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Improving acceptance of pharmacogenetic testing in patient care

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P.C.D. Bank

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Improving acceptance of pharmacogenetic testing in patient care

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Voor pap & mam

*“You inspire me
Like I inspire you”*

Mister And Mississippi - In Between

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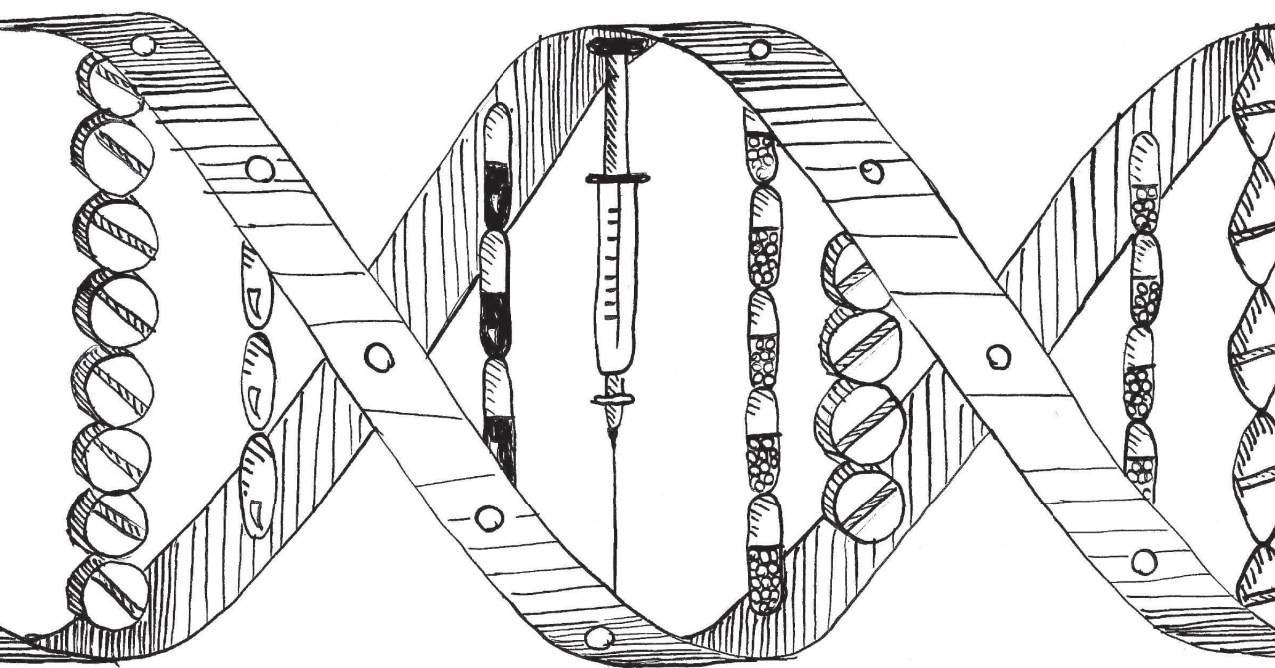
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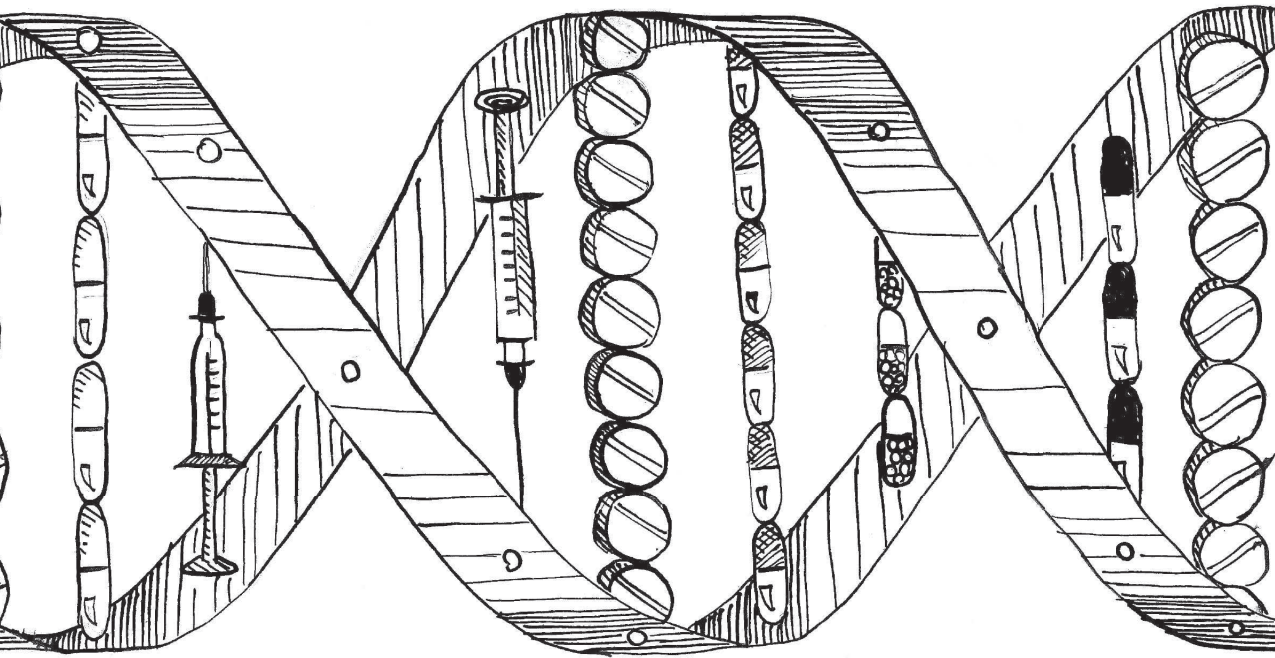
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Chapter 1

General introduction



In recent years evidence has become available that drug response is a complex trait and as a consequence a one-size-fits-all approach in drug therapy is unsuitable. Indeed, a large inter-patient variability in drug efficacy exists and ranges between 25% to 80% (1). In addition to variable drug efficacy, individuals treated without tailoring of therapy may experience drug induced side-effects which in turn can lead to drug-related morbidity and mortality (2-4). Evidence that drug response is at least partly heritable has been available since the early 1930's (5-10). In the following decades new discoveries in the emerging field of genetics and molecular biology have shown that drug efficacy, the absorption, distribution, metabolism and excretion of drugs and the risk of adverse drug events can be explained by genetics in some extent (11-14). Pharmacogenetics (PGx) is the area within the field of genetics that investigates the influence of heritability on drug response. Recent studies have shown that the use of PGx can lead to improved efficacy and a decrease in drug related morbidity and mortality (15-17). However, before PGx could be implemented in clinical care a number of barriers had to be addressed such as the lack of clinical grade tests, the lack of evidence-based therapeutic recommendations and the integration of PGx into the workflow of healthcare professionals (18). In the last decade numerous clinical grade test have become available, the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have developed dosing guidelines for well-known gene-drug pairs and in the Netherlands the therapeutic recommendations of the DWPG are incorporated into electronic drug prescribing and -dispensing systems used by physicians and pharmacists in clinical practice. Currently, pharmacogenetic testing is used by secondary and tertiary care centers to optimize therapy with high risk medication (19). However, up to date the implementation of PGx in primary care has been low, while evidence suggests that patients treated in primary care could benefit from dose optimization based on genetic information. Unlike the field of oncology, where for example only approximately 5% of patients carry a genetic variant in *DPYD* that requires dose optimization of fluoropyrimidines (19, 20), the frequency of patients who receive suboptimal therapy as a result of drug-gene interactions is estimated to be larger. It has been shown that 28.6% of all patients in primary care use at least one drug metabolized by a polymorphic enzyme and 46 of the 53 guidelines issued by the DPWG in 2011 are related to drugs also used in primary care (21-23). These facts support the hypothesis that the adoption of PGx in primary care could lead to a health benefit for patients treated in the first line of health care.

Aim and scope

The aim of this thesis is to investigate the adoption of PGx and the integration of genotype-guided dosing in the workflow of physicians and pharmacists in primary care. This thesis is divided into five parts. The first part (**Chapter 2**) provides an overview of answers to frequently asked questions by clinicians related to the implementation of PGx.

The second part of this covers the implementation of PGx in clinical practice and its impact on a national level. In **Chapter 3** a novel test for the detection of 13 genetic variants in the pharmacogene *CYP2D6*, the Genochip *CYP2D6* macro array, is compared to the gold standard in *CYP2D6* genotyping (the Amplichip *CYP450* test developed by Roche) to investigate whether the Genochip is ready for clinical implementation. **Chapter 4** investigates whether genotype guided dosing in primary care is feasible in a pilot study where 200 patients with an incident prescription for a subset of 10 drugs and historical use are genotyped in a panel based approach for *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *TPMT*, *SLCO1B1* and *VKORC1* and received genotype guided dosing based on recommendations of the DPWG guidelines (the Implementation of Pharmacogenetics into Primary Care Project). In **Chapter 5** an assessment of the clinical impact of PGx in primary care in the Netherlands is provided to predict how many patients that start with a drug currently described in the guidelines of the DPWG require an optimization of therapy. This study was performed with the frequencies of the eight pharmacogenes, obtained from the genotype results of the IP3 study, and combined with national prescription data from a selection of drugs which have a known gene-drug interaction with the selected panel of 8 genes.

Part III covers the harmonization of PGx-test interpretation and therapeutic recommendations. **Chapter 6** presents a counter argument to a proposition by Magnani et al. to reduce fluoropyrimidine dosing even further in heterozygous carriers of the *DPYD**2A variant than currently described in the guidelines of CPIC and DPWG. In **Chapter 7** is investigated which clinically relevant differences in terms of translation from genotype to phenotype and therapeutic recommendations exists between the guidelines of the CPIC and DPWG and whether they can be explained by the methodologies used by the two consortia. As a result of the efforts of the DPWG therapeutic recommendations for patients with known aberrant phenotypes are available at point-of-care through clinical decision support. However, it is currently unknown whether Dutch healthcare professionals feel prepared to guide treatment based on genetic information.

Part IV investigates the knowledge, experience and attitudes towards PGx among (future) healthcare professionals. **Chapter 8** describes a study to benchmark knowledge, experience,

and attitudes of Dutch pharmacists. In **Chapter 9** the question is addressed whether PharmD have received enough training in their curriculum to feel informed about PGx.

