Functional mapping of drug response with pharmacodynamic–pharmacokinetic principles

Kwangmi Ahn¹, Jiangtao Luo¹, Arthur Berg¹, David Keefe² and Rongling Wu¹

¹Center for Statistical Genetics, Pennsylvania State University, Hershey, PA 17033, USA
²Department of Obstetrics and Gynecology, New York University, Langone Medical Center, New York, NY 10016, USA

Recent research in pharmacogenomics has inspired our hope to predict drug response by linking it with DNA information extracted from the human genome. However, many genetic models of drug response do not incorporate biochemical principles of host–drug interactions, limiting the effectiveness of the predictive models. We argue that functional mapping, a computational tool aimed at identifying genes and genetic networks that control dynamic traits, can help explain the detailed genetic architecture of drug response by incorporating pharmacokinetic and pharmacodynamic processes. Functional mapping is particularly powerful in determining the genetic commonality and differences of drug efficacy vs. drug toxicity and drug sensitivity vs. drug resistance. We pinpoint several future directions in which functional mapping can be coupled with systems biology to unravel the genetic and metabolic machinery of drug response.

Challenges and opportunities in pharmacogenetics

There is tremendous variability in drug response among different individuals who receive the same drug treatment [1]. Because genes play a central role in determining this variability, pharmacogenetics or pharmacogenomics, the study of the genetics or genomics of drug response, has now emerged as one of the most widely accepted disciplines in modern medicine [2–4]. The development of pharmacogenetics has been further stimulated by the completion of the human genome sequence in 2005 and its derivative, the HapMap Project, together with rapid improvements in genotyping analysis [5]. These technologies allow geneticists to elucidate the detailed genetic architecture of drug response at the nucleotide level. For example, genome-wide association studies (GWAS) of single nucleotide polymorphisms have identified genes that encode the action of Food and Drug Administration-approved drugs including thiazolidinediones and sulfonylureas (type 2 diabetes) [6], statins (lipid levels) [6] and estrogens (bone density) [7]. In shedding light on the genetic basis of complex traits, GWAS will have an increasing impact on the identification of genes for drug response.

By integrating the dynamic characteristics of drug–body interactions [8], we argue that the genetic control mechanisms of drug response can be studied in an effective way, which could yield a breakthrough that cannot be achieved using current pharmacogenetic approaches based on a single measurement of drug response. Drug response is underscored by two mutually related dynamic processes, pharmacokinetics (PK), which describes what an organism does to a drug, and pharmacodynamics (PD), which describes what a drug does to an organism. To make a comprehensive description of drug response, a series of dynamic measurements should be taken at multiple discrete time points or states. Empirical analyses of PK and PD data suggest that the time-dependent change of drug concentration in the body typically follows a biexponential curve [9,10], whereas drug effects increase with drug dose in a sigmoid manner (i.e. the Emax curve) [11]. These mathematical models that quantify pharmacodynamic/pharmacokinetic reactions can be integrated into a general framework of functional mapping [12–14], pioneered by R. Wu, to better study the interplay between genetic actions and drug response.

Pharmacogenetic studies of drug response are challenged by biochemical complexity in which various pathways interacts with each other through a hierarchic and cross-sectional manner [15]. To better study the behavior of each pathway, drug response can be modeled as a dynamic system, in which pathways are first assayed by analytic approaches and then integrated by synthetic approaches. Observed associations among these pathways could be driven by common genetic factors or can result from physiological interactions. It is of fundamental importance to know whether these pathways share common genetic determinants, which genetic factors are specific to different pathways and what is the nature of the non-genetic interactions among these pathways.

In this article, we show that functional mapping offers an unprecedented potential to study the genetic and genomic architecture of drug response by dissecting it into PD- and PK-governed pathways. We will first outline the biological merit of functional mapping and explain how it can be used to identify genes responsible for drug response. Then, we will show how functional mapping is implemented to a dynamic system of drug response to address fundamental questions about the genetic control of drug...
efficacy and drug toxicity, drug-body interactions and response rhythms. Examples will be presented, although it is not our goal to review these in a comprehensive manner; rather we intend to identify the challenges that must be overcome and their potential solutions through functional mapping. In Box 1, we describe a statistical framework for functional mapping.

