translational research, a pivotal next step in developing theragnostic-guided treatments and health policy (see also section on GAP2).

It is noteworthy that studies aimed at GAP1 knowledge translation can be mistakenly framed as the sole translational research activity on the path from basic biomarker research to individually tailored drug therapy. Although the early phase translational biomarker studies noted above provide preliminary insights into predictive value (e.g., sensitivity/specificity) of theragnostic tests in humans, the complete range of pharmacokinetic and pharmacodynamic variability and attendant predictive performance of theragnostic biomarkers within and among human populations are seldom available at the end of GAP1 translational research. This becomes an acute concern, particularly in the case of theragnostic tests based on genomic, proteomic or other -omic technologies.

An important caveat in pharmacogenomic association studies aimed at personalized medicine 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 [13,15,16]. Consequently, the genetic loci that are reportedly associated with drug response or toxicity may not necessarily correspond to the causal genetic variants. The degree of LD also varies markedly in different regions of the genome, as well as among different populations [17–19]. Thus, unless the causal genetic variants are ascertained, the informativeness of genetic markers identified in small-scale GAP1 translational research for prediction of drug response will be fraught with uncertainty when therapeutic forecasts are extended more broadly to other populations beyond the immediate study sample [20]. Furthermore, due to the multigenic nature of most human diseases and pharmacological traits, pharmacogenomic biomarkers can be, but are not always, population-specific; divergent sets of genes may influence the clinical phenotypes in different populations [20,21]. Attention to a large range of social and environmental factors (e.g., smoking, diet or other lifestyle factors) and gene–environment interactions will also be essential to appreciate individual, geographic and population variability in drug effects. Hence, these considerations collectively call for much larger scale population-based GAP2 translational theragnostic biomarker research.

GAP2: translation from clinical proof-of-concept to treatment guidelines based on theragnostic tests

For theragnostic tests and the personalized medicines to become a reality at point of patient care, a broader scope and types of human genetic (for example, other than single nucleotide polymorphisms), proteomic and metabolomic variation will need to be explained, well beyond what is achievable in small-scale GAP1 translational research studies. This is significant particularly from a clinical standpoint, as noted above, because the only barrier between a patient and severe toxicity or treatment failure will be the theragnostic test itself. In cases where the diagnostic sensitivity/specificity of the test is not sufficiently robust, a number of ethical and legal issues emerge related to knowledge transfer, regulation of novel technologies, commercialization and professional responsibility [7,22,23].

A case in point on the limits of GAP1 translational research is the CYP2D6 drug-metabolizing enzyme that contributes to disposition of several important psychotropic agents. Within the *CYP2D6* gene itself, certain alleles are typified by polymorphisms (for example, insertions/deletions) other than the traditionally investigated common nucleotide substitutions [24]. Attention to rare genetic variants will also be necessary in cases where the test results inform critical decisions on choice of drug prescription or dosage. The required sensitivity and specificity of molecular genetic assays, in a clinical diagnostic context, must be markedly higher than the technical standards acceptable for purely research purposes or biomarker discovery applications. Furthermore, clinicians who are familiar with the rapid turnaround times and relatively low cost of clinical chemistry tests may understandably demand a comparable ease of access, affordability and rapidity of test result (e.g., within several days or ideally by the end of each patient's visit). With the exception of a few specialized research centers and tertiary care centers in developed countries, these 'diagnostic standards' are simply not achievable or are well beyond the present capacity of public healthcare systems in many countries [8].

Another avenue for GAP2 translational theregnostics research, and one that has thus far been overlooked, is the use of population databases such as UK Biobank, the Estonian Genome Project, the Icelandic Healthcare Database and the proposed Quebec CARTaGENE project [10–12]. Thus far, the primary focus of these population databases has been the identification of

disease susceptibility genes with applications towards drug target discovery or disease risk assessment [25,26]. Conceivably, these biological and phenotypic/epidemiologic repositories can also contribute to the identification and/or validation of theragnostic tests to individualize drug treatment regimens. Potential benefits of population biobanks, and the means or research methodologies to achieve them over the long term, still remain ill-defined. The data contained in biobanks are quite variable in terms of content and quality, as well as the type of consent obtained from participating subjects. There is little harmonization or standardization of data collection and banking procedures amongst biobanks [103], making the exchange and sharing of data practically and financially difficult, a situation further compounded by common professional tendencies in biomedicine and human genetics research towards data withholding [10,27–29]. It would be timely to initiate key stakeholder meetings and wider community consultations to examine the impact of biobanks and theragnostic testing on medical practice, health professionals' education, awareness, professional responsibilities and how best to communicate and translate findings related to new theragnostic markers identified or validated in biobanks.

It is still unclear whether the dual objectives of biomarker validation for disease susceptibility and drug response variation are both achievable within the constraints of a single population biobank. For instance, disease phenotypes can be ascertained dichotomously as 'present' or absent'. In contrast, for drug response phenotypes to be clinically meaningful, they may require a higher resolution definition with continuous measures and repeated observations over time. Drug response may also fluctuate due to drug–drug interactions or time-dependent changes in physiological states (e.g., diurnal rhythms or menstrual cycle). Another more focused application of population biobanks could be the identification of gene–environment interactions in the context of drug therapy. Populations of patients who are tracked for their drug response over long periods of time can help to discover and validate rare but serious drug side effects during postmarketing safety assessments. Consider, for example, the relatively uncommon but lethal cardiac side effects of the selective cyclooxygenase 2 (COX-2) inhibitor rofecoxib (Vioxx®) that could not be detected reliably in small-scale early phase premarketing clinical trials. However, given the global nature of contemporary bioscience research, drug development, and marketing of new medicines, it is very likely that a coordinated multi-biobank approach to theragnostic applications will be necessary.

Technical, bioinformatic and phenomic integration in theragnostics: rationale for centralized translational clinical research centers

Success in translational theragnostic research depends on expertise in three fundamental domains:

- Core technical expertise to generate high-throughput biomarker data;
- Collection of large volumes of phenotypic data from patients treated with drugs;
- Ability to perform correlative bioinformatics analyses between biomarker data and drug related phenotypes.

Due to the rapidly declining cost of genotyping and other biomarker genotypic technologies, availability of phenotypic data is now the most crucial and rate limiting step among these three domains [30]. This creates a statistical conundrum: in order to attain adequate statistical power to allow correction for multiple testing and association analyses among multiple biomarkers and clinical end points, researchers require an increasingly larger number of human subjects or biological specimens (for example, tumor biopsy material) to accompany the high-throughput theragnostic biomarker data [29,30]. Therefore, in addition to the technical integration, there is an acute need to establish local, national and international 'phenomic' databases that can integrate drug-related phenotypes across a broad range of treatment outcomes in different therapeutic areas, using both public and privately-sponsored pharmaceutical research and clinical trial data (a significant challenge given the proprietary, and thus secret, nature of such data).

To the extent that integration across technical (for example, amongst genomic–proteomic–metabolomic divides) and phenotypic dimensions is an emerging and timely theme in translational theragnostic research, what are some of the optimal research strategies that can deliver on this goal? We submit that one of the internationally recognized integrated models for translational clinical research is the General Clinical Research Centers (GCRCs), a national US network of approximately 78 centers, mostly located within the research hospitals of academic medical centers. The primary mission

of the GCRCs is to provide a research infrastructure for clinically oriented investigators. Furthermore, GCRCs act as an important link between molecular research and clinical practice, allowing investigators to translate knowledge gained through basic research into the development of new or improved diagnostics and therapeutics for patient care. With the emergence of theragnostics and increasing public demands for personalized medicine, it would be timely to amend the existing GCRC research infrastructure to accommodate integrated biomarker research towards the eventual goal of individually-tailored drug therapy. Conceivably, theragnostic-oriented GCRC networks can also serve to pool phenotypic information derived from industry-sponsored clinical trials (assuming stricter requirements for data disclosure) along with publicly funded academic pharmaceutical research across medical disciplines both at institutional, national and international levels.