Part V contains the general discussion (**Chapter 10**) along with the English and Dutch summaries.

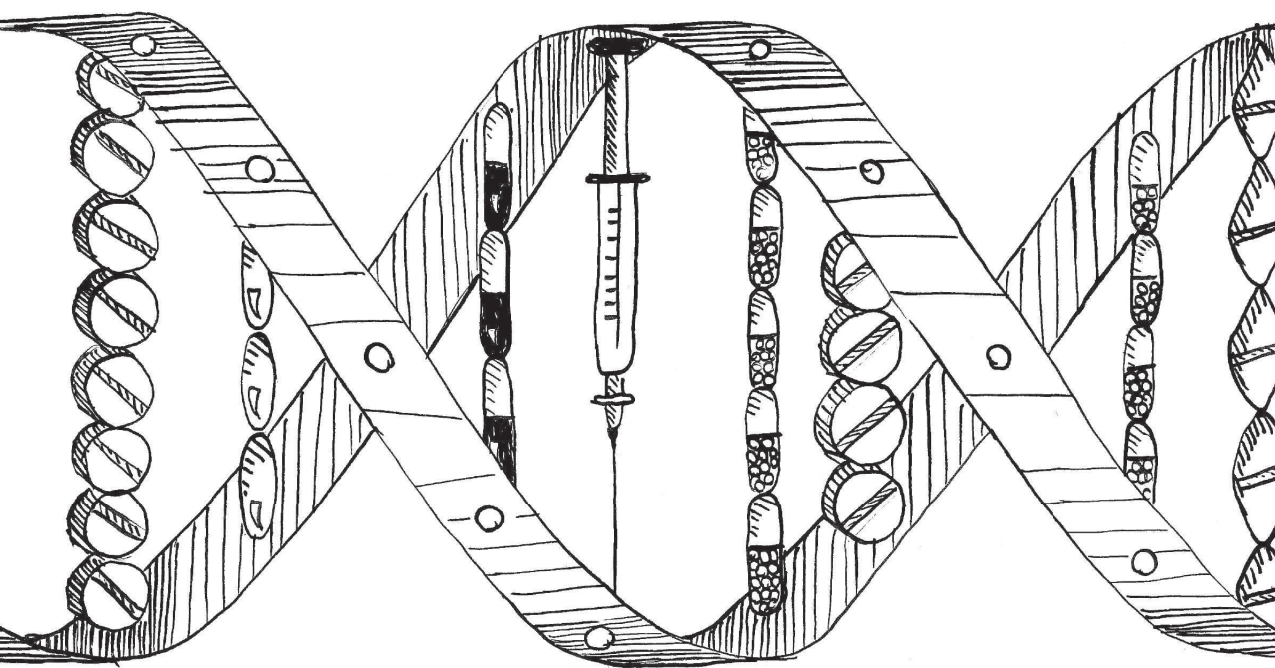
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Part I

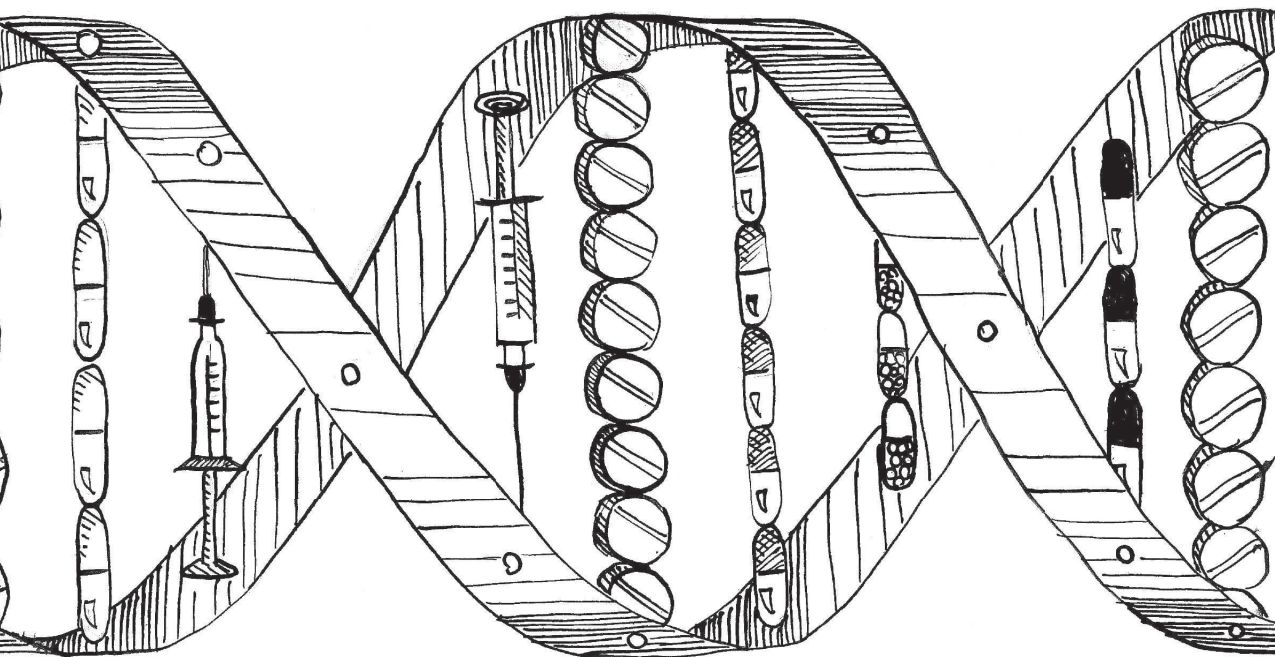
Answers to frequently asked
question by clinicians
regarding PGx



Chapter 2

Pharmacogenetic biomarkers for predicting drug response

Bank PCD, Swen JJ, Guchelaar HJ



Abstract

Drug response shows significant interpatient variability and evidence that genetics influences outcome of drug therapy has been known for more than five decades. However, the translation of this knowledge to clinical practice remains slow. Using examples from clinical practice six considerations about the implementation of pharmacogenetics (PGx) into routine care are discussed: the need for PGx biomarkers; the sources of genetic variability in drug response; the amount of variability explained by PGx; whether PGx test results are actionable; the level of evidence needed for implementation of PGx and the sources of information regarding interpretation of PGx data.

Introduction

Since the completion of the Human Genome Project, pharmacogenetics (PGx) has been heralded as one of the first clinical applications arising from this landmark project. Indeed, in 2001, it was envisioned that within 10 years physicians would use genomic information to guide the treatment of their patients (1, 2). However, currently we can conclude that adoption of PGx in clinical practice is limited and slow. Here, we present six considerations related to implementation of PGx in clinical practice. Each of the considerations is illustrated with clinical examples and potential strategies to improve implementation are discussed.

Case example of the potential effect of genetic variability

Three 2-year-old children are prescribed 10–12.5 mg codeine orally every 4 to 6 h following a successful adenotonsillectomy (3, 4). The following day, the parents of one of the children consult the pediatrician because their child continues to experience pain. The pediatrician stops codeine and prescribes ibuprofen, which successfully lowers the pain (5-7). Routine follow-up with the second child shows a good response to codeine. On the third day after the adenotonsillectomy, the pediatrician receives a report by the paramedics who responded to an emergency call from the parents of the third child. The parents had found their child in the morning with decreased vital signs. The child had been wheezing and had developed fever the previous night. Efforts by the paramedics to resuscitate the child failed. Postmortem analysis showed blood levels of 0.70 mg codeine and 32 ng morphine per milliliter. Autopsy further revealed aspiration of food particles and bilateral consolidation in the lungs consistent with bronchopneumonia (8, 9). Genetic analyses showed that the cytochrome P-450 (*CYP*) 2D6 genotypes could explain the different responses to codeine between the three children (5-9). This extreme example shows that variations in genes coding for metabolizing enzymes can lead to a significant difference in drug efficacy and safety. In this case, *CYP2D6* genotype guided dosing may have prevented nonresponse of the first child and prevented the fatal outcome of the third child.

Do we need (pharmacogenetic) biomarker to predict drug response?

In the current era of evidence-based medicine, patients are treated according to evidence-based guidelines, which are ideally based upon results of multiple, double-blind, randomized controlled trials (RCTs). However, these RCTs often include highly selected individuals and participants are subjected to controlled procedures. In contrast, the actual patient population in clinical practice is highly heterogeneous and includes the elderly, patients with impaired organ function and patients with comorbidities (10). As a result, drug efficacy and toxicity in patients in clinical practice differs from outcomes of drug therapy observed in the clinical studies. Indeed, the variability of drug response among the major drug classes is high. It is estimated that drug efficacy ranges from 25 to 80%, with most of the drugs falling in the interval of 50–75% (11).

In addition, there is also the risk of adverse drug events. Although intense efforts are made by regulatory agencies to provide safe pharmacotherapy for the general population, it was reported that 1.8 million people were hospitalized for adverse drug events in the USA in 1994 (12). Genetic variability contributes to this variability in drug toxicity and efficacy. It has been reported that 60% of all the drugs that caused adverse events were metabolized by enzymes that are under control of polymorphic genes (13). Furthermore, an analysis of 607 patients from three US-based primary care centers showed that 28.6% used at least one drug that is metabolized by an enzyme that is under control of a polymorphic gene (14). The result of the variability in drug efficacy and toxicity is that it is difficult to prescribe the most effective and most safe drug to an individual patient. In fact, the current practice of prescribing could still be considered largely ‘trial and error.’ After diagnosis, a standardized drug and dosage are selected from a (evidence-based) guideline. The dose or drug choice may be individualized to some extent based upon the patients’ characteristics such as comorbidity, comedication, age or organ function. Then drug efficacy and toxicity are monitored by using (surrogate) markers such as blood pressure, cholesterol levels, tumor size or liver enzymes. When the therapy appears to be ineffective or causes side effects, the treatment is modified by adjusting the dose or selected drug.

