and may be modified by environmental factors. High-throughput of the RDA method brings the idea of obtaining heritability estimates in various populations, or under different environmental and therapeutic settings into a practical realm.

In spite of the importance of multifactorial or multigenic variation, we cannot afford to neglect monogenic differences of drug responses. In many cases, the variable gene is known and can be tested in the patient's DNA. That is, the accumulating knowledgebase is increasingly lending evidence on the role of genetic tests to allow prediction of a patient's drug response, or to permit optimization of the drug dose for the patient. The patient does not have to take the drug to test the drug's suitability for her or him.

Furthermore, if a variation in a gene is known to influence drug exposure or response, it may affect the choice of chemicals which the pharmaceutical industry tries to develop into drugs. If a chemical would show useful therapeutic effects in some patients while causing toxicity to others, it could still be released as a drug if it comes with instructions as to who might use it. This is not currently done, but could be logical practice in the future.

If a drug response has been shown to be genetically variable, the frequency of variation may differ between ethnic groups, and so may the type of genetic variant. This means that the suitability of a new drug has to be verified through clinical testing in different populations, unless the variable genes and their effects are known and testable. Interethnic differences may also be seen among multifactorially controlled drugs. In that case, the simple determination of the mean responses in two populations and calculation of the difference in terms of standard deviations may have limited clinical meaning; differences between the edges of the distribution curves may need to be considered. The degree of changes in population edges may serve as an additional reference framework to guide the policy decisions on the clinical relevance of multifactorial differences in drug effects between populations identified by pharmacogenomic tests.

Studies on the interindividual variation in the rate of drug metabolism were initially only of academic interest. Today, however, the US FDA and similar authorities require documentation of the metabolism of a new drug before registration. The knowledge of how a drug is metabolized and which enzymes are involved may help to predict drug-drug interactions and how fast an individual patient may metabolize a specific drug [Bertilsson, 2001].

The pharmaceutical industry uses today high-throughput techniques to screen for drug candidates, which are metabolized by and/or inhibitors of polymorphic enzymes such as CYP2D6. Such drugs are not further developed by many companies. If this had been the case in the past, we would not have many of the important drugs available today. We see a danger in deleting potential drugs from development, if CYP2D6 or other polymorphic drug disposition pathways are involved in their metabolism. Therapeutic drug monitoring with use of pharmacogenetic tests is a valuable tool for personalized drug dosing.

In summary, our understanding of pharmacogenomic problems is still in its beginnings, but it is constantly improving. The greatest difficulty is the introduction and *translation* of molecular pharmacogenomic knowledge into clinical practice and rational drug development. This is in part attributable to the lack of adequate tools for assessment of the *extent* of heritability of pharmacological variations as well as the limitations of existing conceptual frameworks for interpretation of the clinical significance of multigenic pharmacogenomic biomarkers. It is noteworthy that technological advances have thus far been a primary focus in pharmacogenomics over the past several years. This emphasis on technology is now slowly shifting towards a focus back on biology and pharmacology, and the recognition that genetic components are not physical constants. The pharmaceutical industry and the regulatory

scientists are also keenly aware that pharmacogenomics can make a difference in drug development but it is not yet clear exactly how some level of priority can be established for pharmacogenomic research in regards to various new therapeutic candidates under early phase clinical evaluation or late-stage development. This commentary extends the applications of the RDA method from pharmacokinetics to the realm of pharmacodynamics, clinical drug development and regulatory decision-making for multigenic biomarkers. The RDA analyses in various populations, or under different environmental conditions, may contribute to dissection of the relative roles of genetic and environmental factors in pharmacological phenotypes, as a baseline measurement prior to extensive investments in molecular genetic work. Once a particular genetic test for a quantitative and multifactorial phenotype is identified, consideration of differences in the size of (sub)population edges identified by the test may be informative for the interpretation of its clinical relevance, and scaling of the risk from an individual patient to a population level. The latter population-based risk conferred by a genetic test or biomarker may complement decisions bearing on therapeutic policies for predictive pharmacogenomic testing in the clinic. It should also be emphasized that an open dialogue and transparent exchange of information among all stakeholders including the academic institutions, the pharmaceutical-biotechnology industries and the regulatory agencies will serve as a crucial and accountable foundation to secure commitment and sustainable progress towards pharmaco-genomic-guided customized therapies in the near future. During this process, there will be an ever increasing role for, and significant contributions made by, bioethicists and researchers in social sciences and humanities as well [Nuffield Council on Bioethics, 2003]. The time scale for the routine application of pharmacogenomics in medical practice and clinical drug development may be in the order of years or decades, but it will be achieved, and it will contribute to more effective and safer drugs and science-based therapeutics.

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ABBREVIATIONS

AIDS = Acquired Immunodeficiency Syndrome

ANOVA = Analysis of variance

AUC = Area under the curve

CDER = Center for Drug Evaluation and Research, Food and Drug Administration, U.S. Department of Health and Human Services

DZ = Dizygotic

EOP2A = End of Phase 2A

FDA = Food and Drug Administration

IND = Investigational New Drug

LD = Linkage disequilibrium

MDR1 = Multidrug resistance protein 1

MZ = Monozygotic

NAT2 = N-acetyl transferase 2

NDA = New drug application

NME = New molecular entity

SNP = Single nucleotide polymorphism

RDA = Repeated drug administration

r_{GC} = Genetic component

SD = Standard deviation

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