

A Journey from Pediatric Pharmacokinetics to Pharmacogenomics

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I am honored to have been selected as the 2004 Yaffe Award recipient by the PPAG, and to join the list of distinguished prior recipients of this award. Having known Dr. Sumner Yaffe for all of my career, and having been motivated by his passion for pediatric pharmacology and therapeutics, I am pleased to receive an award that recognizes his many outstanding accomplishments and his leadership in our field.

My interest in pursuing a research career in pediatric pharmacology had a serendipitous beginning; first, I was exposed to clinical research because every PharmD student was required to complete a research project in the old days (ca. 1970s). Absent that exposure, I may never have known that I enjoyed pursuing the unknown, and may well be now running an apothecary shop in Clarksville, Tennessee. That curricular requirement also steered me to St. Jude Children's Research Hospital (SJCRH), where I found an enormously supportive major professor for my research, Larry Barker. St. Jude also provided an environment that encouraged ideas from all corners, including students and non-physicians, and fostered success through a dedicated team of senior faculty who enjoyed helping their junior colleagues. Over the course of the last 30 years, my research has had a central thread ("pharmaco-"), but has moved through several dimensions beginning with pharmacokinetics, then evolving to pharmacodynamics, pharmacogenetics, and most

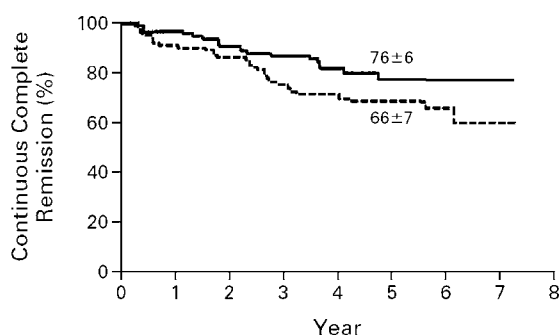


Figure 1. Kaplan-Meier Estimates of Continuous Complete Remission in Patients with B-Lineage Acute Lymphoblastic Leukemia.

The estimated rate of continuous complete remission was significantly higher in the 69 patients receiving individualized treatment than in the 74 receiving conventional treatment. The estimated (\pm SE) rate of continuous complete remission at five years is shown for both groups ($P = .02$). Individualized treatment (—) and conventional (---). Reprinted with permission from reference 6.

recently pharmacogenomics.

It was my interest in mathematics and chemistry that led me to pharmacy, and there I found something called "pharmacokinetics" that allowed me to apply both sciences in the context of therapeutics. There also was not a formal clinical program in "therapeutic drug monitoring" at SJCRH in the mid-1970s, nor a lab performing drug assays for patient care or research. Walter Hughes, MD, who was chair of Infectious Diseases at SJCRH, and Charles Pratt, MD, who led clinical pharmacology, provided me with my initial lab space ("on loan") and an opportunity to apply pharmacokinetics to antibiotics (e.g., aminoglycosides)¹ and anti-cancer agents (e.g., methotrexate).²

Gary Levy, PharmD, Les Benet, PhD, and others advised me that pharmacokinetics was largely a tool, and it is best applied when one is trying to understand the basis for inter-

