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Mini Review

A Family Physician's Digest of Pharmacogenomics A Case-based Syllabus

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Abstract

This is a mini-review of applied pharmacogenomics that is composed for clinicians, especially those providing primary care. In the first half, office scenarios of 7 best known and most clinically relevant drug-gene pairs are presented in brief format, with main references attached to each. In the second half, the background knowledge and current implementations are introduced as a primer leading towards further exploration by interested individuals.

Keywords: Pharmacogenomics; Implementation; Primary care

"CPICguidelines are designed to help clinicians understand HOW available genetic test results should be used to optimize drug therapy, rather than WHETHER tests should be ordered. A key assumption underlying the CPIC guidelines is that clinical high-throughput and pre-emptive genotyping will become more widespread, and that clinicians will be faced with having patients' genotypes available even if they have not explicitly ordered a test with a specific drug in mind. " —— Pharmgkb.org

Introduction

Have you been wondering why codeine appears so often in adverse drug reaction (ADR) profile? Have you ever felt frustrated by a patient with a long list of active medications and a longer list of ADRs who needs another something? Have you always wished to know how likely the alerted event is actually going to happen? I try to shed some light on Pharmacogenomics (PGx), a relatively obscure subject to clinicians, yet a soon-to-be vital tool of day-to-day practice of medicine. A series of clinical scenarios common in primary care are composed to demonstrate the drug-gene pairs best known to today's applied PGx. This is followed by an outline of applied PGx: its foundation-laying technologies, cumulating knowledge, blooming industries and forthcoming clinical implementation.

Part 1. Best Known Drug-Gene Pairs In Primary Care

I have no reason to doubt that this will be my daily routines within 5 years. Actually it is already the look of some Dutch physician's life.

Scenario 1

Ms.Ms. A is a 23 year-old college student who complains of bad bronchitis cough depriving her sleep right prior to her final exams. She requires "something more potent than the over-the-counters". As I attempt to prescribe guaifenesin-codeine, an alert pops out, "Caution! CYP2D6[2] ultrarapid metabolizer (UM) *1A/*2×N[3]. See recommendations (Table 1)." I counsel her to avoid codeine and inform future prescribers of her CYP2D6 genotype.

Core literatures: FDA released its strongest warning in 2013 that alerts the danger of CYP2D6 ultrarapid metabolizers taking codeine, shortly after the deaths of 3 children in

[1] clinical pharmacogenetics implementation consortium

[2]D6 subfamily genes of cytochrome P450

[3]one copy of normal function allele *1A and N copies of normal function allele *2, see Human Cytochrome P450 Allele Nomenclature Database

[4] PubMed Identifier

2012 breast-fed by post-cesarean mothers (PMID[4] 22492761, 19692698, 15625333, 16920476, 23614474, 23709324).

Table 1. Pop-up clinical decision support message for CYP2D6 UM	
Genotype	An individual carrying more than two copies of functional alleles.
Metabolizer Status	Ultrarapid metabolizer (prevalence ~1-2%)
Activity Score	>2.0
Implications	Increased formation of morphine leading to higher risk of toxicity
Recommendations (Strength: Strong)	Avoid codeine use. Alternatives that are not affected by this CYP2D6 genotype include morphine and non-opioid analgesics. Tramadol, and to a lesser extent hydrocodone and oxycodone, are not good alternatives because their metabolism is also affected by CYP2D6 activity.

All 3 PGx associations published dosing guidelines on codeine-*CYP2D6* (pharmgkb.org > codeine > dosing guidelines; PMID: 24214521, 24458010, 21412232). Mechanism of this classic interaction can be simply put as a genetic amplification of CYP2D6 metabolized codeine-to-morphine transformation; CPIC guideline has an excellent illustrated interpretation of it (PMID: 24458010). Similar mechanism is shared by tramadol, oxycodone and hydrocodone, though to a lesser extent (PMID: 18713907, 12920424).