Expert commentary & future outlook

Personalized drug therapy is not a new concept [15,16,31]. However, theragnostic testing is beginning to transform medical practice in a fundamental manner by placing a greater emphasis on the notion of probability [7,32], instead of traditional expectations about definitive prediction of treatment outcomes. The scope of research in this field has changed over the past several years with the availability of new technical and methodological approaches such as proteomics and metabolomics. At the moment, these promising technologies are best suited for exploratory research and remain to be validated both in terms of sensitivity/specificity of the data they generate and their mechanistic relevance in explaining variability in treatment outcomes in a population context. In parallel to these new technologies, the precision of existing technologies in applied genomics (i.e., high-throughput genotyping and gene-expression analysis) has increased while the unit cost of assays has markedly decreased.

Arguably, all these technical advances reflect an emerging 'engineering triumph' in biomarker research and more broadly, in diagnostic medicine [33]. However, for this to translate into a 'biological triumph' in a clinically meaningful manner, there is an acute need for the integration of biomarker data. However, our fear is that continued reliance on a singular biomarker technology platform by different stakeholders may result in an artificial compartmentalization (or fragmentation) of biomarker research. For example, human geneticists and pharmacogenomics researchers may favor genotyping and gene expression analyses, while biochemists may primarily utilize proteomic methods. On the other hand, drug effects are determined multifactorially, and the human genome is subject to poorly understood plasticity. Thus an integrated and promiscuous approach to biomarker technology platforms – whether they rely on genomic, proteomic and/or other methodologies – should be adopted so long as it explains individual differences in drug efficacy and safety in a mechanistic and clinically meaningful manner. It is against this need for technical and phenotypic integration that the new subspecialty of theragnostics and the attendant requirement for translational research centers are emerging.

Despite considerable efforts in GAP1 translational biomarker research, there remains a large and serious gap in further translation of biomarker data obtained in FIH pharmacogenomic proof-of-concept studies to a population level for the development of personalized treatment guidelines using genetic or other types of theragnostic tests. Large-scale biobanks are being developed in several countries around the world to meet these objectives. These databases concern the general population as opposed to particular patient groups or families. The amount of information gathered on the individual, as well as the types of diseases studied, constitute a divergence from the genetic registers of the past as well as from the gene-hunting (or discovery) research of today. Another change in the research paradigm is the desire for public consultation. These databases depend on public participation and assent. Therefore, it is important to encourage a free, open and useful dialogue among all stakeholders involved.

Due to the inherent focus on theragnostic 'product development', whether it be in biobanks or GAP1 translational research, there may be cause for concern over how much weight will be given to more fundamental research that may not directly have an application in the clinic [29]. Such concerns coincide with a shift in the perceived mission of academe and medical research, particularly with regards to the applied sciences. In addition to being sites of advanced teaching and research (the university's 'first' and 'second' missions), universities must now engage in knowledge transfer that leads to technology development and economic growth (the 'third mission'), a role that has proven popular with

governments, industries and universities worldwide [1,29]. To facilitate this third mission (and some would argue, to transform universities into 'entrepreneurial' institutions), laws and policies have been implemented to ensure strong protection of intellectual property rights and facilitate commercialization and technology transfer. Such patents can still have serious negative consequences for the conduct of academic research and free sharing of data amongst population biobanks [3,29].

Advances in theragnostics will likely take place in small but significant steps. Development of the necessary research resources – i.e., interdisciplinary research centers, harmonized large-scale biobanks, and so on – to enable the integration of molecular biomarker data with the attendant environmental factors, and the subsequent translation into clinical practice and regulatory frameworks needs to be planned much sooner. There is a clear need for translational clinical research centers that can integrate the full range of biomarker data from different levels of the biology and technology platforms (e.g., genomic, proteomic and metabolomic) as well as a broad range of pharmacological phenotypes (i.e., phenomics) in a way that is meaningful from both the physicians' and patients' individual perspectives.

Highlights

- In the context of theragnostics, translational research is clearly a complex and multistage process.
- Inadequate recognition of two major bottlenecks impedes the translation of current theragnostics research to the point of patient care: translation from basic science to first-in-human proof-of-concept; and translation from clinical proof-of-concept to development of evidence-based personalized treatment guidelines.
- The need to harmonize large-scale population biobanks to enable translation of theragnostics research is fraught with scientific, technical, social and political challenges. However, these challenges are not insurmountable.
- Broad public and stakeholder engagement is essential for the development of effective and socially acceptable biobanks that can allow a deeper understanding of both disease pathophysiology and individual determinants of variability in drug response and toxicity.
- The application of theragnostics at the point of patient care, i.e., the dream of personalized medicines, requires broad scale interdisciplinary collaboration along the development pathway, from rigorous basic and applied -omics research to the ethical implementation and delivery of safe and effective therapeutics.
- There is an acute need for resource development, for example, translational clinical research centers, for integration of biomarker data from different levels of the biology and technology platforms, as well as a broad range of pharmacological phenotypes in a way that is meaningful from both the physicians' and patients' individual perspectives.

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ORIGINAL INVESTIGATION

Asymmetry in Scientific Method and Limits to Cross-Disciplinary Dialogue: Toward a Shared Language and Science Policy in Pharmacogenomics and Human Disease Genetics

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Pharmacogenomics is a hybrid field of experimental science at the intersection of human disease genetics and clinical pharmacology sharing applications of the new genomic technologies. But this hybrid field is not yet stable or fully integrated, nor is science policy in pharmacogenomics fully equipped to resolve the challenges of this emerging hybrid field. The disciplines of human disease genetics and clinical pharmacology contain significant differences in their scientific practices. Whereas clinical pharmacology originates as an experimental science, human disease genetics is primarily observational in nature. The result is a significant asymmetry in scientific method that can differentially impact the degree to which gene-environment interactions are discerned and, by extension, the study sample size required in each discipline. Because the number of subjects enrolled in observational genetic studies of diseases is characteristically viewed as an important criterion of scientific validity and reliability, failure to recognize discipline-specific requirements for sample size may lead to inappropriate dismissal or silencing of meritorious, although smaller-scale, craft-based pharmacogenomic investigations using an experimental study design. Importantly, the recognition that pharmacogenomics is an experimental science creates an avenue for systematic policy response to the ethical imperative to prospectively pursue genetically customized therapies before regulatory approval of pharmaceuticals. To this end, we discuss the critical role of interdisciplinary engagement between medical sciences, policy, and social science. We emphasize the need for development of shared standards across scientific, methodologic, and socioethical epistemologic divides in the hybrid field of pharmacogenomics to best serve the interests of public health.

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Coalescence of Clinical Pharmacology and Human Disease Genetics by Shared Application of New Genomic Technologies

The scope of scientific inquiry in clinical pharmacology and human disease genetics has expanded over the past several years with the development of population-based databases (eg, UK Biobank, the Estonian Genome Project, GenomEUtwin, CARTaGENE) and the introduction of new genomic technologies, such as high-throughput analysis of gene expression.[1–5] These genomic technology platforms aim to characterize multiple genes, often on the order of tens of thousands, to enable an integrated view of genetics and its role for drug efficacy and safety. The origin of the genomic technologies is not, however, rooted in pharmacology but can be traced back to advances made on the heels of the Human Genome Project.[6–8]

Intensive deoxyribonucleic acid (DNA) sequencing efforts in the late 1990s, facilitated by the coalescence of traditional methodologies used in human genetics and cell biology, resulted in technology platforms capable of generating large volumes of data in very short time frames. Genomic technologies are now increasingly adopted in pharmacologic sciences, with an attendant expansion of the scientific process. These advances start with the view that a broader investigation of the multiple components of a complex biologic pathway targeted by a pharmaceutical compound may provide better insights into the mechanisms of drug action and ultimately allow individualization of drug therapy.[9] Hence, clinical pharmacology and human genetics research are rapidly coalescing, in part owing to such broad and shared applications of genomic technologies.