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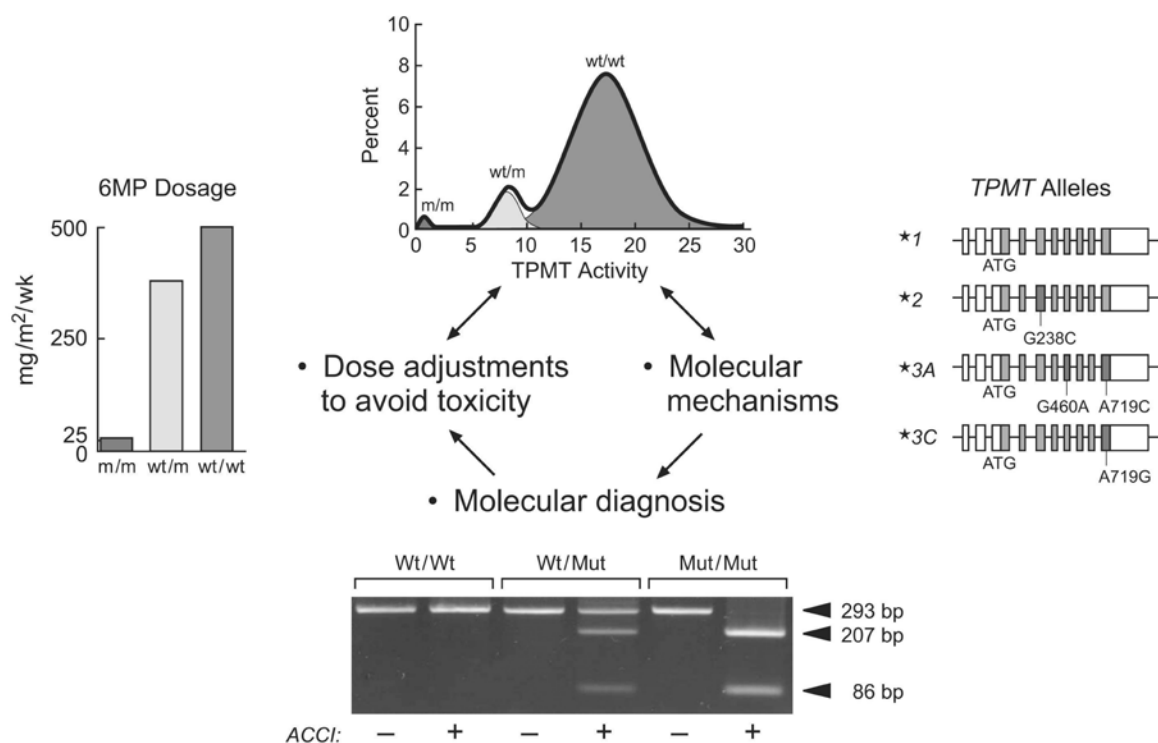


Figure 2. Genetic polymorphism of TPMT activity and thiopurine therapy, from bench to bedside and back.

Top panel: Distribution of TPMT activity in most populations. Right panel: Structure (10 exons) and positions of mutations (in exon 5 for *TPMT**2, and in exons 7 and 10 for *TPMT**3A, and in exons 10 for *TPMT**3C) in the most prevalent mutant alleles of *TPMT*, in Caucasians (more than six others now have been reported in various ethnic groups). Bottom panel: Results of PCR-based techniques for detection of inactivating mutations at the human *TPMT* gene locus. Left panel: Potential extent of MP dosage to avoid toxicity in patients with inherited deficiency of TPMT activity. Reprinted with permission from reference 8.

individual differences in drug response. This stimulated my initial interests in pharmacodynamics, and led me to focus on childhood acute lymphoblastic leukemia (ALL) as the disease context for these studies. Childhood ALL turned out to be a great choice for several reasons; (1) it is the most common type of childhood cancer, (2) it was drug-sensitive and in the 1970s was curable with chemotherapy in about 50% of children, and (3) it is a “liquid tumor,” thus permitting one to obtain samples of the target tissue for characterization in the lab. This led to a series of pharmacodynamic studies of antileukemic agents, that revealed concentration-effect relationships³⁻⁵ and led to a randomized study demonstrating that the use of pharmacokinetics to individualize doses of antileukemic agents could improve treatment outcome (Figure 1).⁶

SJCRH and the University of Tennessee also provided another important opportunity to take my research in a new direction when I was given a sabbatical year in the lab of Urs Meyer, MD, at the University of Basel (1987-88). This allowed my research to take on a

more molecular nature, providing a foundation for pharmacogenetic studies of antileukemic agents. It was at once educational and exciting to be in Urs’ lab when he and Frank Gonzalez, PhD, were publishing the first cloning and molecular characterization of a human drug metabolizing enzyme that exhibited genetic polymorphism (i.e., *CYP2D6*).⁷ My group was able to subsequently apply these strategies to identify the major genetic polymorphisms in the human thiopurine S-methyltransferase (*TPMT*) gene that are responsible for inherited differences in the activity of this enzyme (Figure 2).⁸⁻¹¹ This led to a molecular diagnostic to identify *TPMT*-deficient patients based on their genotype¹² and to link this polymorphism to the risk of severe hematopoietic toxicity¹³ as well as the risk of irradiation-induced brain cancers.¹⁴ The *TPMT* genotype became the first CLIA-certified pharmacogenetic diagnostic available from national reference labs and is now increasingly used to determine the appropriate dosage of thiopurine medications (e.g., mercaptopurine, azathioprine).

