

CLINICAL PHARMACOGENOMICS AND CONCEPT OF PERSONALIZED MEDICINE

KLINIČKA FARMAKOGENOMIKA I KONCEPT PERSONALIZOVANE MEDICINE

Nikolina Babić

University of Chicago, Pritzker School of Medicine, Department of Pathology, Chicago, IL, USA

Summary: The term »personalized medicine« (PM) was coined in the late 1990s, but was not introduced to general US public until about a decade later, through Genomics and Personalized Medicine Act. According to this act, PM is defined as any clinical practice model that utilizes genomic and family history information to customize diagnostic and therapeutic interventions and improve health outcomes. One of the emerging disciplines essential for implementation of PM is clinical pharmacogenomics (PGx), where patient's genetic information is utilized to personalize drug therapy. PGx testing includes mostly detection of small DNA variations, such as single nucleotide polymorphisms (SNPs), insertions, and deletions in the genes encoding the drug transporters, receptors and metabolizing enzymes. By providing the right drug at the optimal dose to each patient, PGx promises to significantly improve drug efficacy and prevent adverse drug reactions. In the early 2000s, the US Food and Drug Administration joined scientists and laboratorians in their efforts to translate recent genetic advances into clinical practice by requiring the drug manufacturers to include genetic information on their product labels. To date several drugs including irinotecan, warfarin, abacavir and clopidogrel are labeled with the information relating different enzymatic polymorphisms with the adverse drug effects or the impaired drug efficacy. The majority of PGx testing involves SNP detection within the family of Cytochrome (CYP) P450 enzymes responsible for metabolism of most drugs, such as anti-depressants (e.g. CYP2D6) and anticoagulants (e.g. CYP2C9, 2C19) to name a few. PGx tests are still very low volume tests and it is not clear how and to what extent genotyping information is being utilized in the clinical practice, mostly due to the lack of outcome studies demonstrating the clinical utility of PGx testing. For instance, it is well known that approximately 30% of Caucasian population carries a polymorphic CYP2C9 allele that predisposes them to higher

Kratak sadržaj: Izraz »personalizovana medicina« (PM) je nastao kasnih 1990-ih, ali je uveden u opštu upotrebu u SAD tek deceniju kasnije, kroz Akt o genomici i personalizovanoj medicini. Prema ovom aktu, PM je definisana kao bilo koji model kliničke prakse koji koristi genomičke i informacije iz porodične istorije za prilagođavanje dijagnostičkih i terapijskih intervencija i poboljšanje ishoda. Jedna od novih disciplina esencijalnih za implementaciju PM je klinička farmakogenomika (PGx), gde se genetičke informacije pacijenta koriste za personalizaciju farmakoterapije. PGx ispitivanja uključuju uglavnom detekciju malih varijacija DNK, takvih polimorfizama pojedinačnih nukleotida (SNP), insercija i delecija u genima koji kodiraju transportere za lekove, receptore i metabolišuće enzime. Davanjem odgovarajućeg leka svakom pacijentu u optimalnoj dozi, PGx obećava značajno poboljšanje efikasnosti leka i sprečavanje neželjenih efekata. U ranim 2000.-im, Američka administracija za hranu i lekove se pridružila naučnicima i laboratorijskim stručnjacima u njihovim naporima da privedu nedavna otkrića na polju genetike u kliničku praksu zahtevom da proizvođači lekova uključe genetske informacije u prateće podatke za svoje proizvode. Do sada su za nekoliko lekova, uključujući irinotekan, varfarin, abakavir i klopidogrel dodate informacije koje povezuju različite enzimske polimorfizme sa neželjenim efektima ili smanjenom efikasnošću. Većina PGx ispitivanja uključuje detekciju SNP unutar familije enzima citohroma (CYP) P450 odgovornih za metabolizam većine lekova, kao što su na primer antidepresivi (npr. CYP2D6) i antikoagulanasi (npr. CYP2C9, 2C19). Zahtevi za PGx testovima su još uvek vrlo retki i nije jasno kako i u kom stepenu će se informacije o genotipu koristiti u kliničkoj praksi, uglavnom zbog nedostatka studija ishoda koje pokazuju kliničku korist PGx ispitivanja. Na primer, dobro je poznato da oko 30 % populacije belaca poseduje polimorfni alel CYP2C9 koji predstavlja predispoziciju za veću osetljivost prema varfarinu i time za

Address for correspondence:

Nikolina Babić
University of Chicago, Pritzker School of Medicine
Department of Pathology, Chicago, IL, USA

warfarin sensitivity and thus to increased bleeding risk. However, there are no large, randomized outcome studies that conclusively demonstrate reduction of bleeding events or decrease in hospitalization rates in population dosed based on genotype information. The clinicians are thus reluctant to incorporate warfarin genotyping into their practice. Despite the attention PGx has received in recent years, the adoption of PGx into routine clinical testing is still far from being commonplace. The barriers to wider adoption and implementation of PGx include lack of education and understanding by prescribing physicians regarding the available tests, lack of consensus guidelines on interpretation and use of genotype results and scarcity of randomized controlled trials demonstrating the clinical utility of PGx testing. However, as genetic testing is becoming increasingly patient driven thought direct-to-consumer testing, clinicians and laboratorians must continue to work toward full implementation of PGx testing into routine clinical practice.

Keywords: personalized medicine, pharmacogenomics, direct-to-consumer testing

Introduction

»Personalized DNA testing is coming to a Walgreens near you this Friday,« read announcement on abc channel news in May 2010 (1). Although Food and Drug Administration (FDA) halted this drug store giant's intent to proceed with over-the-counter sale of unregulated DNA collection kit, the event is still a sobering reminder to all clinical practitioners just how fast and how far has technology advanced. The future we dreamt of, where we can just walk into the drug store, purchase a kit for \$20–30 and have science reveal our risks of developing cancer, heart disease, or any other ailment one could imagine, has arrived.

In the Genomics and Personalized Medicine Act of 2010, the term »personalized medicine« is defined as any clinical practice model that utilizes genomic and family history information to customize diagnostic and therapeutic interventions and improve health outcomes (2). The goal of personalized medicine is to use molecular markers to detect disease risk or presence before clinical signs and symptoms appear. This will shift the focus of medicine on prevention and early intervention rather than reaction to and treatment of already advanced disease. New biomarkers are being discovered and translated for clinical use on an ongoing basis. Some examples include genetic tests for BRCA1 and BRCA2 mutations to assess woman's risk of breast and ovarian cancer (3, 4), or recent efforts in predicting the efficacy of anti-EGFR agents using KRAS mutation testing for patients with metastatic colon cancers (5).

The Role of Pharmacogenomics (PGx)

PGx role in personalizing medicine is to use genetic information to provide the right dose of the

povećani rizik od krvarenja. Međutim, ne postoje velike, randomizirane studije ishoda koje pokazuju redukciju krvarenja ili smanjenje stope hospitalizacije u populaciji u kojoj je doziranje zasnovano na genotipskim informacijama. Zato kliničari nerado inkorporiraju genotipizaciju varfarina u svoju praksu. Uprkos pažnji posvećenoj PGx poslednjih godina, uvođenje PGx u rutinska klinička ispitivanja je daleko od uobičajenog. Prepreke širem prihvatanju i implementaciji PGx uključuju i nedostatak edukacije i razumevanja raspoloživih testova od strane lekara, nedostatak konsenzus smernica za interpretaciju i upotrebu rezultata genotipizacije i oskudica randomiziranih kontrolisanih ispitivanja koja pokazuju kliničku korist PGx ispitivanja. Međutim, kako genetička ispitivanja postaju sve više usmerena ka pacijentu, iako spadaju u ispitivanja »direktno do potrošača«, kliničari i laboratorijski stručnjaci moraju da nastave da rade na potpunoj implementaciji PGx ispitivanja u rutinsku kliničku praksu.

Ključne reči: personalizovana medicina, farmakogenomika, ispitivanje »direktno do potrošača«

drug to the right person at the right time. Vogel introduced the term »pharmacogenetics« as the study of various genetic variations relevant to a drug's disposition or effect (6). PGx testing includes mostly detection of small DNA variations, such as single nucleotide polymorphisms (SNPs), insertions, and deletions in the genes that regulate phase I oxidative drug metabolism (e.g. cytochrome P450, CYP family of enzymes such as CYP2D6, CYP2C19 and so on), phase II drug conjugation enzymes (e.g., glucuronosyltransferases and N-acetyltransferases), drug transporter proteins (e.g., organic anion transporters) and receptors (7). A good example of the role of PGx testing in tailoring the therapy is a case of clopidogrel, a drug widely used to prevent stent thrombosis following the percutaneous coronary angioplasty. Two recent studies published in the New England Journal of Medicine showed that, among the patients on clopidogrel therapy, those individuals carrying CYP2C19 loss-of-function alleles (e.g. *2, *3, *4, or *5) had significantly lower concentrations of the active drug metabolite and reduced platelet inhibition, resulting in 3-fold increase in risk of the stent restenosis and a 3.6 fold increase in rate of the cardiovascular events (8, 9). In these individuals, an alternate platelet inhibitor, such as prasugrel, should be considered. Thus CYP2C19 genotyping to predict individual's response to clopidogrel could be a useful biomarker for tailoring this expensive drug to minimize therapeutic failure and reduce the risk of future cardiac events.