When scientific disciplines meet toward a common goal, both technical expertise and expectations of practitioners for what constitutes scientific merit inevitably struggle for position. The extent of similarities and discrepancies among the views of scientists from the respective disciplines and the ensuing critical debate on new hypotheses or technologies in a given field often serve as catalysts for the rejection or wide adoption of new hypotheses and technologies.[10] Important innovations emerge from creative interdisciplinary sharing of methods and concepts, yet it is essential that precautionary principles are adhered to in standards for scientific validity and reliability.[11–14]

Whereas clinical pharmacology is an experimental science, most genetics research on human diseases uses a scientific approach that is primarily observational. This results in an asymmetry in scientific method that can differentially impact the degree to which environmental components of phenotypic variability are controlled, including the sample size requirements of each discipline. The number of subjects participating in observational genetic studies of diseases is often used as a key criterion of attendant scientific value; it is also a significant driver of which 'disease gene' discovery is worthy of further policy-oriented translational research or application at the point of patient care. Because environmental factors (and the attendant confounding) are difficult to discern or control in observational study designs, there is an expectation, particularly on the part of the policy makers familiar with population health and large-scale epidemiologic studies, of a large sample size (eg, from several hundreds to thousands) in genetic studies on disease predisposition. Yet these requirements do not necessarily apply to experimental study designs.

Environmental confounding can (and we suggest should) be monitored more readily by scientists in experimental sciences (eg, in pharmacology or pharmacogenomics) prior to or during the execution of the study. Failure to discern such discipline-specific nuances for differential environmental confounding in genetic studies rooted in either pharmacology or disease predisposition will bias expectations for sample size requirements, along with perceptions of the merit of new genomic discoveries. Such interdisciplinary differences in norms and expectations regarding scientific merit may lead to inadvertent dismissal of methodologically sound small-scale exploratory pharmacogenomic studies as new policies are being developed for genomics research in population-based databases. Some of these pharmacogenomic studies may well have appropriate statistical power to detect genetic components of pharmacologic variability.

Pharmacogenomics is usually defined as the study of variability in drug response using information from the entire genome of a given individual patient.[1,2,4] Pharmacogenetics, by contrast, is hypothesis driven

and focuses on a limited set of candidate genes selected based on a priori observations of disease susceptibility, drug absorption, metabolism, transport, and excretion, as well as drug targets, as opposed to a genome-wide hypothesis-free approach in pharmacogenomics. It is noteworthy that pharmacogenetics and pharmacogenomics are also interdependent: once a novel gene(s) of relevance for mechanism of drug action is identified through the genome-wide pharmacogenomics search, such individual genetic biomarkers require further validation and follow-up by pharmacogenetics before they can be routinely applied in clinical medicine. For the purpose of the present discussion, we use the term *pharmacogenomics*, but many of the concepts discussed herein will also be applicable to pharmacogenetic investigations.

The objective of the present comparative analysis is to identify and elaborate on these significant asymmetries between clinical pharmacology and human disease genetics in the hybrid field of clinical pharmacogenomics. We emphasize the importance of recognizing pharmacogenomics as an experimental form of science. This broader view of pharmacogenomics addresses an ethical and science policy imperative to favor prospective clinical pharmacogenomic investigations over the ad hoc retrospective biomarker investigations that have, thus far, typified biomarker applications at the point of patient care or late-stage drug development.

Expectations and Challenges for Policy Making in Interdisciplinary Science

Expectations about the merit or promise of a biotechnology or a new scientific field evolve through a complex and subtle interaction of (1) media interest and consumer demand in the society (eg, patients, caregivers, and physicians) for better therapeutic products and services; (2) dialogue among scientists, governments, and policy makers to ensure that the latest scientific standards are met and empirically grounded interdisciplinary science policies are developed; and (3) corporate or private sector marketing of resulting technologies.

Within the process of policy making, there may be increased complexity (and unpredictable outcomes) when disciplinary boundaries are crossed by individual regulators or scientists investigating the broad application of a novel discovery or technology in multiple fields of scientific inquiry. This situation is particularly evident with the application of genomic, proteomic, or other high-throughput '-omics' technologies in fundamental and applied bioscience research. Such cross-disciplinary journeys are not without their challenges. Scientists regularly encounter stigma and resistance to novel hypotheses or methods, and collaborations can reach an impasse when the norms governing scientific merit in a discipline are not mutually reconciled or renegotiated in light of the particular attributes of each field of inquiry. Thus, while evaluating new technologies and concepts borrowed from diverse but complementary disciplines, regulators engaged in policy making need to employ multiple lenses to discern disciplinary nuances.[15–17] This is a timely consideration for, as noted earlier, many countries and the private sector in applied genomics are in the process of developing large-scale genomic databases and biobanks.[3,18,19] When drawing conclusions on the public health significance of new genetic discoveries and their potential for application in patient care, identification of the particular characteristics of human disease genetics and pharmacogenomics that strengthen or weaken the credibility of the resulting methods or products should be taken into account.

Contrast between Observational and Experimental Study Designs: Why Is This Relevant to Interdisciplinary Policy Development for Pharmacogenomics?

Since the late 1990s, the idea of exploring pharmacologic phenotypes (eg, drug effectiveness and side effects) as another promising dimension of genetic research has attracted a number of human geneticists to the field of clinical pharmacology and vice versa. This bidirectional exchange of scientific expertise benefited and complemented the classic pharmacologic approaches to questions of variability in pharmacokinetics and pharmacodynamics. At the same time, there has been a tendency to view pharmacologic responses akin to disease phenotypes. There are, however, several fundamental differences between human disease genetics research and clinical pharmacogenomics that require particular attention for a balanced interpretation of scientific merit in genetic studies of pharmacologic phenotypes (Table 1).

A fundamental goal of human genetics research is to establish the causal links between genes and disease phenotypes or characteristics. Yet most common complex human diseases initiate and progress over a considerable period of time before clinical signs and symptoms manifest. This means that environmental contributions to disease phenotypes are difficult to determine without longitudinal studies. It can be prohibitively expensive to discern disease-environment interactions when long-term observation and follow-

Table 1 Distinctions in Scientific Method (Experimental vss Observational) between the Disciplines of Clinical Pharmacogenomics and Human Genetics, Respectively, that May Differentially Influence the Sample Size Requirements and the Attendant Perceptions on Scientific Merit

Discipline-Specific Attribute	*Clinical Pharmacogenomics*	*Genetics of Common Complex Human Diseases*
Study design considerations		
Most common design	Experimental; the investigator can actively manipulate the drug dose or exposure	Observational; the investigator does not induce the disease and instead quantifies phenotypes, usually after disease is clinically manifested
Within-subject study design	Feasible	Not feasible or can be unethical
Reduction of bias in study design with use of randomization	Feasible	Not feasible; disease susceptibility is not subject to assignment and, rather, is observed
Phenotype considerations*		
Temporal attributes of phenotype	Both prospective and retrospective samplings are feasible	Often retrospective sampling of disease phenotypes is required or the only feasible option
Repeated measures data collection to enrich phenotypic characterization	Feasible	In most cases, it can be prohibitively expensive owing to long time frames required for clinical manifestation of disease signs and symptoms
Environmental contribution to phenotypes	Calculable	Often incalculable; difficult to control or eliminate when calculable
Baseline phenotypes	Discernible prior to drug administration; this allows unequivocal calculation of the net drug-related phenotypes by subtracting the predrug phenotypes from the composite phenotypes obtained post–drug administration	Often not discernible owing to slow initiation and progression of most common complex human diseases over many years
Rechallenge/challenge with independent variable (ie, drug treatment or disease induction or susceptibility)	Phenotype ascertainment and its 'drug-relatedness' can be further strengthened by discontinuation of drug treatment followed by subsequent rechallenge with drug treatment	Disease processes often cannot be experimentally switched 'on' or 'off' to ascertain the attendant clinical phenotypes
Other distinctions		
Feasibility of in vitro studies to estimate the scope of allelic or locus genetic heterogeneity	Drug itself can be used as a 'probe' by virtue of its physicochemical interactions with drug-metabolizing enzymes, transporters, or molecular targets for efficacy to discern the high-priority candidate pharmacokinetic and pharmacodynamic pathways and the attendant locus and allelic genetic heterogeneity In vitro studies are feasible to estimate the upper-bound limit on the number of plausible candidate genes, particularly in the case of pharmacokinetic pathways or molecular drug targets	Often no biologic or physicochemical probe is available to empirically discern the type or the number of disease-related biologic pathways (with the exception of certain environmentally induced cancers or diseases)