Scenario 2

Mrs. B is a 75 year-old retired nurse who is admitted for an ischemic stroke that is attributed to extensive atherosclerotic vascular disease. She has been on simvastatin 20mg daily without side effects for many years. Alert appears while simvastatin is being re-dosed: *"SLCO1B1 diplotype *1A/*5 translates to an intermediate risk for myopathy due to one of two copies being decreased function allele (*5)."* She is counseled on the better chance for tolerance if lower dose of a more potent statin less affected by low SLCO1B1 activity is given instead of increasing simvastatin to 80mg; rosuvastatin 20mg is chosen. Enclosed in the alert is an interpretation by PGx consultants: "*SLCO1B1 transports statins into hepatocytes and its dysfunction slows down statin clearance leading to increased myopathy. While *1A allele has normal function, *5 contains "C" at rs4149056[5], encoding a decreased activity transporter.*"

Core literatures In 2008, the single nucleotide variant *SLCO1B1*5* was detected with solid significance by genome-wide sequencing, and then verified in large number of myopathy cases to be associated to simvastatin-induced myopathy (PMID: 18650507). CPIC dosing guideline focuses on simvastatin for it is the most affected, though other statins also are influenced to a lesser degree (pharmgkb.org > simvastatin > dosing guidelines; PMID: 24918167). Pravastatin was shown in a randomized study to be a reasonable first choice statin for carriers of the *SLCO1B1*5* allele; and women may benefit from increased surveillance for symptoms (PMID: 19833260).

Scenario 3

I receive a letter from the cardiologist of my patient Mr. C, a 64 year-old chef. It is a summary of the patient's hospitalization for acute MI, coronary artery stenting 2 days ago and plan of post-MI care. A literature review is attached to the letter and starts as this: *"Clopidogrel is a pro-drug that needs activation by CYP2C19 for platelet suppression effect. Poor metabolizers (PM) of this enzyme should receive alternative anti-platelet agents e.g. prasugrel or ticagrelor, due to the risk of increased re-thrombosis."* A consent is also attached and signed by patient to disclose *CYP2C19* genotype.

Core literatures Cases of antithrombosis failure due to lack of clopidogrel activation by CYP2C19 have explained all (PMID: 21270785, 21215696). FDA released boxed warning in 2009 that recommends CYP2C19 genotyping prior to prescribing clopidogrel (pharmgkb.org > clopidogrel > drug labels). Both CPIC and DPWG provide dosing guidelines (pharmgkb.org > clopidogrel > dosing guidelines; PMID: 23698643, 21412232).

Scenario 4

Mrs. D, a 42 year-old high school teacher with breast cancer is here to receive PGx testing results. She has a history of estrogen-positive cancer, has been taking tamoxifen for 3 years since lumpectomy and chemo-radiation therapy and is doing well. She did develop chemo-induced menopause despite young age. As I open her report, several recommendations are displayed with one highlighted at the top: *"Studies with good quality suggest increased risk of breast cancer relapse when tamoxifen is used on CYP2D6 poor or intermediate metabolizers (IM), avoid concomitant use of CYP2D6 inhibitors and consider aromatase inhibitors (AI: anastrozole, letrozole, or exemestane) in postmenopausal women."* I explained above message to her. A return visit with her oncologist to discuss alternative hormone suppression agents is requested. **Core literatures** Tamoxifen is another pro-drug activated by CYP2D6 (to endoxifen). Evidences are not as sufficient and strong, maybe due to long follow-up needed to reach significant difference in outcomes, and also need for larger sample to reveal findings in subset analysis. CPIC has not recommended any actions based on *CYP2D6* genotype, meta-analysis (PMID: 24329190) .