**Functional mapping**

**Biological merit**

All biological traits experience a dynamic or developmental process. Traditional approaches for genetic mapping of dynamic traits have been to associate markers with phenotypes for different ages or stages of development and to compare the differences across these stages, or to use multiple-trait mapping where the same character is repeatedly measured at different times. In either case, these approaches do not capture the dynamic structure and pattern of the process, which greatly limits the scope of inference about its genetic architecture. To overcome these limitations, functional mapping, that is the integration of the mathematical aspects of the biological mechanisms and processes of the trait with the statistical genetic mapping framework, is the natural way to approach the genetics of dynamic traits by mapping the underlying quantitative trait loci (QTLs) [12–14]. The combination of statistical modeling, genetics and developmental biology is able to address many questions, such as the patterns of genetic control over development, the duration of QTL effects, as well as what causes developmental trajectories to change or stop changing. Functional mapping provides a quantitative and testable framework for assessing the interplay between gene action and development.

The genetic dissection of a biological process through functional mapping will not only identify genes that regulate the final form of the trait but also characterize the dynamic pattern of genetic control in time. Furthermore, functional mapping capitalizes on the parsimonious modeling of longitudinal mean–covariance structures and, therefore, substantially increases the statistical power and robustness of genetic mapping.

**Box 1. Functional mapping**

Functional mapping is founded on finite mixture models, a statistical model that represents a heterogeneous population as a mixture of homogeneous components, where each component follows a certain distribution and the components aggregate to yield the mixture distribution. Mixture models are employed to model genotypic segregation of specific genes that determine dynamic traits such as drug response [47]. For a dynamic trait, each subject is described by a curve that is observed by a finite set of measurements with T time points, arrayed in $y_i=[y_i(1), \ldots, y_i(T)]$. According to the mixture models, each curve is assumed to have arisen from one of the components, where each component is modeled by a multivariate (usually normal) distribution. Assuming there are $J$ genotypes that contribute to the variation among different curves, such a mixture model is expressed as

$$y_i \sim p(y_i|\omega, \varphi, \eta) = \sum_{j=1}^{J} \omega_j f_j(y_i; \varphi_j, \eta_j)$$  \hspace{1cm} (1)$$

where $\omega=(\omega_1, \ldots, \omega_J)$ are the mixture proportions (i.e. genotype frequencies) which are constrained to be non-negative and sum to unity; $\varphi=(\varphi_1, \ldots, \varphi_J)$ are the component- (or genotype-) specific parameters, with $\varphi_j$ being specific to component $j$; $\eta$ are parameters which are common to all components; and $f_j(y; \varphi_j, \eta)$ is a multivariate normal distribution with mean vector

$$u_j = [u_j(1), \ldots, u_j(T)]$$  \hspace{1cm} (2)$$

and covariance matrix

$$\Sigma_j = \begin{bmatrix} \sigma^2_{11} & \cdots & \sigma_{1T} \\ \vdots & \ddots & \vdots \\ \sigma_{T1} & \cdots & \sigma^2_{TT} \end{bmatrix}$$  \hspace{1cm} (3)$$

Traditional genetic mapping approaches estimate all $JT$ time-specific means in the mean vector [2] and $T(T+1)/2$ variances and covariances in the matrix in Equation 3. Instead of estimating all these parameters, functional mapping estimates the parameters that model the mean structure and covariance structure based on biological and statistical principles. In particular, genotype-specific parameters, $\varphi$, are used to model the time-dependent changes of genotypic means, whereas common parameters, $\eta$, are used to model the structure of the longitudinal covariance matrix. The detailed description of functional mapping used to map the dynamic change of genetic control is given in Ma et al. [12], Wang et al. [16] and Zhao et al. [48] extended functional mapping to model the temporal pattern of genetic variance and correlation between bivariate dynamic traits that jointly change as a function of the same independent variable.

As a demonstration, we used bivariate functional mapping, as shown in Wang et al. [16], to detect QTLs for drug response with a small data set collected from the AIDS Clinical Trials Group (ACTG) Protocol 315 [17]. In this trial, 53 HIV-1-infected patients were periodically measured for their HIV-1 and CD4+ cell contents on days 0, 2, 7, 10, 14, 21, 28 and weeks 8 and 12 after the treatment of highly active antiretroviral therapy (HAART) [18]. According to previous research, we used a biexponential function to model HIV-1 cell dynamics [17] and a quadratic function to model CD4+ dynamics [19]. Functional mapping was used to analyze the ACTG data by estimating genotype-specific parameters that define these dynamic curves and testing their differences among different genotypes. A major QTL was detected for HIV and CD4+ cell dynamics [20]. The estimated curve parameters for the three genotypes at this major QTL graphically illustrate pronounced differences in HIV-1 (Figure 1a) and CD4+ profiles (Figure 1b).