*Our comparative analyses should not suggest that clinical pharmacogenomics, as a discipline, is uniformly at a greater advantage in achieving optimal phenotype ascertainment and study design than human disease genetics research. Instead, the distinctions highlighted are context specific and emanate primarily from the differences in the scientific method between the two disciplines (experimental vs observational, respectively). Moreover, phenotypic ascertainment of certain pharmacologic phenotypes, particularly in the case of categorical treatment outcomes (eg, responders and nonresponders), can meet with discordance among physicians, whereas the availability of disease diagnostic criteria (eg, *International Classification of Diseases*) may facilitate uniformity in phenotype ascertainment in human disease genetics research.

up are required in ostensibly healthy individuals who are predicted to develop a disease phenotype in the far too distant future. By contrast, as an experimental science, clinical pharmacology is able to elicit phenotypes (in a controlled laboratory or hospital setting) within a matter of a few minutes (eg, antihypertensive drugs), days, or weeks (eg, anticancer medications), during which it is feasible to measure and account (to a certain extent) for environmental components of pharmacologic variability. Seen in this light, it is possible to understand drug effects as an acquired form of biologic variance.[20]

The measurability of drug effects and the recognition that drugs are well-characterized modifiers of normal life processes or (patho)physiologic events led, nearly 50 years ago, to establishment of the origins of pharmacogenomics as a new medical subspecialty.[1,2,9] The technical advances over the past decade have, in effect, blurred the interdisciplinary boundaries in pharmacogenomics research. For example, even though the observational and experimental nature of human disease genetics and pharmacogenomics, respectively, may allow different degrees of control over environmental influences, such disciplinary nuances are not always recognized. This recognition is important since sample size requirements to achieve an optimal signal to noise ratio for discovery of genetic markers of pharmacologic phenotypes and disease-related traits can markedly differ.

It should be stressed that reproducibility of new genetic findings in independent samples is required in both human disease genetics research and pharmacogenomics, in part owing to population-to-population differences in the type and frequency of genetic susceptibility loci for a given phenotype in the human genome. In addition, large sample sizes are often required to detect the small individual effects of numerous genes and their complex gene-gene/gene-environment interactions on drug response or disease phenotypes. We suggest, however, that a smaller sample size is sufficient for such replication studies in clinical pharmacogenomics owing to greater control of environmental confounding in pharmacologic phenotypes.

In the late nineteenth century, Paul Ehrlich proposed the presence of "chemoreceptors" on microorganisms and cancer cells that differ from the host organism—a precursor to the current concept of molecular drug targets and selective toxicity of modern medicines.[21] The presence of discernible targets suggests that drugs can serve as invaluable probes to guide the identification of plausible pharmacokinetic or pharmacodynamic biologic pathways. One concrete example is in vitro drug metabolism studies that reliably identify the CYP-450 enzymes that may contribute to clinical pharmacokinetics of a new therapeutic candidate. Because only a handful of CYP-450 enzymes are responsible for drug metabolism, these in vitro approaches can provide a practical upper-bound limit on the number of candidate genetic loci and, by extension, the scope of genetic heterogeneity causally related to variability in a clinical pharmacology phenotype.[17,22]

These theoretical and applied nuances collectively underscore the fact that environmental factors and genetic heterogeneity can be discerned or controlled more readily (although never totally controlled) in clinical pharmacogenomics than human genetics by virtue of pharmacology's nature as an experimental science (see Table 1).[23] Hence, for a given sample size, our ability to detect genetic markers may be significantly enhanced by careful consideration and accounting for environmental effects through experimental study designs in pharmacogenomics. Additionally, the application of randomized and prospective pharmacogenomic studies is an entirely feasible strategy through which confounding by environmental factors can be further reduced.

A rational strategy is needed to assign priority to drugs that are subject to a higher degree of genetic regulation.[22] This would enhance the signal to noise ratio for genetic factors and could permit pharmacogenomic association studies in smaller number of subjects. Typically, heritability estimates are obtained using the twin method. Twin studies are very useful to establish the genetic components for common complex disease phenotypes (eg, breast cancer) but have limited applicability in pharmacologic responses to drugs. Some of these limitations include difficulties in recruitment of twins and 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. To remedy the difficulties associated with the twin approach, a repeated drug administration (RDA) method was proposed by Werner Kalow wherein between- and within-subject variances in drug efficacy or safety are compared.[22,24,25] The RDA method requires the following considerations. 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 biologic variation, random and nonrandom (eg, 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 by Kalow and colleagues,[24] the genetic component (r_{GC}) of variability in a time-dependent pharmacokinetic or pharmacodynamic occurrence can be estimated with the following equation:

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

The r_{GC} values approach 1.0 point to overwhelming genetic control, whereas those close to zero suggest that environmental factors dominate. In essence, any dynamic biologic process exhibiting time-dependent decay and negligible carryover effects between repeat observations can be amenable to RDA studies to dissect the genetic contribution to interindividual variability in the corresponding biologic phenotype.[22] Recent applications of the RDA method demonstrate that genetics plays a paramount role in pharmacologic traits hitherto not subjected to pharmacogenomic analysis, such as renal drug disposition and pharmacokinetic variability of the antiretroviral drug didanosine.[26,27]

In our focused comparison of clinical pharmacogenomics and human disease genetics research, it should be clear that despite the application of prospective design, clinical pharmacogenomics cannot completely account for the diverse socioeconomic and environmental factors (eg, other medications, alcohol, diet, workplace, etc.) that will actually affect the patient and potentially result in adverse drug reactions in their day-to-day use of the medication.[28] Moreover, phenotypic measurement of drug effects remains particularly problematic in fields such as psychopharmacology, even in the presence of strict monitoring of environmental effects. The temporal and geographic plasticity of human behaviors (independent from drug treatment) and limitations of clinical rating scales to capture nuanced changes in behavioral responses to drugs introduce uncertainty in ascertainment of pharmacologic phenotypes in psychiatric pharmacogenomics.

Increased Ability to Generate High-Throughput Genomic Data Creates New Sociotechnical Actors and Control Points in the Scientific Process

High-throughput genomic technologies can generate large volumes of genetic data, but they also create a particular statistical conundrum. To attain adequate statistical power and to allow association analysis between multiple genetic factors and clinical phenotypes, researchers require an increasingly larger number of human subjects or biologic specimens (eg, biopsy material from cancerous tissue) to match the high-throughput data generated by new genomic technologies. At first glance, this may come across solely as a logistical issue concerning subject recruitment for clinical pharmacogenomic investigations. Indeed, subject recruitment is, and has always been, an important barrier to successful execution of clinical investigations, whether they are in the area of human disease genetics or pharmaceutical research. However, present throughput of the data generated by genomic methods is vastly greater, by at least several orders of magnitude, compared with only a decade ago.