[5]"rs" stands for reference SNP, is the reference number assigned to a specific single-nucleotide polymorphism (SNP) site in human genome; "C" is cytosine base instead of thymine; so the diplotype at rs4149056 site is "C/T" while the diplotype for the gene of SCLO1B1 is *1A/*5.

but DPWG has its dosing guideline (pharmgkb.org > tamoxifen > dosing guidelines; PMID: 21412232). Studies thus far important are copied here (PMID: 19809024,18024866, 16361630, 23213055,23570465, 23764426) including a meta-analysis (PMID: 24329190).

Scenario 5

Coumadin is initiated on Mr. E, an 80-year-old caucasian gentleman for newly discovered atrial fibrillation. *CYP2C9* and *VKORC1* genotypes, **1/*3* and *"A/A"* at rs9923231, along with other indices auto-populate into the electronic form used to initiate coumadin clinic enrollment. As I complete and sign the order, the recommended warfarin daily dose is provided: *"Highly sensitive to warfarin. Maintenance dose of 1.8mg with optional first day loading dose of 2.7mg is recommended"*. For easier operation, I prescribe 2.5mg on day 1 and 2.0mg on day 2 and day 3. On day 4 I receive his first INR from home monitor along with the recommended dosing for the next 5 days; it is signed and sent back to Mr. E.

Core literatures Genotype-guided coumadin dosing is probably the single one example of PGx implementation that has gone the farthest. Several large-scale randomized controlled trials comparing genotype-guided to traditional dosing have been completed (PMID: 24251360, 24251361, 24251363). Currently the most widely used algorithm was developed by Washington University and is free for public use (www.warfarindosing.org; PMID: 18305455). CPIC dosing guideline (pharmgkb.org > warfarin > dosing guidelines; PMID: 21900891) and main references (PMID: 19031075; 19300499, 19228618, 18574025) are collected here.

Scenario 6

Mrs. F, a 47-year-old Chinese descent is here for physical exam. A new drug, carbamazepine, is reflected as interval change of her medication profile. It was started 1 month ago by a neurologist for trigeminal neuralgia, and she had not ever used carbamazepine before that. As I update the active medication list in EMR[G], a message jumps out: *"HLA-B*15:02 and HLA-A*31:01 both significantly increase the risk of carbamazepine-induced hypersensitivity reactions including Steven-Johnson Syndrome / Toxic Epidermal Necrolysis (SJS/TEN). Individuals carrying at least one copy of either allele are not recommended to start carbamazepine if naïve to the medication."* Mrs. F reports that a genetic test was done by her neurologist prior to starting carbamazepine and she brings me a copy of the report. A panel of clinically relevant HLA alleles are tested: *"Mrs. F carries one copy of HLA-B*58:01 (heterozygote for the allele) which renders her susceptible to allopurinol-induced severe cutaneous adverse reactions (SCAR). All other alleles tested are negative including HLA-B*15:02 and HLA-A*31:01.* The report is sent to our PGx consultants to be EMR-charted for future reference.

Core literatures Association of *HLA-B*15:02* and carbamazepine-induced SJS/TEN (but not other reactions) was first reported in Han-Chinese (PMID: 15057820), then replicated quickly by multiple asian ethnic groups (PMID: 23132554). *HLA-A*31.01* was discovered to be related to carbamazepine-induced hypersensitivity reactions (not limited to SJS/TEN) via GWAS in 2011 in two different ethnic groups (European and Japanese) by two different teams (PMID: 21428769, 21149285). In 2007, FDA released boxed warning that requires *HLA-B*15:02* screening prior to carbamazepine prescription; information on *HLA-A*31:01* was added in 2013; drug package inserts in Japan also contain the warning on *HLA-B*15:02* (pharmgkb.org > carbamazepine > drug labels; PMID: 23895776). CPIC and CPNDS guidelines were published in 2013 and 2014 successively (pharmgkb.org > carbamazepine > dosing guidelines; PMID: 23695185, 24597466). There are only few drugs by far that have a known HLA allele that confers clinically significant outcomes. Among them, allopurinol-*HLA-B*58:01* pair is the most pertinent to primary care, therefore main articles are listed for reference (PMID: 15743917, 18192896, 19933789, 21912425). Anti-HIV drug abacavir causes systemic hypersensitivity syndrome (SHS) with extremely high mortality in carriers of *HLA-B*57:01*; preemptive testing is obligated by FDA regardless of ethnicity (pharmgkb.org > abacavir > drug labels)