Another important role of PGx testing is in reduction of the adverse drug reactions (ADRs) associated with the failure to use the right drug at the right dose. Several studies found that approximately 5% of all hospital admissions are associated with ADRs (10–14). Incidence of serious ADRs is estimated to be 2 million per year in the US and cause 100,000 deaths (12). One very important contributor to ADRs

is failure of drug metabolizing enzyme to properly metabolize the drug, which can occur if, for instance, one or both of the alleles coding for such an enzyme contain activating or inactivating variant(s). In such a patient, relative to general population, the drug will be metabolized either faster and its effects exacerbated or slower rendering the therapy inefficient. PGx testing is invaluable in these situations because it could assist the physician in selecting the appropriate drug therapy optimize the drug therapeutic index. Desire to improve therapeutic index of drugs stems from the knowledge that efficacy of the most widely used drugs in the US today averages around 50% with a range of 25% for chemotherapeutics and up to 80% for analgesics (15). While errors associated with the incorrect prescriptions or patient non-compliance will not be corrected by PGx testing, adverse events due to sub-optimal dose or selection of an inappropriate therapeutic agent may be minimized by this testing. For example, implementation of pharmacogenomic testing for abacavir (16) or carbamazepine (17, 18) promises to greatly reduce the incidence of potentially life-threatening, delayed cutaneous hypersensitivity reactions.

PGx Testing Methodologies

Commercial platforms available for PGx testing include multiplexed DNA arrays, real-time PCR and recently whole genome sequencers.

Real-time PCR testing involves the homogenous amplification with variant DNA detection by hybridization of fluorescent probes. Since each PCR reaction tube can detect one SNP, this methodology is not amenable to multiplexing. Focused DNA arrays can be arranged onto silicon-based »gene chip« or color-coded beads enabling simultaneous detection of multiple SNPs. Examples of commercially available automated platforms include AutoGenomics (INFINITI®), Luminex (xTAG® assays) and GenMark Dx™ (eSensor®). Most multiplexed systems combine allele-specific primer extension (ASPE) and detection of the generated fluorescent signal (19).

There are advantages and limitations for each analytical platform. Factors like sample type (whole blood, buccal swab), system footprint, random-access capability, total assay time, automation, frequency of no-calls, and complexity of technologist training will affect which system is a better fit for a particular clinical laboratory. The three platforms mentioned above have comparable analysis times, taking 4–8 hours from DNA isolation-to-genotyping results and they are all fairly compact benchtop analyzers (19, 20). The eSensor has the smallest footprint, fastest analysis time and is the only true random access analyzer and thus very convenient for the routine clinical laboratory. However, the other two platforms are more customizable to individual laboratory needs.

With development of the next generation sequencing (NGS), the multiplexing platforms are slowly being replaced with the whole genome-wide arrays that can simultaneously detect hundreds of thousands of SNPs. NGS platforms in clinical use today include Roche 454 GS Junior (Roche Diagnostics), Ion Torrent (Life Technologies™) and MiSeq® (Illumina) analyzers. Until last year, the \$50 000 cost of the whole genome sequencing was cost prohibitive for clinical applications (21). In May 2011, Illumina announced cost reduction of the whole genome sequencing down to \$5000 (22), approaching more realistic levels for clinical applications. Most genotyping tests are still in the category of laboratory-developed tests and the costs associated with developing these tests and running the larger arrays will likely be higher than for focused DNA chips for quite some time. Furthermore, technical expertise required to analyze and interpret results of such testing is not trivial.

Clinical Implementation of PGx

Despite clear advantages of molecular testing, the implementation of PGx at bedside or in the physician office has been slow. Various steps and challenges in implementing the clinical pharmacogenetic testing were recently reviewed by Grossman (23). The barriers to wider adoption and implementation include lack of education and understanding by prescribing physicians regarding available PGx tests, lack of consensus guidelines on interpretation and use of genotyping results, regulatory issues, technology access, cost and reimbursement issues, and demonstration of cost-effectiveness. Recently, the US FDA has approved revised labeling requirements for selected drugs where the polymorphisms have been linked to either a reduction in drug efficacy or an increased incidence of adverse events (19) (Table I), an effort that should help accelerate the implementation of clinical PGx. Proper clinical utilization of PGx promises to incrementally improve therapeutics.