Reflecting on the three key components of scientific process, from (1) conception of new ideas or study design and (2) execution of a study protocol (eg, including subject recruitment) to (3) analysis and interpretation of new findings, it becomes evident that subject recruitment or collection of clinical phenotypic data is increasingly the de facto critical rate-limiting step or bottleneck in pharmacogenomics.[29,30] The cost of genotyping or other genomic methods has declined markedly, and sophisticated but affordable bioinformatics software and trained personnel are available for association analysis to establish the link between genomic data and clinical phenotypes. This, then, invariably affects the nature of stakeholders and the attendant sociotechnical networks.[29] The role of scientists as gatekeepers in genomic science is being fundamentally altered.[29] In particular, those scientists with small-scale innovative laboratories with limited subject recruitment infrastructure are particularly vulnerable to this new type of large-scale recruitment-driven genomic science. New sociotechnical actors and research coordinators who are not necessarily grounded in human genetics, pharmacology, or social sciences may thus become influential in subject recruitment and, by extension, in research governance.[29,30]

Returning to genomics and science policy, it is noteworthy that the present emphasis on large study sample sizes in clinical pharmacogenomics in part reflects the expectations carried over from observational genetic studies on disease susceptibility as the two disciplines coalesce around shared genomic technologies. If the experimental nature of clinical pharmacogenomic inquiries and the attendant ability to better control or eliminate environmental contributions are not fully appreciated, there will be a risk of premature dismissal of small sample–sized pharmacogenomic studies, even though, as noted earlier, they may have adequate statistical power. Thus, the differences in scientific method in clinical pharmacogenomics and human disease genetics present challenges to practitioners in both research fields. There are

also, however, untapped opportunities to increase adoption and acceptance of genomic technologies at the point of patient care. In particular, the recognition that pharmacogenomics is an experimental science creates an avenue for a systematic policy response to the ethical imperative to prospectively pursue genetically customized therapies before regulatory approval of pharmaceuticals.

Visions of Pharmacology as an Experimental Science: An Ethical Obligation to Conduct Prospective Pharmacogenomic Studies?

In general, the drug development process spans between 10 and 15 years from the discovery of a new drug molecule to regulatory approval for the drug to be marketed to the public. Understandably, a lag period is anticipated before new therapeutics developed with the use of -omics technologies, such as pharmacogenomics or proteomics, will be available in the clinic. For drugs that are presently in clinical use, one might expect that pharmacogenomics would have been already adopted prospectively in phase 4 clinical trials (ie, postmarketing studies of large patient populations) as there has been a dramatic increase in the availability of -omics technologies in biomedical research laboratories over the past decade.[6,11] It is interesting to note, then, that there is an acute shortage of prospective clinical studies designed to individualize drug labels, that is, formally limit a drug's target population to those people with a certain genotype.[8,15,31]

To date, most pharmacogenomic studies have been conducted in clinical trials designed for another purpose: to demonstrate efficacy or safety for drug registration by regulatory bodies such as the US Food and Drug Administration (FDA). The highly structured time frames in these trials may not always permit adequate scientific rigor or flexibility for exploratory research oriented toward genetic test development for individualization of drug therapy. In certain cases, this may lead to an ad hoc retrospective sampling of clinical trial data (eg, only when or if a compound displays toxicity after introduction into the market), even though, as noted earlier, prospective study designs are entirely feasible in pharmacology. By contrast, an abundance of discovery-oriented research (ie, remote from direct clinical applications to customize drug therapy) with genomic technologies is taking place for identification of new drug targets or proof of concept in early-phase clinical trials.[15] But this early-phase upstream basic research does not necessarily guarantee the eventual downstream access to genetic testing or delivery of personalized medicines at the point of patient care.[15,31–33] A number of concerns, such as small market sizes in narrowly defined therapeutic fields, have been presented in the past as an explanation for the obvious trepidation associated with the prospective development of pharmacogenomic tests at the point of care.[31,32,34]

We suggest that the motivations for prospective clinical pharmacogenomic applications to proactively influence drug labels and prescriptions may also be shaped by the type of pharmaceutical associated with specific pharmacogenomic tests. In 2004, of the 113 new drug applications (ie, marketing approval) approved by the FDA, only 17 (15%) were considered significant improvements compared with already marketed products.[35] Although there is much to be celebrated in terms of singular success stories on selected innovative medicines developed by the pharmaceutical industry, many of the pharmacotherapies introduced into the market every year are 'me-too' drugs, displaying comparable efficacy and safety profiles with already existing medicines (Figure 1).[35–37] These me-too drugs may be economically very profitable and in some cases will even constitute 'blockbusters' that generate billions of dollars in revenue. But for our purposes, it is important to note that in the context of customized therapeutics, me-too drugs (whether blockbuster or not) may adversely influence motivations for pharmacogenomic testing in the clinic in ways that were previously unanticipated.

Consider a hypothetical therapeutic area (eg, statins to reduce blood cholesterol or selective serotonin reuptake inhibitor antidepressants) that is characterized by an abundance of me-too drugs, with 60 to 80% of the available drugs exhibiting a similar pharmacologic mode of action or efficacy or safety profile. A pharmacogenomic test for a me-too drug may be equally predictive of treatment outcomes for most, if not all, drugs within the same me-too category, redistributing the financial gains made on the diagnostic test from an individual pharmaceutical company holding the pharmacogenomic patent to multiple firms that manufacture similar me-too drugs. Hence, the past and present focus on me-too drug development may serve as a barrier to both innovation in pharmacotherapy and the development of targeted therapies in conjunction with pharmacogenomic tests.

Another hitherto overlooked consideration is the significant reduction over the past decade in the duration of tenure and increased turnover of chief executive officers (CEOs) in various multinational corporations. For example, in a survey of CEO succession at the world's largest 2,500 publicly traded companies, Lucier and colleagues found that 14.2% of

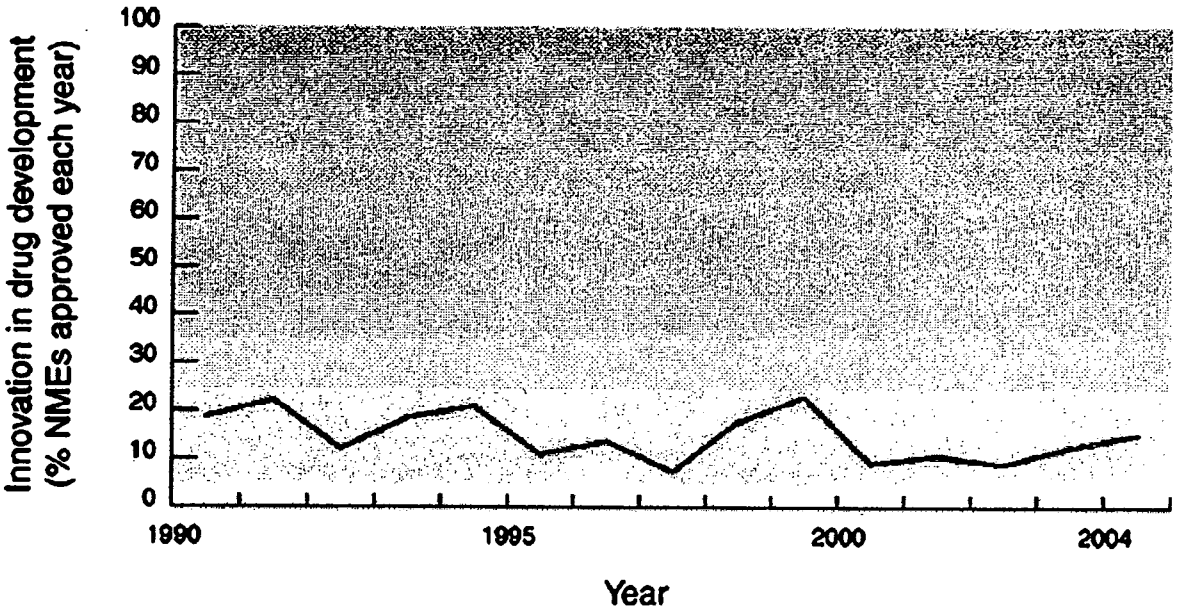

Figure 1 New drug applications (NDAs) approved in calendar years 1990–2004 by the US Food and Drug Administration (FDA) and the new molecular entities (NMEs) subjected to priority regulatory review while offering a significant improvement compared with marketed products in the treatment, diagnosis, or prevention of a disease. Innovation in drug development, as defined by the percentage of these breakthrough NMEs in relation to all NDAs approved in each calendar year, remained low for more than a decade. This further underscores the importance of recognizing (1) pharmacology and pharmacogenomics as experimental lines of scientific inquiry and (2) the attendant ethical obligation to prospectively pursue pharmacogenomics-guided drug development models (instead of the traditional 'wait-and-see' approach) that can improve innovation rates in drug development. Reproduced with permission from Ozdemir V.[15] [2]