Scenario 7

As I preview the chart of my next patient Miss. G, a 35 year-old graduate student of sociology with a history of bipolar type II with major depressive episodes, anxiety and fibromyalgia, as a message catches attention: *"More than 3 ADRs detected share a common CYP450 enzyme. Consider genotyping for minor metabolizer status."* There are 9 medications listed under She tells me during the interview that she stops taking medications all together, and struggles to manage her mood and pain by counseling and exercise. Genotyping of *CYP450* panel comes back in 3 days; diplotypes of crucial *CYPs* are translated into metabolism phenotypes with the help of PGx consultants (Table 2B). A new list of drugs addressing her problems with no or minimal dependence on deficient CYPs are constructed (Table 2C). Gabapentin, desvenlafaxine, lamotrigine and clonazepam are selected and planned to be initiated in an orderly fashion under close observation.

Core literatures: Though knowing *CYP2* genotypes does not prove to benefit an individual drug's therapeutic outcome, a finely designed trial comparing genotype-guided versus traditional prescription of all medications used for major depression patients reflects overall benefit (PMID: 24018772). This reversed the failed initial attempt to guide psychotropic drug dosing, as it turned out to be an issue of medication selection rather than dose adjustment. DPWG guidelines feature most of

[6] both chromosomes have Adenine base (confers high sensitivity to vitamin K antagonism) instead of Guanine base at SNP site rs9923231

the widely used SSRIs, SNRIs, TCAs and antipsychotics whose metabolism largely rely on *CYP2* family enzymes (Pharmgkb.org > PGx drug dosing guidelines > DPWG).

Part 2. Brief Digest Of Applied PGx

Pharmacogenetics (PGt) studies how drug response is affected by interindividual variations, and PGx is simply PGt on genomic scale. Box 1 takes an overview at a half century's history of PGt-PGx.

Table 2. CYP dependence analysis provided by PGx consult

A. Medications not tolerated

Drug \ CYP	*1A2*	*2B6*	*2C9*	*2C19*	*2D6*	*3A4/5*	
Amitriptyline	□		□	■	■✡	□ ✡	☹
Duloxetine	■				■		☹
Bupropion		■✡		□			☹
Citalopram				■	□	■	☹
Fluoxetine			■	□	■✡	□	☹
Sertraline		■	□	□	□	□	☹
Venlafaxine					■✡	□	☹
Aripiprazole					■	■	☹
Trazodone				□	■		☹
B. CYP450 functional status							
Diplotype			**1/*3*	**1/*2*	**4/*4*	**1*1/*3*3*	
Phenotype			*IM**	*IM**	*PM**	*EM**	
C. Medications considered for use							
Gabapentin							✌
Mirtazapine	□				■	■	〰
Desvenlafaxine						□	✌
Quetiapine					□	■	〰
Olanzepine	■				□		〰
Lamotrigine							✌
Diazepam				■		■	〰
Alprazolam						■	〰
Clonazepam							✌
Lorazepam							✌

■major pathway □ minor pathway ✡activation ☹adverse reaction or intolerance 〰with caution ✌good option

*EM: extensive metabolizer *PM: poor metabolizer *IM: intermediate metabolizer

Box 1. Milestones of pharmacogenetics-pharmacogenomics

1957 Pharmacogenetics described and named

1960 Differential elimination of isoniazid related to slow or rapid acetylation which is under genetic control

1975 *CYP2D6* polymorphism described after wide interindividual range of plasma concentrations of debrisoquine and sparteine cause orthostatic hypotension and fetal death respectively