Functional mapping has the power to detect QTLs that pleiotropically or separately affect HIV-1 and CD4+ dynamics. Hypothesis tests indicated that this detected QTL exerts a significant pleiotropic effect on both function-valued traits. The favorable allele (denoted by A) that causes a rapid decrease in viral loads and a rapid increase in CD4+ cell counts occurs with rare frequency; its frequency is estimated as 0.25, compared with 0.769 for the unfavorable alternative (denoted by a). The heterozygote (Aa) and homozygote (aa) for the unfavorable allele together account for an overwhelming majority of patients (0.93). The detected QTL operates in an overdominant manner. In both the cases of HIV-1 and CD4+ dynamics, the heterozygote seems to have an unfavorable effect on the progression to AIDS. Patients who carry such a genotype display a smaller decay rate of viral loads in long-lived and/or latently infected cell compartments (Figure 1a) and smaller increase rates in CD4+ cell counts (Figure 1b) than the patients who carry the other two genotypes.
several interconnected compartments through which the drug is distributed and absorbed at certain rates. This method can quantify distributional behaviors of a drug between the plasma and other tissues or organs in the body. Such a compartmental analysis has benefited from a model built by ordinary differential equations (ODEs) that describes a continuous biological process. Numerous ODEs have been derived to describe changes in drug concentrations and action in each compartment at a rate proportional to the flow of blood to that compartment [22].

Box 2 provides such an example in which two coupled systems of ODEs are used to define a one-compartment model with the first-order absorption and Michaelis–Menten elimination, and a one-compartment indirect response model when the drug stimulates production of the effect used, respectively [21].

Luo et al. [23] developed a mathematical algorithm to solve ODE parameters that define functional mapping. Luo et al.’s approach can be used to estimate genotype-specific ODE parameters that define the system. By testing genotype-specific differences in these parameters, functional mapping can empower geneticists to identify the genetic control of PK and PD.

**Drug–body interaction models**

The flow of drugs among different organs could affect the pharmacological (favorable or adverse) effects of the drugs. To characterize the elimination of a drug called warfarin from the plasma and its excretion in the bile, a physiological flow model was formulated by a system of ODEs for several inter-related compartments that describe drug absorption, distribution, and elimination.

Functional mapping for a pharmacokinetic and pharmacodynamic system

Biochemical networks in a system for drug response consist of chemical reactions, such as association, dissociation, degradation and synthesis. The dynamics of biochemical networks obey the rules of mechanics that regulate their ability to organize movement and biological functions. Given the complexity of a biological system due to massive amounts of interactions, we need a sufficiently sophisticated analysis that can deal with such complexity. Mathematical models using kinetic theory provide a robust approach for describing a complex system that encompasses principles of non-equilibrium statistical mechanics.

The models for system dynamics based on the generalized Boltzmann equation can describe and predict the population dynamics of several interacting elements [21].

When a drug is administered into a patient’s body, the concentration of the drug will decay with time due to drug absorption, distribution and elimination (i.e. metabolism) and excretion [8]. PK is the study of the rate processes observed over time for a given dosage of a drug. Absorbed by the body, the drug will trigger its effect on the body, which is described by PD. Whereas PK concerns the time-course of drug concentration, PD studies the time-course and intensity of drug action or response.

PK and PD reflect many biochemical properties of drug–body interactions directly or indirectly related to drug effects. One way to model and predict the dynamic behavior of these two processes is to view the body as a system with

---

**Box 2. Differential equations for drug response**

**Pharmacokinetics:** Wang et al. [22] show that the one-compartment model with the first-order absorption and Michaelis–Menten elimination can be described by a coupled system of ordinary differential equations (ODEs)

\[
\begin{align*}
\frac{dQ}{dt} &= -k_A Q \\
\frac{dC}{dt} &= k_A Q - \frac{V_{max} C}{V + k_m} - k_{out} C
\end{align*}
\]

where time \( t \) is the independent variable varying between 0 and 300 min, \( Q \) is the amount (mg) of drug in the gastrointestinal tract, \( C \) is the plasma drug concentration (mg/l), \( V \) is the distribution volume, \( k_A \) is the rate decay of the drug, \( k_m \) is the Michaelis–Menten constant, \( V_{max} \) is the maximum rate of the reaction. According to the Michaelis–Menten equation that describes the initial rate of reaction as a function of the substrate concentration, the reaction reaches a certain maximum rate as the substrate concentration increases.