CEOs left office in 2004, a 300% increase in CEO departures since 1995.[38] Within the health care sector in 2004, CEO dismissals rose to 16.2%.[38] Nearly a third of all CEO resignations in 2004 were related to failure to meet demands for financial returns by increasingly impatient shareholders. Notably, the CEOs removed for inadequate performance had a median tenure of 5.2 years in the United States; in Europe, the situation was more difficult, with poorly performing CEOs remaining only for a median of 2.5 years. According to Lucier and colleagues, corporations "have reached a tipping point, in which power in the corporation is permanently shifting away from chief executives." In this climate of risk-averse and demanding shareholders and CEOs increasingly anxious about maximizing returns on a quarter-by-quarter basis, new pharmacogenomic technologies are being implemented.[39,40] Thus, it is difficult to reconcile the short-lived (2.5–5.2 years) tenure of the CEOs with new health technologies (eg, -omics biomarker platforms) that require long-term investment before tangible financial returns can be observed.

What incentives, then, can be put in place for corporate directors (as well as shareholders) to voluntarily exhibit socially responsible commitments to genomic technologies to achieve targeted therapeutics that, while potentially reducing short-term revenues,[34] may increase long-term retention of products (ie, safe and effective drugs) in the market? In the case of new genomic technologies, important social structural aspects,[15,32,33,38–40] such as those discussed above (eg, increased executive turnover and shareholder demands in favor of expediency), that can impact commercial or academic pharmacogenomic research and professional conduct may be dismissed or mistakenly ignored in the framing and future projections of these technologies.[41,42] To this end, a multidisciplinary learned society, such as the American Federation for Medical Research (AFMR), would be uniquely positioned to play a pivotal leadership role in facilitating dialogue across different professional languages and norms at the intersections of social sciences, research governance in public and private sectors, and professional practice of clinical

pharmacology and human genetics research to best realize the dream of pharmacogenomics-guided personalized medicines.

Regardless of the various sociologic, technology-based, or commercial factors and motivations that impede or facilitate the development of pharmacogenomic tests at the point of care, the fact is that the traditional model of drug development, with its focus on finding 'the next blockbuster drug,' is increasingly viewed as no longer realistic or viable.[37] Often overlooked is the fact that most recent blockbuster drugs were likely the 'lower-hanging fruits' resulting from rational and scientific drug development in the second half of the twentieth century. Further, many blockbuster drugs initially developed for broad use in the population have, on prescription in larger patient samples, been withdrawn from the market because of serious toxicity, a lack of effectiveness, or adverse drug-drug interactions. In effect, drug development without accompanying clinical biomarkers to customize prescriptions amounts to a statistical time bomb: when drug exposure exceeds the 1,000 to 3,000 patients collectively enrolled in typical premarketing clinical trials, members of the broader patient population who do not reflect the 'average' biologic or demographic attributes of trial participants are invariably exposed, leading to adverse drug-related events.

Exposing patients in clinical trials or during the postmarketing phase to partially preventable risks becomes a more acute and palpable social and ethical concern, especially when we consider that pharmacology is an experimental science amenable to proactive and prospective biomarker applications long before drug-related problems emerge. We submit that it is essential for both drug developers and regulators to adopt a longer-term vision that projects beyond the immediate goal of obtaining regulatory approval toward an enhancement of the entire life cycle and quality of a medicinal product. That is, prompt and timely introduction of new drugs to patients should be balanced against their sustainable use in the clinic, without postregistration withdrawal.[43]

Introducing noncustomized drugs in the clinic does not, in the long run, benefit many of the key actors in knowledge-based economies, whether they are patients or industry shareholders. Any costs incurred for postmarketing safety monitoring of drugs, such as frequent liver or kidney function tests, are ultimately transferred from the drug manufacturer to the patients and the payors.[44] Looking through the lens of global public health,[45] unfavorable perceptions about the societal commitment of a drug manufacturer on a given product withdrawn from the clinic will also have multiple detrimental effects on other compounds in their drug development pipeline: employee morale may suffer, thereby seriously undermining corporate initiatives to develop an equitable and attractive workplace environment that will retain highly trained and costly staff, whereas the broader mission of creating public benefit and ultimately safeguarding corporate and fiduciary responsibilities toward shareholders will be jeopardized.[46–48]

Future Outlook

As noted by David and Foray, commenting on the evolution of knowledge-based economies and civil societies, "[d]iscoveries in many domains are...made in the course of unplanned journeys through information space."[49] The genealogy of scientific progress can be even more complex in the case of interdisciplinary dialogues and experiments. Simply 'chunking' pharmacogenomics and human genetics together in conceptual proximity as two identical disciplines would be inadequate for a balanced reconciliation of their nuanced differences in science policy. Nor would such an approach acknowledge how these two fields might, in turn, impact both real and perceived expectations, for example, on sample size requirements in studies on the development of genetic tests for customization of drug therapy. More in-depth and realistic projections of their codevelopment as a new hybrid and intellectually richer discipline necessitate self-reflection that extends beyond the classic disciplinary boundaries. Hence, although the fields of clinical pharmacogenomics and human genetics research are increasingly coalescing through technology and knowledge transfer, it is critical to discern the ways in which discipline-specific traditions, tacit knowledge, and expectations of practitioners may influence the course of scientific dialogue and collaboration at their disciplinary boundaries and interdisciplinary junctions.

As academic institutions move increasingly toward serving a dual role as engines for economic growth and a knowledge commons (research and teaching),[50–52] future public policy debates on pharmacogenomics, genetic testing, and personalized medicine will need to be reframed to incorporate these subtle but significant characteristics (see Table 1). Ultimately, the recognition that pharmacology is an experimental science should also elevate the ethical standards and accentuate the moral obligation to develop pharmacogenomic or other biomarkers prospectively before obtaining marketing approval. For drugs that have already been in clinical use, an equal effort should be made to facilitate

their targeted use for individuals and patient populations. Blockbuster drugs may increase the profits in selected cases, but they also unethically concentrate the risks of drug development in specific groups and communities.[53]

The expansion in scope of scientific research enabled by new genomic technologies may soon result in fragmented but more diversified and narrowly defined therapeutic fields or markets for drugs that will ultimately benefit patients while also shaping the varied expectations for long-term and sustainable growth in the pharmaceutical industry. This expansion also creates new control points and sociotechnical actors in academic research governance. By contextualizing genomic technologies as important technical and social sources of momentum that unites human geneticists and pharmacologists, one sees the future of personalized medicine or clinical pharmacogenomics contingent on often indeterminate or multifactorial events.[54,55] Yet while the future remains undecided and uncertain, there is arguably an actual ethical responsibility on the part of regulatory scientists, human genetics, molecular medicine, pharmacogenomics, and social science researchers to engage in a sustained interdisciplinary, open, accountable, and transparent dialogue aimed at the development of shared standards and science policies that demonstrate optimal methodologic rigor to favorably advance discoveries and serve the best interests of patients' and public health.