1993 *CYP2D6* ultrarapid metabolizer reported

1994 *CYP2C9* and *2C19* reduced activity alleles discovered

2000 PharmGKB founded

2001 *CYP2D6, 2C19* and *2C9* genotypes-based dosing guidelines of psychotropic drugs published

2005 First-generation haplotype map of human genome completed by International HapMap project

2006 AmpliChip® became first commercial PGx array approved by FDA

2006 DPWG incorporated G-Standard into nationwide EMR in Netherland

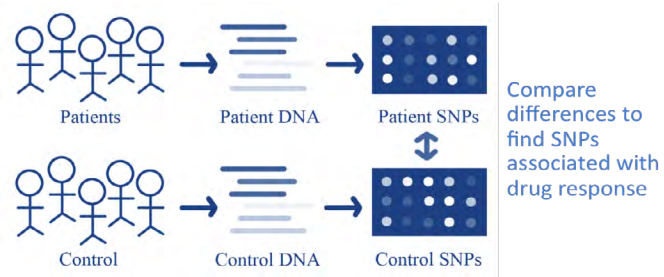
2007 FDA released warning on clopidogrel and *CYP2C19.PM*

2011 CPIC published first set of dosing guidelines

2011 GWAS related *HLA-A*13:01* to carbamazepine-induced hypersensitivity reactions

2013 FDA released warning on codeine and *CYP2D6.UM*

PGx research starts with looking for an association between a drug response trait and a polymorphism (majority SNP[1]). Traditionally a candidate SNP was tested in a case-control study; if the SNP's frequency was significantly different between cases and controls, an association was identified. Upon advent of the era of next-generation sequencing, thousands of randomly selected SNPs scattered throughout human genome can be tested within a short time and an affordable budget, revealing SNP biomarkers that have never been hypothesized to play a role in the process. This is called genome-wide association study, or GWAS, as illustrated in Box 2.



Box 2. Genome-wide association study

Clinical applications of PGx are not new to today's medicine: much so popular have been targeted therapies for cancers and autoimmune disorders in the past decade. There are however a lot more to it, on the road of translation into clinics and hospitals. Box 3 demonstrates the spectrum of drug-geneintertions, by an artificial classification.

Box 3. Spectrum of PGx interactions

		Outcome	
		Safety	Efficacy
Mechanism	PK	**Metoprolol-*CYP2D6*** *(UM)*-decreased exposure to active drug due to fast deactivation **Tamoxifen-*CYP2D6*** *(PM)*-decreased production of active metabolite, endoxifen **Clopidogrel-*CYP2C19*** *(PM)*-decreased production of active metabolite for antiplatelet effect	**Codeine-*CYP2D6*** *(UM)*-increased exposure to active metabolite, morphine **Warfarin-*CYP2C9*** *(PM)*-increased exposure to warfarin due to slow deactivation **Simvastatin-*SCLO1B1*** *(C@rs4149056)*-increased exposure to simvastatin due to reduced intracellular transportation
	PD	Vast majority of targeted drugs in oncology and rheumatology	**Warfarin-*VKORC1*** *(A@ rs9923231)*-increased drug target sensitivity
	HLA	Theoretically possible but clinically relevant examples not available	**Carbamazepine-*HLA-B*** *(*15:02) or* ***HLA-A*** *(*13:01)* **Allopurinol-*HLA-B*** *(*58:01)*

PD: pharmacodynamic **PK:** pharmacokinetic **HLA:** immunogenic, sometimes called idiosyncratic; also in this category: abacavir, phenytoin (Pharmgkb.org > PGx drug dosing guidelines).
Besides single drug-gene interactions, drug-drug-gene interactions (DDGI) result in significant outcomes when the 2nd drug add to the genetic effect usually via CYP inhibition. Attached is a case report for example (PMID: 20837591)

Our knowledge is more advanced in PK interactions. Among all PK genes, *CYPs* have received highest attention due to the vital role P450s play in biotransformation of about 80% of all drugs in clinical use, and also because of their extensive polymorphism.