As mentioned previously, most of the PGx tests used clinically today involve identification of genetic variants in the complex CYP 450 system and correlation to individual's phenotype. Depending on the variant identified, patients have been traditionally categorized as either slow, intermediate, fast, or ultra drug metabolizers (24). Phenotype prediction could become quite complicated in individuals who are heterozygous for different variants and even more difficult with the identification of new allelic variants and sub-variants. For instance, as of December 2011, over a hundred allelic variants and sub-variants have been identified for CYP2D6 (25). This has led some investigators to develop alternate schemes to relate genotyping to metabolizing capability. Gaedigk et al. (26) computed an »activity score« for CYP2D6, based

Table 1 Selected Personalized Medicine Drugs and Corresponding PGx Tests (adapted from The Case for Personalized Medicine, 3rd Edition (28). The drugs listed below are US FDA-approved drugs with PGx information listed in their label.

Drug	Biomarker	Indication	Drug Label*
Warfarin	<i>CYP2C9</i> <i>VKORC1</i>	Cardiovascular disease	Genotyping is recommended to establish the appropriate initiation dose
Clopidogrel	<i>CYP2C19</i>	Cardiovascular disease	Information only
Irinotecan	<i>UGT1A1</i>	Colon cancer	Genotyping is recommended to establish the appropriate starting dose
Cetuximab Panitumumab	<i>BRAF</i>	Colon cancer	Information only
Cetuximab Panitumumab Gefitinib Erlotinib	<i>KRAS</i>	Colon Cancer	Information only
Gefitinib Erlotinib	<i>KRAS</i>	Lung Cancer	Information only
Imatinib	Philadelphia C hromosome (<i>BCR-ABL</i>)	Leukemia	Imatinib is indicated for treatment of newly diagnosed Philadelphia Chromosome positive patients
Mercaptopurine Thioguanine Azathioprine	TPMT	Leukemia	Genotyping is recommended for dose adjustment
Carbamazepine	<i>HLA-B*1502</i>	Epilepsy Bipolar disorder	Genotyping is recommended to assess the risk of dermatologic reactions
Abacavir	<i>HLA-B*5701</i>	HIV	Genotyping is recommended to assess the risk of hypersensitivity reaction
Celecoxib	<i>CYP2C9</i>	Pain	Information only

*complete information regarding product labeling of these drugs can be found on FDA website (<http://www.fda.gov/Drugs/ScienceResearch/ResearchAreas/Pharmacogenetics/>)

on the ability of individuals with various genotypes to metabolize dextromethorphan, a CYP2D6 substrate. This scoring system classifies individuals into just a few manageable categories using the experimental data. All the complexity described above indicates that a consensus guideline for standardizing the genotype-phenotype prediction is crucial before the adoption of such testing into more widespread clinical practice can occur (27, 28).

Drug specific dosing models will also be needed in the future if PGx is to become part of the routine clinical practice. Theoretically, an algorithm can be developed for any drug that is influenced by PGx variables, where a therapeutic drug concentration range has been established. PGx testing will be most useful

if the drug has a narrow therapeutic range and a significant proportion of the variation in response in the population tested is predicted by genotyping. A good example of such drug is an anti-coagulant warfarin, a drug with a very narrow therapeutic index. It has been suggested that a dosing algorithm based on genotype information can be used to successfully predict correct initial dose of warfarin and thereby reduce the risk of bleeding events (29).

However, large, randomized outcome studies that conclusively demonstrate reduction of the bleeding events or decrease in hospitalization rates in population dosed based on genotype information are still lacking. The clinicians are thus reluctant to incorporate warfarin genotyping into their practice.

Future Perspectives

Personalized medicine and PGx are currently attracting a lot of attention from patients and legislators. This is largely due to avalanche of web-based *direct-to-consumer DNA testing* services that promise a wealth of genetic information, cost less than \$1000, and are now widely marketed to the average consumer.

One can easily envision the future with PM embedded in every hospital, clinic and medical practice, with patient's complete genotypes readily available in their medical records enabling the physician to order tailored blood and tissue tests aimed at very early and precise diagnosis. This scenario is quickly

becoming our reality especially with constantly decreasing costs of the genomic sequencing and already very affordable direct-to-consumer testing. The time has come when the patient is taking initiative to make more informed healthier lifestyle choices and driving their own diagnosis and therapy. With such rapid and monumental developments, it is imperative for the health care system and clinical laboratories to co-evolve with the technology.

Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

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