**Pharmacodynamics:** The one-compartment indirect response model that describes how a drug stimulates the production of the effect is expressed [22] as

\[
\begin{align*}
\frac{dC}{dt} &= -\frac{C}{C_i} - \frac{E_{max} C}{E_{C50} + C} - k_{out} R \\
\frac{dR}{dt} &= k_{in} \left( 1 + \frac{E_{max} C}{E_{C50} + C} \right) - k_{out} R
\end{align*}
\]

where time \( t \) is the independent variable varying between 0 and 300 min, \( C \) is the plasma drug concentration (mg/l), \( R \) is drug response that can be viewed as any physiological phenotypes related to drug efficacy and toxicity, \( V \) is the distribution volume, \( C_i \) is the clearance rate, \( k_m \) and \( k_{out} \) are the zero-order and the first-order rate constant for production and loss of an effect, respectively, and \( E_{max} \) and \( E_{C50} \) are the maximum effect of the drug and the drug concentration producing 50% of the maximum stimulation, respectively.
each organ including plasma, liver, kidney, muscle, skin, gut and bile, aimed to model the elimination of warfarin from the plasma and its excretion in the bile [24]. The model is based on organ weights or volumes, rates of blood flow to the organs and the ratio ($R$) of the concentrations of drugs in the different tissues to the concentration in plasma (Figure 2). These parameters contained in the differential equations (Figure 2) describe the distribution, action and elimination of the drug in different organs.

A system of differential equations for each organ (Figure 2) can be organized into the framework of functional mapping, allowing the test of how genes control the flow of drugs into different organs in a dynamic system. Luo et al. [23] provided the derivation of algorithms for estimating mathematical parameters and the stability tests of these parameter estimates. Many of these types of studies are conducted in experimental animals such as the rat because the kinetic data and tissue concentrations required cannot be obtained safely in man.

Drug efficacy and drug toxicity

The same medications often produce remarkably different effects among patients with the same illness. It is possible that a specific medication produces both the desired therapeutic effects (efficacy) and a risk for an adverse drug response (toxicity) both of which vary based on an individual genetic makeup [25]. In Figure 3, the first patient (a) displays greater drug efficacy but lower drug toxicity, thus having a wider window for the safe use of the drug, compared with the second patient (b).

Mathematical equations have been established to model the PK and PD reactions of drug efficacy and drug toxicity (Box 3). For example, a system of differential equations...
was used to quantify the flows of oxaliplatin, a type of drug used to treat human colorectal cancer, between different compartments [26]. After intravenous or intraperitoneal injection, oxaliplatin diffuses according to order 1 kinetics firstly in the plasma, then to healthy tissues and to tumors. Oxaliplatin triggers its efficacy and toxicity by inhibiting cell population growth in tumoral and healthy issues, respectively. The mean drug activity of oxaliplatin in the tumor, concentration in the healthy tissue, and tumor were found to affect patients’ circadian rhythms through simple to complex oscillatory behavior and to delineate the conditions under which they arise [35].

**Box 3. Kinetic equations for drug efficacy vs. toxicity**

The following differential equations can be used to specify the PK processes of drug reactions in different compartments, healthy tissue and tumor [26]

\[
\begin{align*}
\frac{dP}{dt} &= -\lambda P + \left(\frac{l(t)}{V}\right) \\
\frac{dC}{dt} &= -\mu C + P \\
\frac{dD}{dt} &= -\nu C + P 
\end{align*}
\]

where \(P\) is the free Pt plasma concentration, \(C\) is the total Pt concentration in the healthy tissue, \(D\) is the total Pt concentration in the tumor, \(l(t)\) is the intravenous drug infusion flow (mg/h) at time \(t\), \(V\) is the distribution volume (ml), \(\lambda, \mu, \nu\) are the diffusion parameters calculated after the half-life (ln2/half-life) of the drug in each compartment.

The PK process in the healthy tissue is described by drug toxicity by

\[
f(C, t) = F \left(\frac{C}{C_{50}}\right)^{g_S} \left[1 + \cos2\pi \left(\frac{t - \phi_S}{T}\right)\right]^{n}\]

where \(g_S\) is the Hill coefficient, \(C_{50}\) is the half-saturation concentration, \(T\) (24 h) is the period of drug sensitivity variation, \(\phi_S\) is the maximum toxicity phase (h), \(F\) is the half-maximum toxicity [26].