In increasingly overspecialized, hypercompetitive, and fragmented biomedical research with semantic and disciplinary discontinuities,[56,57] the only assurance for continuity and objectivity in interdisciplinary fields of inquiry (eg, pharmacogenomics) will thus depend on certain human qualities in scientific professional practice and, more broadly, in public health research. These qualities include an open recognition of our own discipline-specific biases and shortcomings, giving credence to (at least noticing) hitherto disenfranchised professional viewpoints and the boundaries surrounding each discipline or individual scientific methodologies.[58,59] Reductionist conceptual juxtapositions of one discipline next to another (ie, pharmacology and human disease genetics presented as pharmacogenomics) or borrowing technologies from one discipline and applying in another without adequate reflection, in the best of circumstances, may only lead to multidisciplinary summation of scientific inquiries. But this is not necessarily equivalent to interdisciplinary synthesis and reasoned reconciliation of norms at disciplinary intersections. It is only when we comfortably place ourselves in that interdisciplinary space and acknowledge the attendant semantic and methodologic uncertainties that we can begin to dispassionately learn from other disciplines while building a more certain and ethical future for pharmacogenomics, personalized medicine, and equitable public health policies.

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Dose-Dependent Effects of the 3435 C>T Genotype of *ABCB1* Gene on the Steady-State Plasma Concentration of Fluvoxamine in Psychiatric Patients

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Abstract: This study investigated effects of the 3435 C>T genotype of the adenosine triphosphate–binding cassette subfamily B member 1 (ABCB1, MDR1) gene on the steady-state plasma concentration of fluvoxamine (FLV).

Methods: Sixty-two psychiatric patients were treated with different doses (50, 100, 150, and 200 mg/d) of FLV. Blood samples were collected after at least 2 weeks of treatment with the same daily dose to obtain steady-state concentrations of FLV, and 3435 C>T genotype was determined by polymerase chain reaction.

Results: FLV concentration-to-dose ratio was significantly different among 3435 C>T genotype groups at the 200 mg/d dose ($P = 0.019$). A post-hoc analysis revealed that FLV concentration-to-dose ratio was significantly higher in the TT genotype group as compared with the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.861 vs 0.434, $P = 0.026$). FLV concentration-to-dose ratio was significantly higher in the CT + TT genotype group than the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.618 vs 0.434, $P = 0.031$). At 50, 100, and 150 mg/d dose, FLV concentration-to-dose ratios were not significantly different among 3435 C>T genotype groups. At 50, 100, and 150 mg/d dose, no significant differences were found in FLV concentration-to-dose ratios between the CT + TT genotype group and CC genotype group.

Conclusions: This study suggests that pharmacokinetics of FLV depend on *ABCB1* gene polymorphism only at the 200 mg/d dose.

Key Words: ABCB1 (MDR1/P-glycoprotein), gene polymorphism, antidepressants, SSRIs, plasma fluvoxamine concentration

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INTRODUCTION

Fluvoxamine (FLV) is a selective serotonin reuptake inhibitor (SSRI), which is used not only for the treatment of depression but also for the treatment of a variety of other psychiatric disorders, such as panic disorder, social anxiety disorder, and obsessive-compulsive disorder. Several studies have shown cytochrome-P450 (CYP) 1A2[1,2] and CYP2D6[2,3] to have a significant impact on FLV pharmacokinetics. However, other studies reported that CYP1A2[4] and CYP2D6[4,5] had no major effects on plasma FLV concentrations. The specific factors involved in the pharmacokinetics of FLV have not been clearly identified.

The adenosine triphosphate (ATP)-binding cassette subfamily B member 1 (ABCB1), which is also known as MDR1 or P-glycoprotein, is an integral membrane protein of 170 Kd and belongs to the ATP-binding cassette superfamily of membrane transporters. It serves as a potent ATP-dependent efflux pump for a wide variety of lipophilic compounds. Overexpression of ABCB1 in tumor cells confers the commonly known phenomenon of multidrug resistance against antineoplastic agents.[6,7] ABCB1 is also expressed in normal tissues such as liver, kidney, and intestine where it contributes to the elimination of xenobiotics and drugs into bile and urine or limits drug absorption from the gastrointestinal tract.[8,9]

Hoffmeyer et al[10] reported that a single nucleotide polymorphism (3435 C>T) in exon 26 of *ABCB1* gene was associated with duodenal expression of ABCB1 and associated function in humans. Carriers homozygous for this polymorphism (TT) showed more than a 2-fold lower ABCB1 expression and higher digoxin plasma concentration than the CC group. Several studies also have reported that the T allele of 3435 C>T relates to higher concentrations of drugs such as digoxin,[10,11] cyclosporine A,[12,13] and tacrolimus.[14,15]

Penetration of amitriptyline into the brain was enhanced in mice lacking P-glycoprotein.[16] The 3435 C>T genotype was also associated with the occurrence of nortriptyline-induced postural hypotension.[17] These studies suggested that ABCB1 might affect the pharmacokinetics of antidepressants. However, there have been few clinical studies investigating effects of the functional status of ABCB1 on pharmacokinetics of antidepressants. In this study, we investigated effects of the *ABCB1* gene polymorphism on steady-state plasma concentration of FLV in patients treated with different doses of FLV.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethics Committee on Genetics of Niigata University School of Medicine.

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All patients received an explanation of the objectives of the study, and only those who gave written consent to participate in this study were enrolled. Demographic data; medical history; and laboratory data including hematology, serology, electrolytes, and urine analysis were collected for each patient. Patients with obvious physical illness were excluded. Patients older than 65 years of age and those younger than 20 years of age were excluded. Smokers (≥20 cigarettes/day) were also excluded because smoking is known to induce the CYP1A2 enzyme and increases the metabolisms of drugs eliminated by this enzyme. Patients concomitantly treated with other drugs, except some benzodiazepines, were excluded. The subjects included 62 Japanese outpatients (25 females and 37 males). The mean age ± standard deviation (SD) was 36.2 ± 11.9 years. Their diagnoses were major depressive disorder (n = 55), dysthymic disorder (n = 1), depressive disorder not otherwise specified (n = 1), bipolar disorder (n = 1), anxiety disorder (n = 1), bulimia nervosa (n = 1), and adjustment disorder (n = 2), according to the criteria of the *Diagnostic and Statistical Manual of Mental Disorders,* Fourth Edition, Text Revision.[18] The subjects received FLV in 2 equally divided doses at 9:00 and 21:00.

Blood Sampling

Patients were maintained on the same daily doses of FLV for at least 2 weeks to obtain steady-state concentrations of FLV. Blood sampling was done using a Venoject tube with ethylene tetraacetic acid (EDTA)-Na (Terumo Japan, Tokyo, Japan) 12 hours after the last dosage. Within 2 hours of collection, samples were centrifuged at 3,000 *g* and aliquots of plasma were drawn out in pipettes for determining plasma levels of fluvoxamine with samples stored at –80°C until assayed.

Determination of Drug Plasma Concentration

Plasma FLV concentration was measured using column-switching high-performance liquid chromatography with ultraviolet detection. Drugs in plasma to which cisapride had been added as an internal standard were extracted with hexane-chloroform. The extract was subjected to an automated column-switching high-performance liquid chromatography using a TSK BSA-C8 precolumn (Tosoh, Tokyo, Japan) for sample cleanup and a TSK gel ODS-80TS column (Tosoh) for separation.

Calibration curves (n = 6) were linear over the concentration ranging from 1.2 to 150 ng/mL ($r > 0.999$) for FLV. Intraday (n = 6) and between-days (n = 6) coefficients variations determined at 3 different concentrations (2.5, 38, and 150 ng/mL) were less than 4.7% and 3.9%, respectively. Recoveries and their coefficients variations (n = 6) determined at 3 different concentrations (1.25, 25, and 100 ng/mL) were 94.9–97.3%, and less than 1%, respectively. The limit of quantification (signal/noise ratio = 5) was 1.0 ng/mL.

Genotyping

Genomic DNA was extracted from peripheral blood using a QIA-amp Blood Kit (Qiagen, California). 3435 C>T of *ABCB1* were genotyped using the TaqMan 5'-exonuclease assay. Primer and probe sets were designed and synthesized by Applied Biosystems (Foster City, California). Polymerase chain reaction amplification was preformed using TaqMan 2x Universal Master Mix, No AmpErase UNG (Applied Biosystems), 5 ng of DNA, 0.9 μmol/L of each primer, and 200 nmol/L of each probe in total volume of 5 μL. Thermal cycler conditions were 95°C for 10 minutes, 40 cycles of 92°C for 15 seconds, and 60°C for 1 minute. Fluorescence and allelic discrimination were measured with a ABI PRISM 7900HT Sequence Detection System using SDS 2.0 software (Applied Biosystems).