Although this process of trial and error prescribing may be laborious and time consuming, it is not a problem for some treatments and conditions. For example, in the case of treatment of hypertension with antihypertensive drugs to lower the risk of cardiovascular disease, treatment efficacy can be monitored simply by measuring the blood pressure after 4 weeks. Results from the Framingham study have shown that a hypertensive patient with additional risk factors has a risk of $\geq 20\%$ of dying from cardiovascular disease in the next

10 years (15). Effective antihypertensive drug treatment results in a relative risk reduction of 20–25% over a 10-year time span and in a patient with a predicted Framingham risk of 25% antihypertensive treatment would lead to an absolute risk reduction of 5.0–6.3% (16, 17). It can be calculated that a 3-month delay of effective treatment due to trial and error prescribing would lead to an increase of only 0.13–0.16% in the absolute risk of cardiovascular-related death in 10 years. Therefore, a delay as a result of ineffective treatment has little, if any, effect on the reduction of cardiovascular events. Obviously, this is different in the field of oncology, where generally patients in a metastatic setting have a short life expectancy. For example, a 3-month delay due to difficulties in finding an effective and safe therapy is not acceptable in the treatment of metastatic colorectal cancer patients, with a median overall survival of only 24–36 months (18).

Therefore, it is of great importance to develop biomarkers for predicting drug response, especially in the following situations: where the drug effect is difficult to assess and presents after months to years; when treatment delay has important clinical consequences; in diseases with a poor prognosis and treatments with severe side effects.

In summary, drug response both with regard to efficacy and toxicity of frequently prescribed drugs is highly variable. Currently, pharmacotherapy is largely based on trial and error selection of drugs and dosages for individual patients. By using predictive PGx biomarkers, a more rational approach could be achieved and drug response could be predicted upfront, resulting in a safer and more efficacious drug treatment for the individual patient.

Which are the sources of genetic variability in drug response?

Drug response is a combination of pharmacokinetics (PK) and pharmacodynamics (PD). PK describes the processes of absorption, distribution, metabolism and excretion and is generally referred to as ‘what the body does to the drug’. PD describes the processes when drugs, or their active metabolites, interact with their target, which can range from a receptor to a drug transporter. Changes in PK or PD contribute to variability of drug response, both with regard to efficacy and toxicity.

Genetic influence on pharmacokinetics

Single nucleotide polymorphisms (SNPs), insertions and deletions in the genes that code for Phase I and II enzymes involved in PK can lead to reduced or even complete absence of enzyme activity. Although less common, increased enzyme activity has also been described, for example, due to allele multiplication (19). Consequently, these genetic variants can

lead to changes in plasma concentrations of drugs (14). Especially for drugs with a narrow therapeutic index, changes in the plasma drug concentration can easily result in failure of therapy or toxicity (see section ‘Case example of the potential effect of genetic variability’).

The effect of SNPs on drug PK can be exemplified by the fluoropyrimidines such as 5-fluorouracil and capecitabine. These drugs are the cornerstones of treatment of many cancers, including colorectal and breast cancer. The most important metabolic step in the PK of fluoropyrimidines is the inactivation by the enzyme dihydropyrimidine dehydrogenase (DPD) in the liver. This enzyme is a product of the autosomal inherited *DPYD* gene and is responsible for > 80% of the metabolism of fluoropyrimidines (20). Normally, 10% of the patients who undergo fluoropyrimidine-based treatment experience severe toxicity and a significant number requires hospitalization for recovery (21). Diminished DPD activity is seen in up to 3% of the population, and treatment of an individual with diminished capacity to metabolize fluoropyrimidines can lead to severe or even lethal gastrointestinal, hematological or neurological toxicity (22-26). It has been shown that multiple SNPs in the *DPYD* gene can lead to an enzyme with a decreased or even absent activity (25-27). The IVS14+1G>A variant is the most studied variant and is present in an intron between exons 14 and 15 in the *DPYD* gene, where it causes the exon skipping of exon 14 and leads to a nonfunctional enzyme and decreased clearance of fluoropyrimidines (25, 26). Other relevant SNPs in *DYPD* that can lead to low or absent DPD activity are the 2846A>T and the 1236G>A mutations. The incidence of grade 3 to 4 diarrhea increases to 71% ($p < 0.01$), 62% ($p < 0.02$) and 50% ($p < 0.006$), respectively, in patients heterozygous for the IVS14+1G>A, 2846A>T or 1236G>A mutation compared to 24% in patients with a wild type *DPYD* gene (28-31).

Genetic influence on pharmacodynamics

Genetic variants in the genes coding for a receptor, a transporter or an enzyme can lead to a change in associated downstream signaling and biochemical processes. These differences can result in an altered drug response.

An example of how genetic variation may affect a drug's PD is the SNPs in the *ADRB2* gene encoding the β_2 -receptor. β_2 -agonists are frequently prescribed drugs in patients with asthma and chronic obstructive pulmonary disease. The *ADRB2* gene is located on q31-q32 of chromosome 5 (32). SNPs in *ADRB2* have been correlated with a difference in downregulation, expression profiles and response to (ant)agonists of the β_2 -adrenoreceptor (33, 34). The SNPs 46G>A and 79C>G result in substitution of arginine to glycine at codon 16 and glutamine to glutamic acid at codon 27, respectively (35). Homozygous carriers of

glutamic acid at codon 27 show a higher venodilatation after exposure to the β_2 -agonist isoproterenol (36). Multiple studies in children and adults with asthma have shown that homozygous carriers of arginine at codon 16 have a better airway response to albuterol compared to heterozygous carriers of arginine and homozygous carriers of glycine at codon 16 (33, 34).

Genetic influence on both pharmacokinetics & pharmacodynamics

Obviously, since genetic variation in genes is abundant and for the majority of drugs multiple genes are involved in the PK and PD pathways, both variation in PK and PD will importantly contribute to variable drug response of most drugs. An example of a group of drugs where genetic variations in both PK and PD have been found related to variability in drug response is the coumarins. This group of drugs influences blood coagulation by targeting a metabolizing enzyme involved in the recycling of vitamin K (37-41).

The majority of the Phase I metabolism of coumarins occurs by the liver enzyme CYP2C9. Currently, over 30 genetic variants of the *CYP2C9* gene have been described (42). The *CYP2C9**2 (420C>T) and *CYP2C9**3 (1075A>C) alleles both lead to changes in the amino acids in the catalytic site of the enzyme, resulting in lower or absent capacity to metabolize coumarins (43, 44). A case report by Steward et al. and a case-control study with 88 patients and 100 controls by Aithal et al. showed that patients with a variant allele of *CYP2C9* require a lower maintenance dose than those with a wild type allele (45, 46). Four individual PGx studies with European patients confirmed these results (47-50). Coumarins target the enzyme vitamin K epoxide reductase multiprotein complex subunit 1 (*VKORC1*). A genome-wide linkage study identified *VKORC1* as the coding gene (51). Sequencing of *VKORC1* in patients with a type 2 deficiency of vitamin-K-dependent clotting factors showed several genetic variants in the exome of the gene that were responsible for decreased activity of the enzyme (52). However, studies in European and American Caucasian patients using coumarins showed that variants in the promoter and an intron of *VKORC1*, the SNPs 1639G>A and 1173C>T, respectively, were more prevalent and showed better correlations with daily doses of coumarins than the found mutations in the exome (53-55). In the case of coumarins, the two genes coding for the enzymes CYP2C9 and *VKORC1* influence PK and PD, respectively, but both contribute to drug response.

Genetic influence on idiosyncratic drug reactions

In some situations, adverse events are not related to the drug exposure. The frequency of these idiosyncratic (or type B) adverse events is lower than exposure-dependent (or

type A) adverse events and they are usually detected at a later stage in the process of drug development or after market approval. Indeed, between 1976 and 2005, 28 drugs were removed from the market because of serious idiosyncratic adverse events in the USA (56, 57). In recent years, genome-wide association (GWA) studies have identified genetic variants related to idiosyncratic reactions of several drugs (57, 58).

Flucloxacillin-induced liver injury is an example of a severe idiosyncratic drug reaction. The antibiotic flucloxacillin causes serious liver injury in < 1 in 10,000 patients. A GWA study of 51 cases of drug-induced liver injury (DILI) caused by flucloxacillin and 282 controls showed that SNPs in the genetic region of the HLA genes are a significant predictor of DILI. The strongest hit, a SNP located in the *HCP5* gene, showed strong linkage disequilibrium with the *B*5701* allele of HLA class I. Further analysis and replication in 16 additional DILI cases showed that individuals who carry the *B*5701* allele have a much higher risk of flucloxacillin liver injury (an OR of 80.6) (59). Alleles that were identified in these GWA studies and candidate gene studies do not appear to influence PK or PD, but seem to modulate immune response to drugs. Similar associations between SNPs in HLA genes and toxicity have been reported for abacavir, allopurinol and carbamazepine (60-62).

How much variability is explained by pharmacogenetics?

Fueled by the classic examples of Mendelian disorders, many clinicians consider PGx an on-or-off phenomenon. However, this phenomenon is rare and to date only a limited number of genes have been reported that have such an effect on the outcome of therapy. In addition to *DPYD* and several variants of *HLA* genes, the PGx biomarker TMPT, coding for the enzyme thiopurine methyltransferase and responsible for the metabolism of thiopurines, is one of the few examples where a large part of the observed variability in toxicity can be explained by genetic variants in a single gene. In general, not only genetics but also multiple nongenetic factors (i.e., age, organ function and weight) contribute to the complex phenotype of drug response and a single PGx biomarker may only be expected to explain a part of the observed interindividual variability in drug response. For most PGx biomarkers, the relative contribution in explaining interindividual response can be quantified as a percentage of explained variance symbolized by R^2 . Using these parameters, the clinical value of a PGx biomarker in selecting the right drug and the right dose can be estimated and compared to the performance of conventional clinical factors used to guide pharmacotherapy.

One of the first to quantify the effect of PGx on the variability in response was the group of Sconce et al. They investigated the effect of PGx variants on warfarin dose and showed

that genetic variants in *VKORC1* and *CYP2C9* could explain 17.5 and 15.0% of the variance in dose, respectively. Combination of the two genotypes with clinical factors such as age and height increased the explained variance in dose by the algorithm to 55% (See Table 2.1) (55). For acenocoumarol and phenprocoumon the two genes *CYP2C9* and *VKORC1* explain 4.5%, 27.2%, 4.6% and 34.1% of the variance in dose requirement respectively (63). Combination of *CYP2C9* and *VKORC1* status with other markers for response such as weight, height, sex, age and concomitant medication improved the explained variance to approximately 52.6% for acenocoumarol and 55.9% for phenprocoumon, respectively (Table 2.1) (63).