By contrast, the PD process in the tumor is described as drug efficacy by

\[
g(C, t) = H \left(\frac{C}{C_{50}}\right)^{g_T} \left[1 + \cos2\pi \left(\frac{t - \phi_T}{T}\right)\right]^{n}\]

where \(g_T\) is the Hill coefficient, \(D_{50}\) is the half-saturation concentration, \(T\) (24 h) is the period of drug sensitivity variation, \(\phi_T\) is the maximum efficacy phase (h), \(H\) is the half-maximum efficacy [26].

**Drug chronotherapy**

In several studies of drug response, so-called clock genes were found to affect patients’ circadian rhythms through clock-controlled transcription factors [27–29], holding a great promise for the determination of an individualized optimal body time for drug administration based on a patient’s genetic makeup. It has been suggested that drug administration at the appropriate body time can improve the outcome of pharmacotherapy by maximizing potency and minimizing the toxicity of the drug [27,30], whereas drug administration at an inappropriate body time can induce severe side effects [31]. In practice, body-time-dependent therapy, termed chronotherapy [27,28,32], can be optimized by implementing the patient’s genes that control expression levels of his/her physiological variables during the course of a day.

The molecular bases of circadian rhythms have been clarified during the past decade by experimental advances, first in *Drosophila* and *Neurospora*, and more recently in cyanobacteria, plants and mammals [33,34]. In view of the large number of variables involved and of the complexity of feedback processes that generate oscillations, mathematical models are necessary to comprehend the transition from simple to complex oscillatory behavior and to delineate the conditions under which they arise [35].

**Future directions: functional mapping merged into systems biology**

Traditional genetic mapping detects genes for drug response based on a direct genotype–phenotype relationship, whereas functional mapping does so through linking biochemical influences to the outcome of drug response in PK and PD pathways. Functional mapping has been extended to incorporate environmental signals into the model, testing genotype × environment interaction effects on biological processes [36,37]. Given that the endpoint outcome of drug response is the consequence of the mutual coordination of multiple factors, such as biochemical, developmental, environmental and stochastic error [38–42], functional mapping should be expanded to model a web of interactions among these factors. The predictive measurement is then used with genetic, biochemical, developmental and environmental parameters obtained from a subject to predict whether that subject will respond to the particular drug or treatment regimen.

Current functional mapping models are based on a direct relationship between genotype and phenotype through statistics, leaving intermediate steps from genotype to phenotype unknown. As a fundamental rule to form, control and direct every aspect of a phenotype, the central dogma of biology, DNA → mRNA → enzyme (inactive) → enzyme (active) → metabolite(s) → metabolism → cellular metabolism → cellular physiology → phenotype, subjected to continuous addendums and modifications in the recent past, can be incorporated into functional mapping. With such incorporation, we are in a better position to unravel the mechanistic and regulatory process of how genes control the phenotype of drug response.

As the cost of methods for measuring mRNA, protein and other indicators continues to fall, it becomes reasonable to design experiments for functional mapping that capture the dynamic processes of phenotypic formation of drug effects on timescales. With these data, a new discipline, systems biology, arises from the study of the transcriptome (the set of RNA transcripts) and the metabolome (the entire range of metabolites taking part in a biological process).
Other omes (sets) that might also be of interest include the interactome (complete set of interactions between proteins or between these and other molecules), the localizome (localization of transcripts, proteins, etc.) or even the phenome (complete set of phenotypes) of a given organism [43,44]. In future research, we need to develop a new functional mapping model for reconstructing biological networks by incorporating interactome, localizome and phenome related to drug response. In particular, the model is capable of mapping specific QTLs that control transcriptional (eQTL), proteomic (pQTL) and metabolomic (mQTL) expressions [45] and their interaction networks among these different types of QTLs. Linking functional mapping with systems biology holds new opportunities to unravel the genetic and developmental machinery of patients’ responses to medications and push forward the frontier of personalized medicine [46].

Acknowledgements
We are grateful to Dr. Zuohe Wang for analyzing the ACTG data using a bivariate functional mapping model. This work is supported by National Institute of Health American Recovery and Reinvestment Act (NIH ARRA) grant 09095.

Conflict of interest
The authors declare no competing financial interests.

References
18 Marschner, I.C. et al. (1998) Use of changes in plasma levels of human immunodeficiency virus type 1 RNA to assess the clinical benefit of antiretroviral therapy. J. Infect. Dis. 177, 40–47