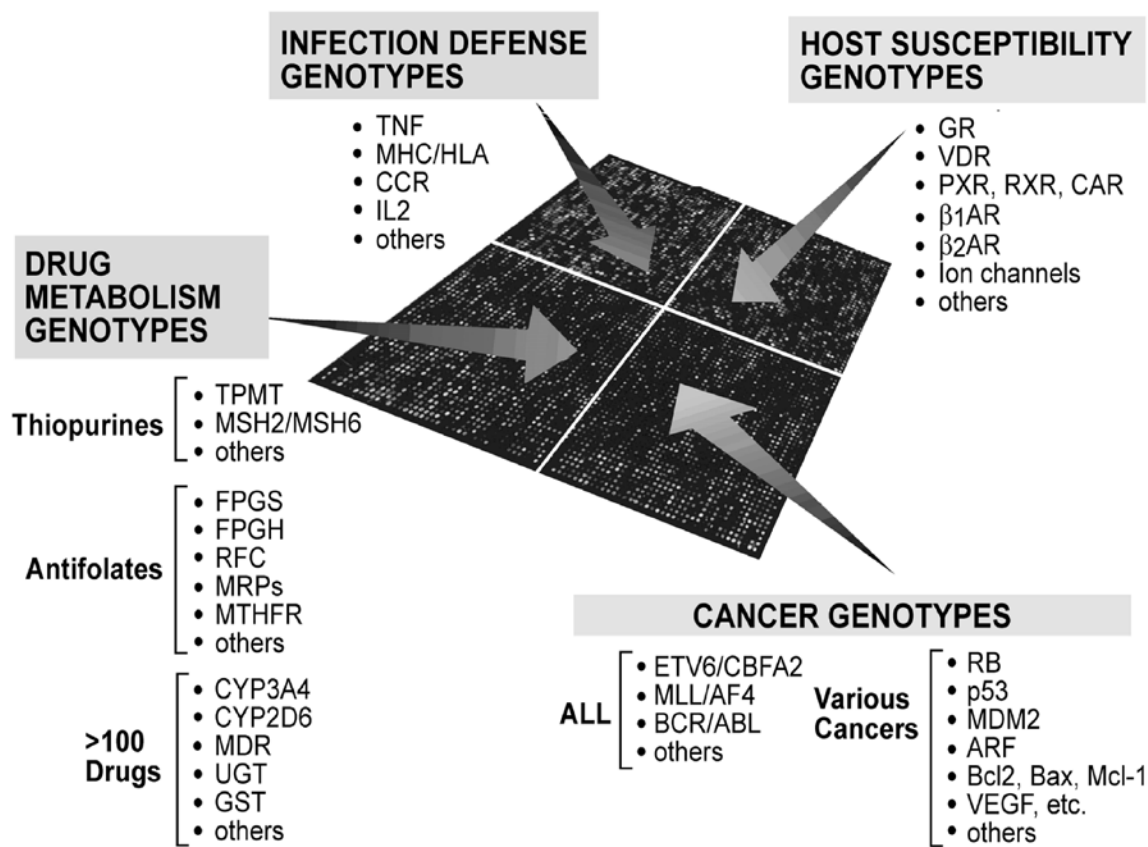


Figure 3. Molecular diagnostics of pharmacogenomic traits.