Similar potential for PGx biomarkers to explain a significant proportion of variability in response to medication is also observed in the field of rheumatology. Patients who are diagnosed with rheumatoid arthritis are treated with methotrexate (MTX), the most widely used disease-modifying antirheumatic drug (64, 65). However, only about half of

Table 2.1: Explained variance in dose requirements, disease activity score response and #3 grade toxicity with and without clinical factors for three coumarins, methotrexate, fluoropyrimidines and irinotecan

Drug	(Genetic) biomarker	R ²	Studied phenotype	References
Warfarin	<i>CYP2C9</i> + <i>VKORC1</i> + Age & height	15.0% 32.5% 55.0%	Dose requirement	(55)
Acenocoumarol	<i>CYP2C9</i> + <i>VKORC1</i> + Age, height, weight, sex, drug-interactions	4.5% 31.7% 52.6%	Dose requirement	(63)
Phenprocoumon	<i>CYP2C9</i> + <i>VKORC1</i> + Age, height, weight, sex, drug-interactions	4.6% 34.1% 55.9%	Dose requirement	(63)
Methotrexate	<i>AMPD1</i> + <i>ATIC</i> + <i>ITPA</i> + <i>AMTHFD1</i> + clinical factors (sex, Rf status, smoking status, baseline DAS)	10% 35%	DAS response	(68)
Capecitabine/ 5-fluoropyrimidine	<i>DPYD</i>	23–38%	Risk of ≥ 3 grade toxicity	(69-72)
Irinotecan	<i>UGT-1A1</i> + SN-38 AUC	22% 49%	Absolute neutrophil count	(82)

AUC: Area under the curve; AMPD1: Adenosine mono- phosphate deaminase; ATIC: Aminoimidazole carboxamide ribonucleotide transformylase; DAS: Disease activity score; ITPA: Inosine triphosphate pyrophosphatase; MTHFD1: Methylenetetrahydrofolate reductase; RF: Rheumatoid factor.

the patients show a good clinical response during treatment and in 30% of patients MTX treatment is stopped as a result of side effects (66, 67). In a study by Wessels et al., it was shown that the clinical factors such as sex, rheumatoid factor (RF) status, smoking and disease activity score (DAS) at baseline could explain approximately 25% of the variance in DAS response to MTX therapy in patients with newly diagnosed rheumatoid arthritis. In this study, genotypes for 17 SNPs in 13 candidate genes related to PK and PD of MTX were also correlated with the response in DAS after 6 months of treatment with MTX. SNPs in the genes adenosine monophosphate deaminase (*AMPD1*), aminoimidazole carboxamide ribonucleotide transformylase (*ATIC*), inosine triphosphate pyrophosphatase (*ITPA*) and methylenetetrahydrofolate reductase (*MTHFD1*) were significantly associated with a DAS response after 6 months of MTX therapy. Combined with the clinical factors, these four PGx biomarkers could explain approximately 35% of the variance in DAS response to MTX (Table 2.1) (68).

As described above, variation in the *DPYD* gene can explain an important part of observed toxicity to fluoropyrimidines. Indeed, multiple studies have shown that 23–38% of the occurrence of ≥ 3 grade toxicity of fluoropyrimidine treatment can be explained by genetic variants in the *DPYD* gene (Table 2.1) (69–72). Deenen et al. have shown that a dose reduction of 50% based on the IVS14+1G>A status can lead to a risk reduction of ≥ 3 grade toxicity from 68% in historical controls to 15% while maintaining fluoropyrimidine exposure comparable to fully dosed noncarriers of the IVS14+1G>A variants (73). However, as with the coumarin example, other clinical factors including treatment regimen and concomitant medication also contribute to the risk of toxicities (69, 74).

Another example of a drug–gene interaction where drug toxicity can be partly explained by PGx is the combination of the gene *UGT-1A1* and irinotecan, an agent used in advanced colorectal cancer. Irinotecan is a prodrug that is metabolized in vivo by CYP3A into inactive metabolites and by carboxylesterase into the active metabolite SN-38. This active metabolite is responsible for the antitumor effect. SN-38 is glucuronidated in the liver by the enzyme UDP-glucuronosyltransferase (UGT) 1A1 to SN-38 glucuronide, which is excreted through bile and urine (75, 76). The *UGT-1A1**28 gene carries a promoter region with a binding site consisting of six TA repeats, which allows the transcription factor IID to bind and upregulate expression of *UGT-1A1**28. In carriers of the *UGT-1A1**28 allele, the binding site contains seven TA repeats instead of the normal six and the expression of the *UGT-1A1* enzyme is reduced (77). The reduced expression of this metabolic enzyme reduces the capacity of this individual to glucuronidate the active metabolite SN-38 which in turn can lead to accumulation and a higher chance of toxicity in the form of diarrhea and leukopenia than an individual with a wild type *UGT-1A1* gene (78–81). A study by

Ramchandani et al. has shown that the decrease in absolute neutrophil count could be explained by 31%, while a combination of genotype with the area under the curve of SN-38 resulted in an explained variance in absolute neutrophil of 49% (82).

Overall, these examples show that genetics can explain an important part of the variability in drug response. However, monogenetic influence on treatment variability is rare and the proportion of explained variance ranges considerably (5–55%) for different drug–gene combinations. Whether or not these numbers are clinically important is highly dependent on the studied phenotype and the examples also illustrate that PGx biomarkers should not be used as standalone markers for drug response, but should rather be combined with known clinical prediction for drug response to maximize the explained variance (R^2).

Are pharmacogenetic test results actionable?

Typical outcomes of current PGx (GWA) studies include odds/hazard ratios (83, 84) and an increased risk or association with an improved or impaired response or negative drug effect (85, 86). However, these results are difficult to apply in treating individual patients.

Surveys among members of the Clinical Pharmacogenetics Implementation Consortium (CPIC) and members of the American Society for Clinical Pharmacology and Therapeutics in 2009 and 2010 have shown that there is a need for clear and curated peer-reviewed guidelines that help health care professionals to guide the interpretation of a PGx test result in a clinical context (87).

In daily practice, clinical factors such as kidney function are already used to adjust drug dosing and this information is typically available in guidelines or package inserts. Yet these guidelines rarely contain actionable information on the use of genetic biomarkers such as dose recommendations for a specific genotype. To make PGx test results actionable for clinicians, results from PGx studies have to be transformed from odds/hazard ratios to algorithms, decision trees or scoring systems.

An example of such a tool is the MTX scoring system developed by Wessels et al. to help rheumatologists select potential nonresponders from their patient population. The MTX scoring system includes the clinical factors such as gender, smoking status and DAS; the immunological biomarker RF and the *MTHFD1*, *AMPD1*, *ITPA* and *ATIC* genotypes. The system operates by assigning points for the presence of certain characteristics. For example, the presence of RF is scored by one point as well as the presence of variant alleles of *MTHFD1*, *AMPD1* and *ATIC*, while the presence of the variant allele of *ITPA* scores two points. The cut-off values for responder and intermediate responder are set at ≤ 3.5

and < 6 , respectively, while patients with a score of ≥ 6 are classified as nonresponders. Analyses with multiple cohorts have shown that this scoring system predicts the phenotype of responders or nonresponders in 79% (95% CI: 72–85%) of patients correctly (68).

Other examples of dosing algorithms can be found in the field of cardiology. In 2005, Sconce et al. evaluated the role of clinical and genetic factors on warfarin clearance in 297 patients using regression and selected age, height and *CYP2C9* and *VKORC1* genotypes for a new dosing algorithm. In a small cohort of 38 unrelated patients, the algorithm was validated and showed a regression of 0.80 ($p < 0.001$) between the modeled dose and the actual dose (55). For acenocoumarol and phenprocoumon, van Schie et al. developed loading and maintenance dose algorithms for these two drugs based on data from populations of 559 and 375 genotyped patients, respectively, based on the parameters *CYP2C9*, *VKORC1*, age, height, weight and use of amiodaron. The algorithm of acenocoumarol was validated in an independent cohort of 707 patients in which all parameters necessary for the algorithm were collected. In the analysis, the algorithm shows an R^2 of 52.7% for the genetic model, which is comparable with the R^2 of 52.6% in the original study (88).

In conclusion, to make PGx test results actionable and to support clinical application, it is essential that results of PGx studies are presented as actionable dosing algorithms or decision trees.

What level of evidence is needed for implementation?

The level of evidence that is required to implement PGx into clinical practice is highly debated (89–91). Current standards of evidence-based medicine require evidence for increased efficacy and safety or the same performance for lower costs, prior to the implementation of new techniques or treatments. If feasible, this evidence should best be obtained from RCTs (92, 93, 94–96). Thus, if PGx-guided therapy is considered an alternative compared to regular care, the evidence-based paradigm dictates the same level of evidence as is required for all other interventions and RCTs should be performed for each individual gene–drug combination.

However, there are also multiple arguments against the demand for RCTs for each gene–drug combination. There is no valid pharmacological argument to consider gene–drug interactions any different from drug–drug interactions or dose adjustments for clinical factors such as for impaired liver or renal function and dosing recommendations. The latter two are typically exclusively based on observational data. Regulatory authorities do not require data from RCTs showing that these recommendations result in improved clinical

outcomes and in clinical practice dosages are frequently adjusted based on observational evidence (91).

A second argument against the demand for RCTs for every single gene–drug interaction is that they are highly expensive and thus simply impossible for every single drug–gene variant. For novel drugs, RCTs are paid for by the pharmaceutical company that holds the patent. However, for existing drugs the cost of conducting a RCT for a certain genetic variant may be considered too high. Furthermore, for many drugs that have a drug–gene interaction the pharmacology is well known. For example, the majority of tricyclic antidepressants (TCAs) are metabolized by one or more highly polymorphic enzymes of the *CYP* family (97, 98). With the knowledge of the pharmacology of TCAs and the amount of clinical experience that has been obtained by clinicians with dosing based on blood levels, it appears to be a safe strategy to guide TCA dosing by PGx biomarkers (91, 99). As an alternative to RCTs for each gene–drug interaction, some have argued that PGx-guided therapy should be approved based on noninferiority (90). However, because PGx is a supplement on clinical care rather than an alternative for routine care, noninferiority is not the best option from a methodological point of view. Furthermore, noninferiority trials are much more sensitive to biases that dilute the differences between the index and control groups (100, 101).