Statistical Analysis

Statistical analysis was conducted with the SPSS II software for Windows (SPSS Japan Inc, Tokyo, Japan). A Mann-Whitney test was used to detect differences in FLV concentration-to-dose ratio between 50 mg/d and 200 mg/d dose treatment groups. Comparison of mean age among 3435 C>T genotypes was performed by analysis of variance (ANOVA) with the Bonferroni's test used as a post-hoc test. Differences in gender distribution among 3435 C>T genotypes were compared with the χ^2-test. Differences in FLV concentration-to-dose ratio among 3435 C>T genotypes were compared using the Kruskal-Wallis analysis with the Scheffe's test used as post-hoc test. Differences in FLV concentration-to-dose ratio between 2 genotype groups were compared by a Mann-Whitney test. A nonparametric ranking sum test (Friedman test) was used to detect differences in FLV concentration-to-dose ratio among 4 doses of FLV. Level of statistical significance was set at $P < 0.05$.

RESULTS

Of the 62 patients, 15 patients had data of FLV concentration at 1 dose, 15 patients had it at 2 different doses, 12 patients had it at 3 different doses, and 20 patients had it at 4 different doses of FLV. Median value of FLV concentration-to-dose ratio was 0.282 (50 mg/d, n = 49), 0.381 (100 mg/d, n = 42), 0.477 (150 mg/d, n = 40), and 0.554 (ng/mL)/(mg/d) (200 mg/d, n = 30); it significantly increased with increasing daily dose (50 gm/d vs 200 mg/d, $P < 0.001$).

3435 C>T genotype frequency was 0.31 (n = 19) for CC, 0.55 (n = 34) for CT, and 0.15 (n = 9) for TT. Genotype frequency was not significantly different from the values expected from the Hardy-Weinberg equilibrium ($\chi^2 = 0.99$, $P = 0.32$, df = 2).

In the 50, 100, 150, and 200 mg/d dose treatment groups, there were no significant differences among 3435 C>T genotype groups regarding mean age ($P = 0.23$, 0.35, 0.27, and 0.09, respectively; Table 1) or gender distribution ($P = 0.08$, 0.18, 0.29, and 0.08, respectively; Table 1). Furthermore, there were no significant sex-related differences in plasma FLV concentration at any doses (data not shown).

FLV concentration-to-dose ratio was significantly different among 3435 C>T genotype groups at the 200 mg/d dose ($P = 0.019$; Table 1). A post-hoc analysis revealed that FLV concentration-to-dose ratio was significantly higher in the TT genotype group than that in the CC genotype group at 200 mg/d dose ($P = 0.026$; Table 1). FLV concentration-to-dose

TABLE 1. Demographics and FLV Concentration-to-Dose Ratio (ng/mL)/(mg/d) of Subjects Classified by *ABCB1* Genotypes

	50 mg/d			100 mg/d		
	CC	CT	TT	CC	CT	TT
Number of subjects	13	29	7	12	25	5
Female/male	6/7	8/21	5/2	6/6	9/16	4/1
Age (year ± SD)	38.3 ± 12.7	37.7 ± 12.1	36.6 ± 12.0	37.8 ± 10.7	35.7 ± 11.5	28.6 ± 5.0
FLV concentration*						
Median	0.268	0.250	0.350	0.391	0.337	0.556
Range	0.088–0.762	0.032–0.776	0.210–0.832	0.105–0.825	0.058–1.888	0.277–2.172
Kruskal-Wallis test		$P = 0.15$			$P = 0.24$	
	150 mg/d			200 mg/d		
	CC	CT	TT	CC	CT	TT
Number of subjects	11	23	6	10	16	4
Female/male	6/5	8/15	4/2	5/5	6/10	4/0
Age (year ± SD)	35.4 ± 8.4	36.9 ± 12.3	28.3 ± 4.5	36.5 ± 6.2	35.2 ± 13.2	27.3 ± 4.6
FLV concentration*						
Median	0.411	0.433	0.548	0.434	0.603	0.861†
Range	0.196–1.009	0.104–2.241	0.255–2.858	0.188–0.667	0.088–2.042	0.588–3.395
Kruskal-Wallis test		$P = 0.55$			$P = 0.019$	

FLV, fluvoxamine; *ABCB1*, ATP-binding cassette subfamily B member 1 gene; SD, standard deviation.
*Concentration-to-dose ratio (ng/mL)/(mg/d).
†Post-hoc analysis revealed that plasma FLV concentration was significantly higher in the TT group than that in the CC group ($P = 0.026$).

ratio was significantly higher in the CT + TT genotype group than in the CC genotype group at the 200 mg/d dose (median value of concentration-to-dose ratio (ng/mL)/(mg/d), 0.618 vs 0.434, $P = 0.031$).

At 50, 100, and 150 mg/d dose, FLV concentration-to-dose ratios were not significantly different among C3435T genotype groups (Table 1). At 50, 100, and 150 mg/d dose, there was no significant difference in FLV concentration-to-dose ratio between the CT + TT genotype and CC genotype groups ($P = 0.96$, 0.91, and 0.55, respectively).

Table 2 shows FLV concentration-to-dose ratios of 20 patients tested at all 4 doses. In these 20 patients, FLV concentration-to-dose ratios significantly increased with increasing daily dose (Table 2). At 50, 100, 150, and 200 mg/d dose, FLV concentration-to-dose ratios were not significantly different among 3435 C>T genotype groups (Table 2). At 50, 100, 150, and 200 mg/d dose, subjects with the TT genotype of 3435 C>T had higher FLV concentration-to-dose ratios than those from the CC + CT genotype group ($P = 0.038$, 0.12, 0.13, and 0.038, respectively).

DISCUSSION

This study suggested that the 3435 C>T genotype in *ABCB1* had a significant effect on steady-state plasma concentration of FLV only at a treatment dose of 200 mg/d. Subjects with the TT genotype of 3435 C>T showed a significantly higher FLV concentration-to-dose ratio than that of subjects with the CC genotype at the 200 mg/d. Although not statistically significant, heterozygous (CT) subjects tended to have higher FLV concentration-to-dose ratios than that of CC subjects at the 200 mg/d dose. This result was consistent with those of Hoffmeyer et al's findings that showed subjects with the CC genotype of 3435 C>T had high intestinal ABCB1 expression and low digoxin concentration in contrast to TT subjects with low intestinal ABCB1 expression and high

TABLE 2. FLV Concentration-to-Dose Ratio (ng/mL)/(mg/d) of 20 Patients Tested at All 4 Doses

ABCB1 Genotype	50 mg/d		100 mg/d		150 mg/d		200 mg/d	
	Median	[Range]	Median	[Range]	Median	[Range]	Median	[Range]
All (n = 20)	0.284*	[0.048–0.832]	0.474*	[0.085–2.172]	0.526*	[0.104–2.858]	0.580*	[0.088–3.395]
CC (n = 5)	0.252	[0.164–0.338]	0.359	[0.132–0.528]	0.411	[0.196–0.751]	0.501	[0.187–0.667]
CT (n = 11)	0.250	[0.048–0.550]	0.458	[0.085–0.899]	0.464	[0.104–0.986]	0.572	[0.088–1.091]
TT (n = 4)	0.476	[0.310–0.832]	0.626	[0.467–2.172]	0.731	[0.490–2.858]	0.861	[0.588–3.395]
Kruskal-Wallis test	$P = 0.091$		$P = 0.18$		$P = 0.25$		$P = 0.072$	

FLV, fluvoxamine; *ABCB1*, ATP-binding cassette subfamily B member 1 gene.
*FLV concentration-to-dose ratio significantly increased with increasing daily dose ($P < 0.001$, Friedman test).