DNA arrays are being made for automated, high-throughput detection of functionally important mutations in genes that are important determinants of drug effects, such as drug-metabolizing enzymes, drug targets (receptors), disease pathogenesis, and other polymorphic genes that influence an individual's susceptibility to drug toxicities or environmental exposures (such as pathogens, carcinogens, and others). This figure exemplifies components of a potential diagnostic DNA array for genes that could influence a patient's response to chemotherapy for acute lymphoblastic leukemia, including genes that determine drug metabolism, disease sensitivity, and the risk of adverse effects of treatment (cardiovascular or endocrine toxicities, infections and so forth). Reprinted with permission from reference 15.

In one of several (too many perhaps) reviews I have helped write on the topic of pharmacogenetics and pharmacogenomics,¹⁵⁻¹⁷ we speculated that additional genes and genetic polymorphisms would ultimately be found that influence the effects of antileukemic agents, expanding beyond the TPMT example (Figure 3), but the question was, How would these be identified? We are now taking several strategies to identify these additional genes and polymorphisms, including candidate gene studies,¹⁸ gene expression analysis,¹⁹⁻²⁰ genome-wide haplotype mapping, and proteomics (Figure 4). These have already begun to reveal additional genes whose expression, function or genetic polymorphism is related to treatment outcome (e.g., disease free survival, drug toxicity).

In each of these phases of research, the focus has been on how drugs behave in children, what makes children different from adults and the

underlying mechanisms for why children differ from one another in the disposition and effects of medications. There are many more unanswered questions than there are answered ones, and we now have a spectacular array of new technologies to drive science forward in a faster and more definitive manner in the future. In that respect, it is a remarkably propitious time to be entering science and the health professions. "Translational research" is no longer a "buzz phrase," rather it is a reality that is impacting patient care and driving therapeutics forward.

Unfortunately, in many ways children remain an "orphan population" in terms of the discovery and development of new medications to treat serious pediatric diseases, particularly molecularly-targeted anticancer agents. Because of the relatively small market size, children are not seen as a profitable population for new drug development by the large pharmaceuti-