Despite the ongoing debate about the necessity for an RCT for each gene–drug interaction, several RCTs for a number of PGx biomarkers have already been performed. Examples are the drug–gene pairs abacavir with *HLA-B*5701* and the combination of coumarines with *CYP2C9* and *VKORC1*. In the case of abacavir, it was shown that PGx-guided treatment results in lower risk of hypersensitivity. Subsequent studies also proved that screening for *HLA-B*5701* is cost-effective (61, 102). However, for the pharmacogenetically guided dosing of coumarins the results are less clear. In recent years, multiple RCTs have been performed where PGx-guided anticoagulant therapy was compared to standard anticoagulant therapy. While several RCTs with warfarin show results with a significant beneficial effect on time in therapeutic range (TTR), reports in 2007, 2009 and 2013 by Anderson et al., Gong et al. and Kimmel et al., respectively, contradict the positive studies (103–108). The results of the EU Pharmacogenetics of Anticoagulant Therapy (EUPACT) trials where PGx-guided therapy of acenocoumarol and phenprocoumon were compared to normal care only showed a modest effect on TTR in the first 4 weeks and no effect on the primary endpoint of TTR in the first 12 weeks. The EU-PACT results also contradict results from previous retrospective studies (63, 88). A clear explanation for this conflicting data remains to be found. A small effect size, a small window (<4 weeks) where PGx-guided therapy can make a significant difference, a difference in frequency of racial origin resulting in

differences in allele frequencies of *CYP2C9* and the use of the genotype-guided algorithm on the first day of the loading dose have all been suggested (103-109).

In our opinion, a small number of positive RCTs with PGx biomarkers would be sufficient to provide evidence that genotype-guided strategy can lead to a better outcome of therapy. Once this proof of principle is established, additional RCTs might not be needed for each individual gene–drug combination. Then sufficient evidence for clinical utility in the form of observational evidence from well-designed and replicated studies should justify implementation of a PGx biomarker into patient care.

Sources for up-to-date information regarding interpretation of pharmacogenetic test results

The interpretation of a PGx test result can be fairly straightforward only when a small number of variants with a well-established effect in one gene and one drug are involved. Based on the allelic variants carried by the individual, the dose can be adjusted (e.g., 6-mercaptopurine and *TPMT* genotypes) with relative ease or the drug can be avoided (e.g., abacavir and *HLA-B*5701*). However, in clinical reality, the situation is often much more complex. For example, the effects of many genetic variants are not known and often drug metabolism involves multiple enzymes. In the case of the drug amitriptyline, which is metabolized by the enzymes *CYP2D6* and *CYP2C19*, the majority of PGx studies on amitriptyline only focus on one of the enzymes. As a result, the prediction of the plasma concentration and effectiveness and tolerability of amitriptyline is difficult in individuals with polymorphisms in both *CYP2D6* and *CYP2C19* (99, 110). Moreover, when a patient uses multiple drugs, the interaction between gene–drug and drug–drug interactions rapidly becomes more complicated.

To facilitate clinicians with the interpretation of gene–drug interactions, the Dutch Pharmacogenetic Working Group (DPWG) developed PGx dose recommendations for 26 drugs based on a systematic review of the literature in 2008 (111). In 2011, these guidelines were updated and contained 53 gene–drug interactions (112). In the US, a similar initiative was undertaken by the CPIC which was formed in 2009 and has since provided guidelines to translate results from genetic tests into results for 23 gene–drug interactions. The guidelines of both groups can be found on pharmgkb.org (113). The guidelines by the CPIC and the DPWG will greatly facilitate the sound interpretation of PGx test results and subsequent actions.

Expert commentary

In the last decade, PGx has transformed from the once anticipated revolution into a steady evolution. Research on SNPs in PK and PD candidate genes has provided us with several actionable variants that have been incorporated into clinical guidelines. In the past few years, technology has advanced significantly and more and more GWA studies and next generation sequencing (NGS) are published, reporting potential PGx biomarkers which can further explain the observed interindividual variability in drug response. In addition, although the number of newly found PGx biomarkers are relatively small compared to the number of genetic biomarkers for diseases, such as Alzheimer disease, depression, diabetes mellitus and cancer, it appears that the PGx biomarkers appear to have much larger effect sizes (114). However, the majority of these newly identified PGx biomarkers are still exploratory and it will take time and additional evidence to incorporate these findings into clinically actionable guidelines. In 2010, the first attempt was made to use whole genome sequencing data in a PGx context (115). A second assessment of whole genome sequencing data, including disease risk and PGx biomarkers, from 12 adults was recently reported (116).

Another way in which hypothesis-free genetic testing could further increase the number of useful PGx biomarkers is genotyping available material from drug registration trials. Linking PGx biomarkers to PK and PD data obtained in the same trials could simultaneously generate information about clinical validity and utility of PGx biomarkers and provide a scientific base for interventions in the population carrying the genetic variant (e.g., for a dose reduction in a patient carrying an allelic variant that decreases the metabolic rate of that drug). Adaptive signatures, adaptive thresholds or Bayesian adaptive two-stage Phase I/II trial designs could help explore and validate PGx biomarkers found through GWA studies and NGS.

In addition, validated PGx biomarkers could be used to reduce the need for large and costly Phase III trials. In the case of a drug that only shows a modest effect on a composite clinical endpoint, a positive outcome of a Phase III trial is less likely, and especially if a small number of major side effects would occur during the trial this could prevent drug approval. Based on the role of the PGx biomarkers in the pharmacology of the drug (e.g., a genetic variation in the genome of the host leading to an altered metabolizing enzyme or a mutation in a tumor cell leading to an altered drug target) and whether the PGx biomarker is prognostic, predictive or both, the trial design incorporating the PGx biomarker can be adapted. In case the mechanism behind a predictive PGx biomarker is well understood and there is sufficient evidence that certain patients with (or without) the PGx biomarker

will not benefit from the drug therapy, an enrichment design could be used. In this trial design, the patient population is screened upfront and only patients who carry the specific PGx biomarker will be included and receive the new treatment or placebo (117, 118). An important aspect of this design is that the assay that is used for patient stratification is accurate and reproducible among participating centers. In case the clinical consequences of the status of the PGx biomarkers are not yet clear, alternative designs are more suitable. The sequential testing design is a method where a new treatment is tested in a general population of patients initially, and subsequently in a second RCT, the treatment is tested in a subgroup of patients with the PGx biomarker. A downside to the sequential testing design is the introduction of multiple testing (119, 120). In the biomarker, by treatment design patients are stratified according to the status of a predictive PGx biomarker and then randomized between treatment arms. This design is similar to the sequential design but encompasses each subgroup in a single RCT. The biomarker strategy design is a trial design in which patients are randomized into the arm where the given treatment is dependent on the status of a predictive PGx biomarker or into the arm where treatment is not dependent on the PGx biomarker. A downside to this design is the overlap between the two arms (each arm contains patients treated with a similar regimen) resulting in a larger trial (121, 122). Finally, the adaptive accrual design is a two-stage trial where primarily only patients harboring the PGx biomarker are randomized over the treatment arms. When an interim analysis of the first stage is positive, all patients, regardless of the PGx biomarker status, are allowed into the second stage of the trial and with a negative result the trial is stopped (123). In a variant to this adaptive accrual design, the first and second stages are reversed (124). Overall, based on the type of PGx biomarker and the available evidence, a trial design encompassing a PGx biomarker is available with each design having pro and cons in the form of multiple testing, overlapping treatment arms or limiting the proof of treatment to a certain subgroup (125-127). Although these designs have not been applied frequently, there are a number of trials where these designs have contributed to registration of drug with a PGx biomarker in the package insert (e.g., trastuzumab, cetuximab, panitumumab, imatinib) (128-132). In some cases, PGx biomarkers even rescued drugs that initially failed to show efficacy in a 'regular' RCT (e.g., erlotinib) (133-135). With the decrease in drugs in development pipelines of the pharmaceutical industry, these alternate designs could prove useful, especially for drugs developed for targets at the biomolecular level such as in oncology (136).

At the other end of the spectrum of implementation, in primary care, the lack of clinical guidelines, a major barrier for the implementation of PGx, is being addressed by the ongoing efforts of the DPWG and the CPIC to create dosing guidelines that enable the translation of genetic laboratory test results into actionable prescribing decisions for specific drugs.

However, a limitation of these guidelines is that they only recommend what to do when a patient's genotype is known and do not indicate which patients should be genotyped. For a few drugs such as abacavir and clopidogrel, indication for genotyping is in the package insert, but for many other drugs this is not clear.

Another important aspect is the PGx knowledge of health care professionals. A recent survey among US physicians concerning the adoption of PGx shows that 97.6% agrees that variation in a person's genome can influence drug response. However, only 29% of the responding physicians had received formal education on PGx and an even smaller fraction (10.3%) felt adequately informed. This may be the reason why only 12.9% of the physicians had ordered a PGx test in the preceding year (137). A survey among Canadian pharmacists showed similar results. In this study, 96.6% of the surveyed pharmacists claimed that they would recommend a genetic test if it could help predict efficacy of a drug. Yet, only 7.7% of the pharmacists reported that they felt comfortable receiving and advising based on the test results (138). This would suggest that more education about PGx biomarkers and the respective dose/drug recommendations is needed.

In addition to the role of health care professionals in adoption of this technique, the willingness of patients is also important for successful implementation. Multiple surveys have shown that acceptance of PGx by patients is dependent on the confidentiality of the data and possibility of information being shared with third parties (139, 140). To date, it is still unclear who the manager of the patients' genome is, who should perform which genetic tests and who should have access to this (pharmaco)genetic data. Some companies have already profited from this ambiguous situation by providing direct-to-consumer genetic testing.

In line with other clinical parameters that can influence the effects of drugs (e.g., kidney failure or an allergy to medication), genetic biomarkers should be incorporated in the patient's medical file (electronic) so that only health care professionals have access to this information to adjust pharmacotherapy (e.g., alternate drug or alternate dose) based on the patients' genetic data. Incorporation of genetic data in a patient's electronic medical file has the advantage that the genetic information can be coupled with a centralized infrastructure, which holds guidelines on drug-gene-interactions and associated recommendations on choice or dose of a drug. In this way, PGx can be integrated into the workflow of physicians and pharmacists.

These arguments may lead to believe that implementation of PGx into clinical practice is still far away; however, the opposite is true. Due to the rapid advancements in the field of molecular biology, genotyping prices have decreased rapidly making them approachable.