Box 4. Cytochrome P450 superfamily

Human *CYP* superfamily has 57 genes and 59 pseudogenes divided among 18 families and 43 subfamilies. Only *CYP1, 2* and *3* families metabolize drugs, or exogenous toxic compounds, principally in the liver; other CYPs participate in endogenous compounds metabolism and are present in most organs of the body. CYPs catalyze majority of phase I reactions, which introduce a polar group into the substrates rendering them hydrophilic for subsequent reactions. Phase I usually deactivates a drug but occasionally it activates a pro-drug. Phase II and III conjugates and excretes the metabolites respectively.

Though accounts for only 2~4% of all hepatic CYPs and largely non-inducible, CYP2D6 is involved in the metabolism of 25% of drugs (PMID: 19817501). *CYP2D6* is the most highly polymorphic CYP gene. This is hypothesized to represent a diversity in plant detoxification that evolved under survival pressure during periods of food constraint. The presence of numerous pseudogenes and high activity at *CYP2D* locus are thought to have produced the large numbers of *CYP2D6* alleles (PMID: 15492763).

CYP3A4/5, the 2 major isoforms in CYP3A subfamily expressed in hepatocytes, are the CYPs present in largest quantity in human adults; it is highly inducible and less polymorphic than CYP2 family genes; it contributes to the metabolism of 50~60% of drugs (Pharmgkb.org > CYP3A4 > VIP, PMID: 24926778).

It has been 9 years since DPWG guidelines were incorporated into G-standard, the Dutch national drug database embedded in the nationwide EMR. In the US, large quantities of evidences have been accumulated and appraised by PharmGKB since 2000, and almost 30 guidelines have been developed by CPIC since 2009 (PMID: 22992668). PGx implementation efforts are unprecedented today. Box 5 introduces the various resources and platforms in a speedily maturing environment for applied PGx

Box 5. Maturing environment for applied PGx

Implementation projects & Institutional pilot programs
List of Projects @ Pharmgkb.org PG4KDS @ St. Jude Children's Research Hospital 1200 Patients Project @ University of Chicago CDS : warfarin and CYP450 @ Mayo Clinic CDS : HLA-B biomarkers @ NIH Clinical Center
Commercial PGx tests & CDS platforms
Genetic Testing Registry @ NCBI YouScript by Genelex Medi-Span by WoltersKluwer Safety-code.org (PMID: 24088121 23345409)
Authority regulatory actions
Table of PGx biomarkers in drug labeling @ fda.gov New standards on pharmaceutical R&D, clinical trials @ fda.gov

PGx trait, like other individual parameters considered in pharmacotherapy including age, renal function, liver function, drug interactions and more, is part of the profile and has its limitations. Nevertheless, like other non-genetic factors, it needs not be perfect to provide useful information to the prescriber. Individualization has always been an ideal of medicine. PGx is necessary but not sufficient to individualized pharmacotherapy.

Box 6. Numbers in pharmacogenomics

383 severe adverse events could have been prevented if PGx information was considered in a PCMH with 52942 patients over a 5-year period (PMID: 22739144)

4000–6000 USD higher costs per year are used to treat an UM or PM compared to an IM or EM patient (PMID: 24018772)

69 drugs, **6%** of all prescription drugs, taken by **25%** of American patients, contained human PGx biomarkers in their FDA labels in 2008; **62%** of these biomarkers were *CYP* genes (PMID: 18657016)

30~40% of interindividual coumadin dosing variations can be attributed to *VKORC1* and CYP2C9 polymorphisms; 13.3 individuals need to be genotyped to prevent one case to get supra- or subtherapeutic warfarin

In the field of PGx, we have come a long way, and it is high timeto face the ground-breaking step forward.