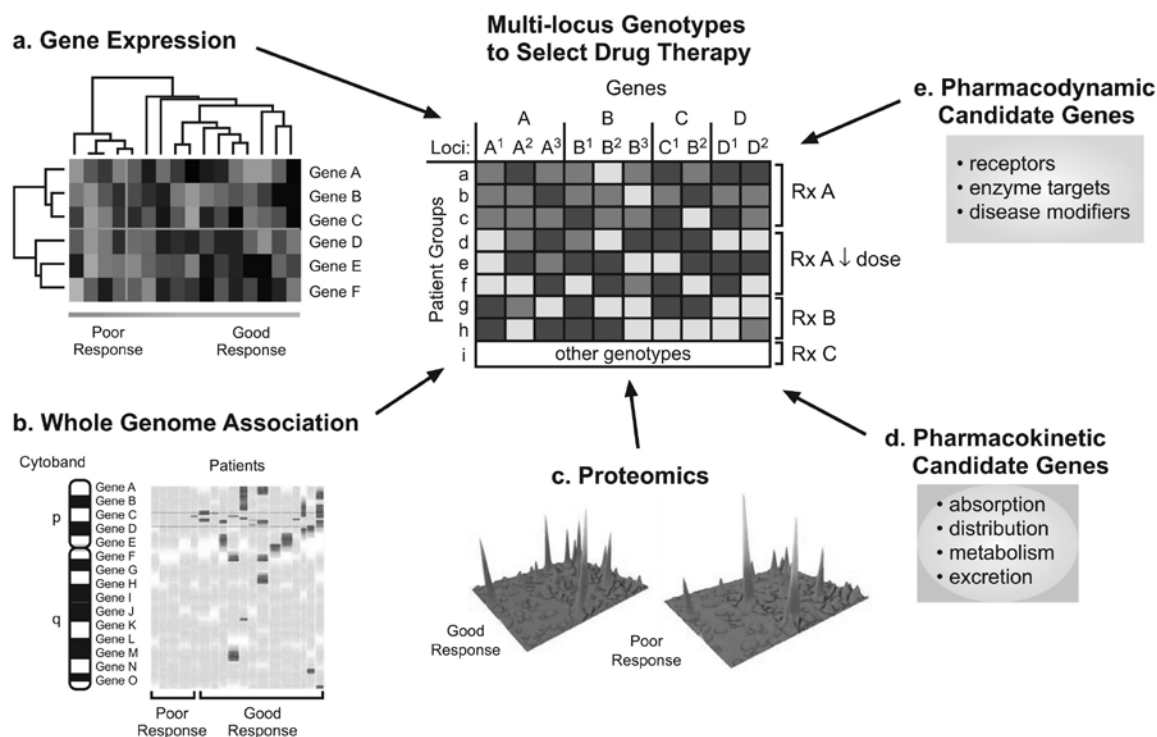


Figure 4. Identifying genes influencing polygenic drug responses.

a-c, Three genome-wide approaches—gene-expression analysis, genome-wide scans and proteomics—can be used to identify potential candidate genes that influence a specific drug response (for example, the promotion of anti-leukaemic effects). a, DNA microarray analysis of cancer cells for 6 genes (rows) in 16 patients (columns), 4 with a poor response and 12 with a good response. b, SNP haplotype map showing 16 gene loci on one chromosome for 5 patients with a poor response and 13 with a good response. c, LC-MS (liquid chromatography/mass spectrometry) analysis of plasma or tumour tissue to identify differences in proteins (see yellow peaks) between good and poor responders. d, e, The conventional ‘candidate gene’ approach, based on clinical pharmacology studies of proteins (for example, receptors) and pathways known to be involved in a drug’s pharmacokinetic or pharmacodynamic response. The middle panel represents a subset of the final product, a panel of multiple genes and loci that collectively segregate patients into groups who respond best to one of several drugs or drug doses. Reprinted with permission from reference 7.

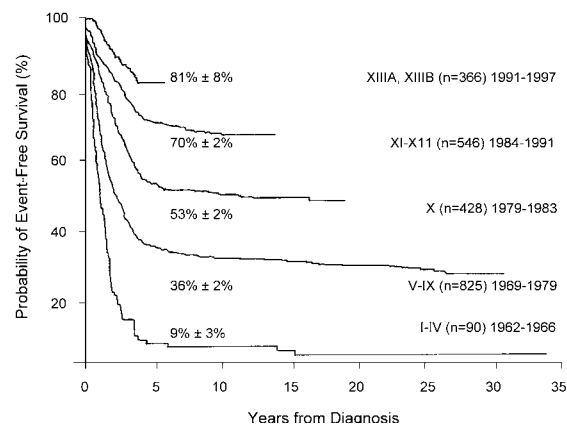


Figure 5. Kaplan-Meier Analyses of Event-free Survival in 2255 Children with ALL in 13 Consecutive Studies Conducted at St. Jude Children’s Research Hospital from 1962 to 1997.

The results demonstrate improvement in survival with the introduction of therapy for subclinical disease of the central nervous system (studies V to IX, 1967 to 1979); with early intensification of systemic chemotherapy, including high-dose methotrexate (study X, 1979 to 1983, and studies XI and XII, 1984 to 1991); and with reinduction treatment, early intensification of intrathecal chemotherapy, and pulsed therapy with dexamethasone and vincristine (studies XIII A and XIII B, 1991 to 1997). The mean (\pm SE) five-year survival probabilities are shown. Reprinted with permission from reference 21.

cal companies and most biotech companies. Even for the most common childhood cancer (ALL), for which the cure rate has been pushed above 80% by using drugs developed largely for adult cancers (Figure 5), there has not been a concerted effort within the pharmaceutical industry to apply modern science and exploit high-throughput technologies to identify molecularly-targeted new agents for this disease. So cancer remains the most common cause of death by disease in US children between 1 and 15 years of age, and the treatment remains largely empirical with drugs developed for adult cancers. Moreover, there are frequently shortages of the antileukemic agents that are needed to achieve an 80% cure rate (e.g., mercaptopurine, L-asparaginase). Therefore, our best efforts are needed on multiple fronts (scientific, clinical, political, and educational), if we are to develop more effective and less toxic therapy for ALL and other childhood cancers. There is much yet to be done.

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