Prospective genotyping is cost-effective for some PGx biomarkers, such as exemplified by *DPYD* with a price of €75 per test (73). In comparison, 3 years ago the assay had an estimated cost of €120 per test, which discouraged routine screening for variants of this gene (141, 142). Currently, 8 of the 80 centers in the Netherlands provide oncological care screen routinely for genetic variants in the *DPYD* gene.

Worldwide, several specialized centers and consortia at the forefront of clinical PGx have initiated large implementation programs (143). In 2011, St. Jude Children's Research Hospital reported that a pharmacist-managed clinical PGx service for *TPMT* and *CYP2D6* was designed and implemented (144). The genotyping service was expanded in 2011 with *CYP2C19* and *SLCO1B1* and more are being added. Recently, it was reported that 1016 patients had been successfully genotyped in the program and that 78% of the genotyped patients carried at least one high-risk allele showing that preemptive genotyping is feasible and clinically useful (145). In 2012, the University of Chicago initiated recruitment for their project of 1200 patients (146). The aim of this project is to determine whether incorporation of a preemptive PGx testing service into routine clinical care is feasible and produces clinically usable results. Recently, it was reported that already 608 patients were genotyped and these genotypes resulted in 367 signals from the genomic prescribing system. Based on these results, preemptive pharmacogenetic testing was considered a success (147). Results from the largest reported prospective genotyping program initiated by Vanderbilt University have been reported recently. Approximately 10,000 patients participating in the PREDICT program have been genotyped for a panel of five drug-gene interactions, including thiopurines and *TPMT*, clopidogrel and *CYP2C19*, simvastatin and *SLCO1B1*, tacrolimus and *CYP3A5*, and warfarin and *CYP2C9* and *VKORC1*. Data indicate that 91% of the patients who were genotyped harbored one or more actionable variants and moreover that prospective testing reduced the testing burden in comparison with reactive genotyping (148). Although some of these programs are still in the research domain and do not represent standard clinical care, centers specialized in cardiology, oncology and transplantation medicine show that PGx can be used to prevent serious adverse drug reactions or guide drug treatment and that routine genotyping for clinical care is possible. Unfortunately, in comparison with secondary and tertiary centers, the implementation of genotype-guided therapy in primary care is still nonexistent. However, adoption of PGx by this field of medicine would probably lead to a major gain of health. The question then remains: Which patients are eligible for standardized genotyping? Algorithms to preemptively assess the patient's exposure to medications that are known to have drug-gene interactions could provide an answer to the question which patients should be genotyped.

Overall, we can conclude that due to the relatively low costs of genotyping techniques, available evidence-based guidelines and integration of genetic data and the guidelines in electronic health care systems, implementation of PGx into clinical care is feasible and will continue to be implemented in the coming years.

Five-year view

The application of novel approaches such as GWA studies and NGS will continue to provide the field of pharmacology with further knowledge and insights into mechanisms and genetic variants involved in drug efficacy and toxicity, which will be incorporated into clinical guidelines. The complex phenotype ‘drug response’ may be largely predictable using a combination of multiple genetic variants and other clinical parameters. In addition, epigenetic research will further help to better understand the molecular mechanisms underlying variability in drug response and safety.

Data from multiple RCTs investigating genotype-guided therapy will become available in the subsequent years and when positive will help clinicians to accept the proof-of-concept of PGx. The cost of genotyping techniques will further decline in the coming years, leading to a larger number of cost-effective PGx tests that improve drug efficacy and safety, making this an increasingly interesting option for health insurers. And with the cost of NGS expected to drop to a few hundred dollars, the personal genome may become a commodity. Ultimately, health care professionals will encounter an increasing number of patients with genetic information in their practice.

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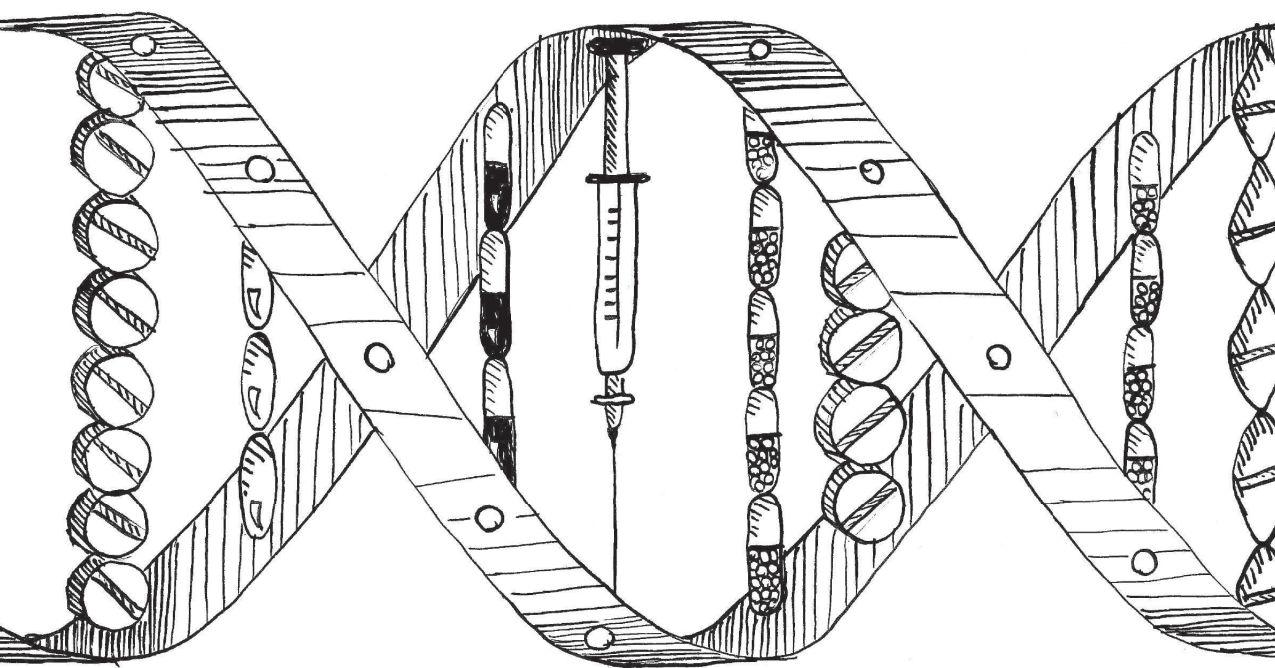
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Part II

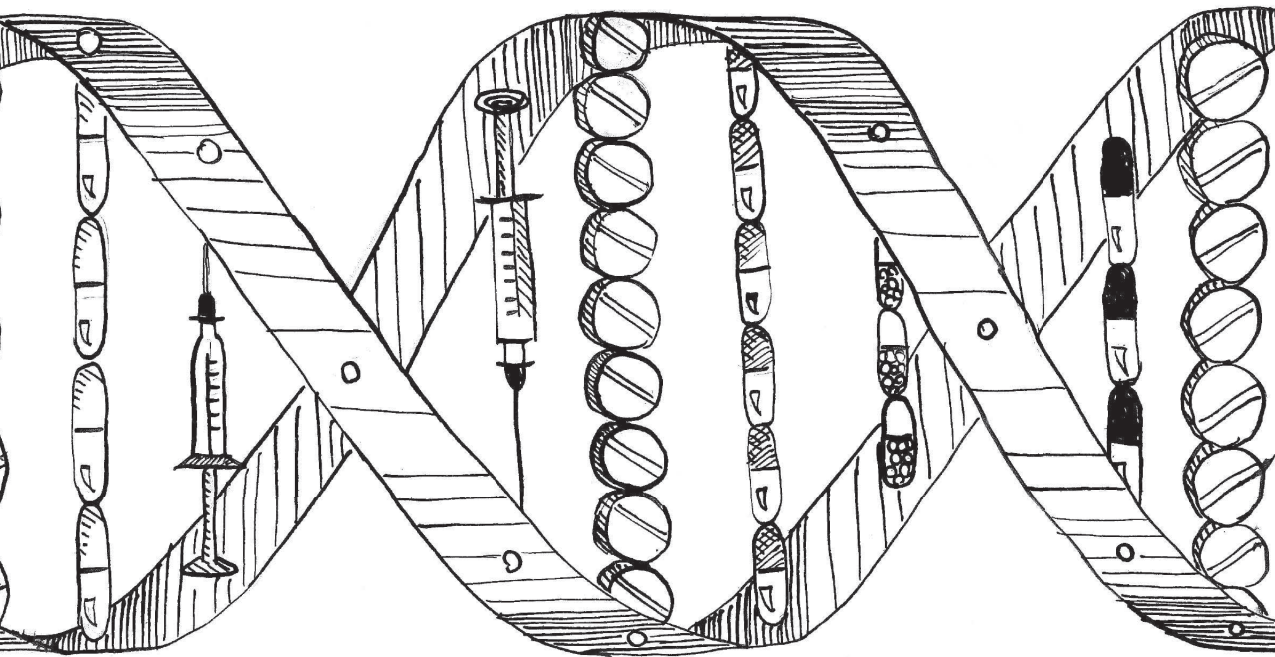
Implementation of PGx in clinical
practice and its impact on a
national level



Chapter 3

GenoChip CYP2D6 macroarray as a method to genotype for *CYP2D6* variants: results of a validation study in a Caucasian population

Bank PCD, Swen JJ, Guchelaar HJ, van der Straaten T



Abstract

The aim of this study was to investigate the concordance between the novel GenoChip CYP2D6 macroarray and the AmpliChip, which is considered the gold-standard in *CYP2D6* genotyping. Germline DNA of 200 patients was genotyped with both the AmpliChip and the GenoChip CYP2D6 macroarray. 198 samples (99%) showed concordance. In two discordant samples the AmpliChip identified a *41 allele while the GenoChip CYP2D6 macroarray did not. Sanger Sequencing showed that the 2988G>A mutation and thus the *41 allele was not present in both samples. We conclude that that the GenoChip CYP2D6 macroarray is a valid method for detecting genetic variants of *CYP2D6* in a Caucasian population.

Introduction

Cytochrome P450 (CYP) 2D6 is a highly polymorphic gene that is involved in the metabolism of 20–25% of the currently marketed drugs (1, 2). Currently more than 80 variants of this gene have been described including single nucleotide polymorphisms (SNPs), insertions and deletions of single or multiple nucleotide(s) and deletion or duplication of the entire gene [<http://www.cypalleles.ki.se/>] (3-5). Based upon the presence of genetic variants in the *CYP2D6* gene, a diplotype can be assigned and translated into extensive metabolizer (EM), intermediate metabolizer (IM), poor metabolizer (PM) or ultra-rapid metabolizer (UM) phenotype to predict the metabolizing capacity of an individual. In 2005 the FDA approved the AmpliChip® (Roche, Indianapolis, US) for clinical use. This platform enables to test for 32 variant alleles of *CYP2D6* and 2 variant alleles of *CYP2C19* and is considered the gold standard in *CYP2D6* genotyping. However, in clinical practice the use of the AmpliChip is limited, potentially due to its relatively high costs. Recently, a macroarray for *CYP2D6* genotyping (GenoChip CYP2D6) was developed by PharmGenomics GmbH (Mainz, Germany) which has a lower cost per test, does not require expensive equipment and is easy to handle. The concept of the macroarray is the formation of a visible pattern on the bottom of the GenoChip CYP2D6 tube using biotin labeled primers, immobilized oligonucleotides and a streptavidin-horseradish peroxidase complex. This pattern then can be captured using a colorimetric camera. The array can detect *3, *4, *6, *7, *8, *9, *10, *11, *17, *29 and *41 alleles using the SNPs rs35742686, rs3892097, rs5030655, rs5030867, rs5030865, rs5030656, rs1065852, rs5030863, rs28371706, rs59421388 and rs28371725 along with deletion (*5) and duplication of the gene. These are the most prevalent alleles with increased, decreased or absent function (6-8). The aim of this study was to investigate the concordance between the GenoChip CYP2D6 macroarray and the AmpliChip.

Materials and methods

DNA samples were obtained from the CYPTAM study (CYPTAM: NTR1509) (9). In brief, the CYPTAM is a prospective multicenter study including 671 female patients with breast cancer in the Netherlands and Belgium. In this study, whole blood samples of 656 patients were collected and DNA was isolated with the Magna Pure system (Roche Diagnostics). The samples were analyzed with the AmpliChip CYP450 array to test for 32 variant alleles of *CYP2D6* and successful genotyping result was produced for 642 patients. The first 200 CYPTAM samples successfully genotyped with the AmpliChip were cross-validated using the PharmGenomics GenoChip CYP2D6 DNA macroarray kit to test for the alleles *3, *4, *6, *7, *8, *9, *10, *11, *17, *29, *41, gene deletion (*5) and gene multiplication according

to the protocol supplied by the manufacturer. Briefly, each DNA sample was amplified using three separate PCR mixes (A, B & C) with biotinylated PCR primers. Mix A was used for amplification of *3, *4, *6, *7, *8, *9, *10, *11, *17, *29, *41. Mix B and C were used for detection of gene deletion (*5) and duplication (*xN), respectively. PCR products were analyzed by gel electrophoresis using a 0.7 % agarose gel to detect the presence of a 4.6 kb fragment in mix A and a 3.5 kb and a 3.2 kb for mix B and C respectively. An aliquot of 3 μ l of each PCR product was then transferred to a single reaction tube containing 60 μ l denaturing mixture and incubated for 2 minutes at 95 °C. Following this step the denatured DNA was transferred to the GenoChip CYP2D6 macroarray where it binds to the oligonucleotide probes immobilized on the bottom of the GenoChip CYP2D6 array tube. By adding a streptavidin-horseradish peroxidase complex and the substrate tetramethyl benzidine a precipitation was formed which resulted in a visible pattern that was captured using a colorimetric camera (see Figure 3.1). Finally, the patterns were analyzed using the software provided by the manufacturer. Diplotypes obtained with the GenoChip CYP2D6 and AmpliChip were converted to genotype predicted phenotypes (gPhenotypes) using the AmpliChip package insert *CYP2D6* genotype to phenotype translation table. The inter-observer difference in the gPhenotypes was analyzed with a Cohen's Kappa analysis using SPSS version 20 (SPSS, Inc., Illinois, USA) and a κ -value larger than 0.95 was considered good concordance. After the primary comparison of the two genotyping methods the DNA of samples with discordant calls were analyzed using Sanger Sequencing along with DNA of positive controls. The DNA of the discordant samples and controls was amplified using 5 pMol forward (5'-GCTGGCTGGCAAGGTCCTA-3') and reverse (5'-GGTGTCCCAGCAAAGTTCA-3') primers using a PCR reaction with 15 minute pre-

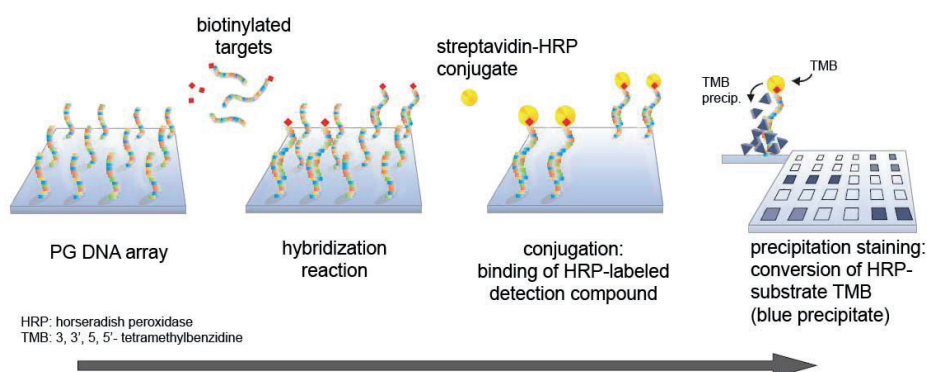


Figure 3.1: A schematic display of the GenoChip CYP2D6.

On the bottom of the GenoChip array oligonucleotides specific to the selection of SNP's are present. During incubation target SNP's are immobilized on the bottom the chip. The hybridized oligonucleotides are labelled using a streptavidin-Horseradish peroxidase conjugate and finally tetramethyl benzidine is added to form a localized pattern. Courtesy of PharmGenomics GmbH.

step of 95 °C, 40 cycles of 95 °C - 55 °C - 72 °C of 30 seconds each, and post step of 10 minutes at 72 °C. 1 µl of each of the PCR products were Sanger sequenced using 10 pMol of forward primer. Data was analyzed using Chromas Software (Technelysium, Brisbane, Australia).

Results

Genotype comparison

The call rate of both the AmpliChip and the GenoChip CYP2D6 was 100%. The concordance between the 200 samples tested with the GenoChip CYP2D6 and AmpliChip was 99 % for the 13 tested *CYP2D6* variants. Results for the alleles *3, *4, *6, *7, *8, *9, *10, *17, *29, gene-deletion (*5) and gene-duplications were 100% concordant. The AmpliChip identified *2 (n = 61) and *35 (n = 18) alleles that are not included in the tested alleles on the GenoChip CYP2D6 array (both alleles are defined as fully functional alleles). A discordance was observed for the *41 allele. The GenoChip CYP2D6 identified 34 *41 alleles compared to 36 *41 alleles identified by the AmpliChip (see Table 3.1). In two samples (3002 and 8008) the AmpliChip identified the *41 allele where the GenoChip CYP2D6 did not, resulting in *29/*41 and *1xN/*41 compared to *1/*29 and *1/*1xN diplotypes (see Table 3.1), respectively.

Comparison of genotype predicted CYP2D6 phenotypes

Based on the used translation table 160 EMs, 21 IMs, 17 PMs and 2 UM were identified by the AmpliChip. In 6 patients a duplication of the *CYP2D6* gene was observed resulting in 2 patients with a genotype predicted Phenotype (gPhenotype) of UM (*1/*1xN and *1/*35xN). Three patients with a duplication with diplotypes of *1xN/*4 (n = 2) and *1xN/*41 were classified as EM and one patient with a *4/*4xN diplotype was classified as PM. A deletion of *CYP2D6* was observed in 26 patients resulting in 14 patients with EM gPhenotype, 3 patients with IM gPhenotype and 9 patients with PM gPhenotype. The translation of the called diplotypes by the GenoChip CYP2D6 using the *CYP2D6* genotype to phenotype translation table resulted in 160 EMs, 20 IMs, 17 PMs, 3 UMs. In two samples (sample 1035 and 5002) the array identified a “*4 mutation heterozygous” and a “gene duplication”. This result could not be interpreted unequivocally and could represent both *1xN/*4 or *1/*4xN diplotype. However, both diplotypes translate into the extensive metabolizer category according to the used translation table. For patient 3002 genotyping with the GenoChip CYP2D6 array resulted in an EM gPhenotype instead of an IM gPhenotype, while for patient 8008 the gPhenotype EM changed to UM. The discordant

Table 3.1: Genotyping results of both methods

Diplotype interpreted with GenoChip CYP2D6 results	Frequency	Genotype Predicted Phenotype	Diplotype detected with AmpliChip	Frequency	Total
*1/*1	60	EM	*1/*1	27	60
		EM	*1/*2	20	
		EM	*1/*35	8	
		EM	*2/*2	3	
		EM	*2/*35	2	
*1/*1xN	3 ^a	UM	*1/*1xN	1	2 ^a
		UM	*1/*35xN	1	
*1xN/*41 or *1/*41xN	0 ^a	EM	*1xN/*41	1 ^a	
*1/*3	2	EM	*1/*3	1	2
		EM	*2/*3	1	
*1/*4	51	EM	*1/*4	31	51
		EM	*2/*4	18	
		EM	*4/*35	2	
*1xN/*4 or *1/*4xN	2	EM	*1xN/*4	2	
*1/*5	14	EM	*1/*5	8	14
		EM	*2/*5	4	
		EM	*5/*35	2	
*1/*6	2	EM	*1/*6	1	2
		EM	*2/*6	1	
*1/*9	3	EM	*2/*9	2	3
		EM	*9/*35	1	
*1/*10	3	EM	*1/*10	3	
*1/*17	1	EM	*1/*17	1	
*1/*29	1 ^a	EM	*1/*29	0 ^a	
*1/*41	21	EM	*1/*41	12	21
		EM	*2/*41	7	
		EM	*35/*41	2	
*3/*3	1	PM	*3/*3	1	
*4/*4	5	PM	*4/*4	5	
*4/*4xN	1	PM	*4/*4xN	1	
*4/*5	9	PM	*4/*5	9	
*4/*6	1	PM	*4/*6	1	
*4/*9	4	IM	*4/*9	4	
*4/*10	2	PM	*4/*10	2	
*4/*41	7	IM	*4/*41	7	
*5/*9	1	PM	*5/*9	1	
*5/*41	2	IM	*5/*41	2	
*9/*41	2	IM	*9/*41	2	
*10/*10	1	IM	*10/*10	1	
*29/*41	0 ^a	IM	*29/*41	1 ^a	
*41/*41	1	IM	*41/*41	1	
	200		Total	200	

The genotyping results of the AmpliChip containing *2 and *35 alleles are clustered with *1 alleles as the GenoChip CYP2D6 does not call the *2 and *35 variants. ^aDifferences in genotyping results excluding *2 and *35 allele.

diplotypes of these two samples and the associated change in gPhenotypes led to the change in the distribution of 160 EMs, 21 IMs, 17 PMs and 2 UMs that were interpreted from the *CYP2D6* diplotypes obtained with the AmpliChip to 160 EMs, 20 IMs, 17 PMs and 3 UMs were interpreted from results of the GenoChip (see also Table 3.1). The Cohen's Kappa analysis of the gPhenotypes of 200 patients showed a high measure of concordance ($\kappa = 0.971$, $p < 0.001$) between the two genotyping methods.

Sanger Sequencing

The Sanger Sequencing results of the two discordant samples showed a homozygous GG (homozygous wildtype) at position 2988 for both samples 3002 and 8008 (see Figure 3.2). The three selected control samples showed 2850CC/2988GG, 2850CT/2988GA and 2850TT/2988AA in the Sanger Sequencing analysis (see Figure 3.3). Based on the results from this analysis it can be concluded that the 2988G>A variation was not present in both samples and these two patients did not carry the *41 allele.

Discussion

The GenoChip CYP2D6 showed a high degree of concordance with the AmpliChip in *CYP2D6* genotyping. Of the 200 tested samples, a difference in alleles called by both methods was only seen in two samples both with discordance in calling the *41 allele. However, Sanger sequencing confirmed the results obtained by the GenoChip CYP2D6 that the *41 allele is not present. The Cohen's Kappa analysis of the gPhenotypes showed a high degree in concordance.

Although there are multiple techniques for *CYP2D6* genotyping available, the analysis of this gene remains complex, as exemplified by a number of reports where a genetic analysis did not produce a result that was usable in the clinical setting (10-12). In line with the findings from this study, problems with *41 genotyping with the AmpliChip have also been reported previously. In a study of 107 genomic DNA samples genotyped for five loci 12 discordant *CYP2D6* diplotypes were observed of which two were identified as *41 allele by the AmpliChip, while the other methods did not (13). In a study of 83 samples comparing the AmpliChip with a Long Range PCR platform combined with Sanger sequencing it was observed that in 9 discordant samples *2 alleles were miscalled as *41 alleles by the AmpliChip (14).

These erroneous results of the *41 allele can be explained by the panel that is interrogated for the *41 allele and the change in definition of the *41 allele over time. The AmpliChip

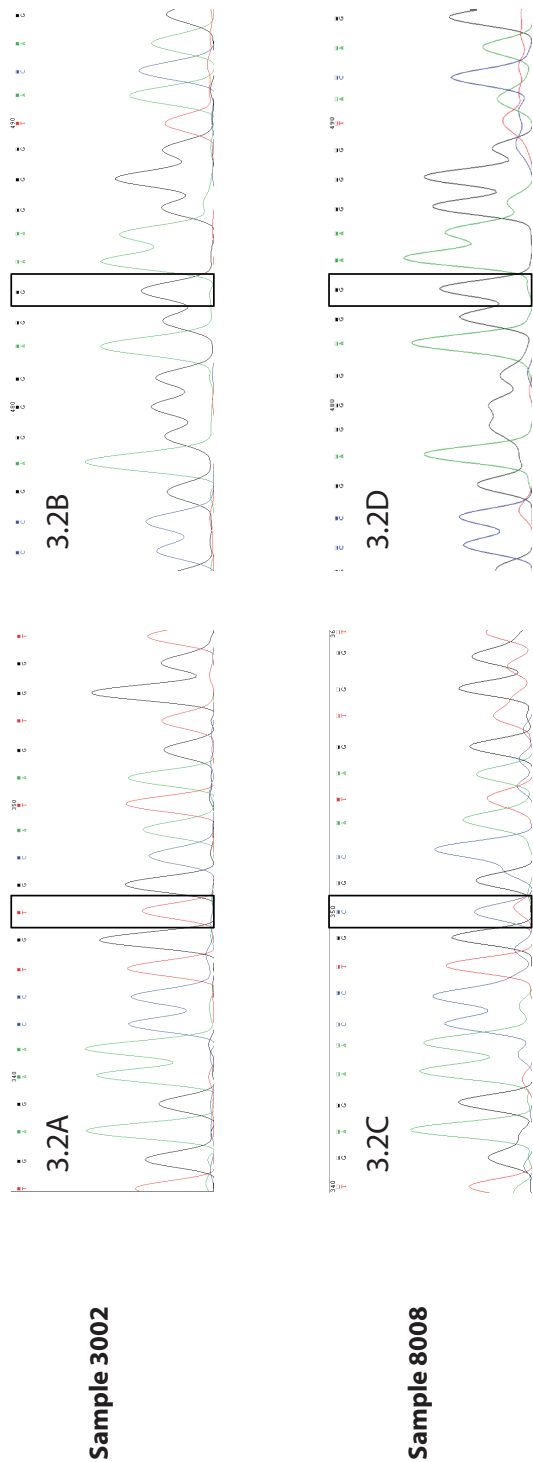


Figure 3.2: Panel of Sanger sequence results of patients 3002 & 8008.

Panel 3.2A and 3.2C represent the sequenced area around nucleotide 2850 to detect the 2850C>T SNP, while panel 3.2B and 3.2D represent the sequenced area around nucleotide 2988 to detect the 2988G>A SNP. As shown in panel 3.2A and 3.2B patient 3002 carries a 2850 TT and 2988 GG, while patient 8008 is carrier of 2850 CT and 2988GG as shown in 3.2C and 3.2D respectively.

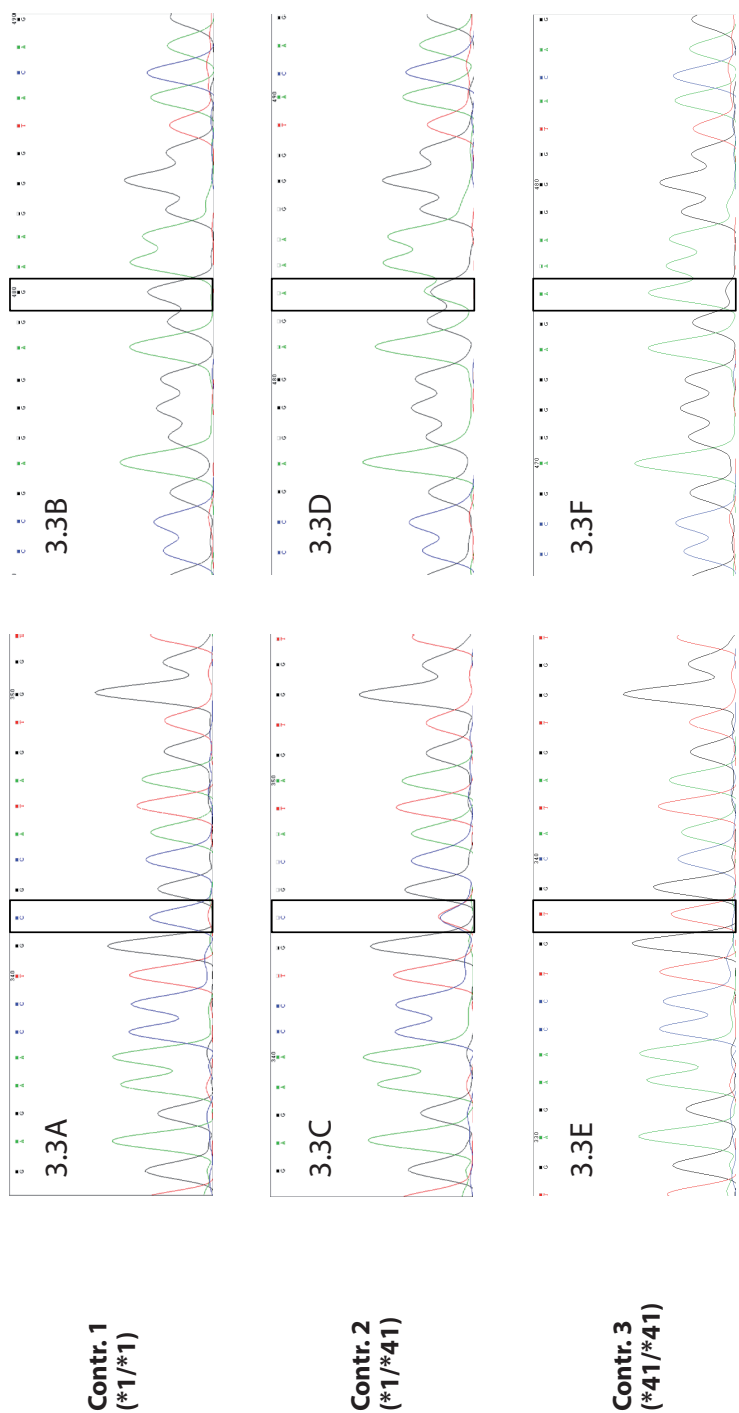


Figure 3.3: Panel of Sanger sequence results of controls 1, 2, 3.
Panel 3.3A, 3.3C and 3.3E represent the sequenced area around nucleotide 2850 to detect the 2850C>T SNP, while panel 3.3B, 3.3D, 3.3F represent the sequenced area around nucleotide 2988 to detect the 2988G>A SNP. As shown in panel 3.3A and 3.3B control 1 with diplotype *1/*1 carries the 2850 CC and 2988 GG variant, control 2 with diplotype *1/*41 carries the 2850 CT and 2988 GA variant as shown in 3.3C and 3.3D respectively and control 3 with diplotype *41/*41 carries the 2850 TT and 2988 AA variant as shown in 3.3E and 3.3F respectively.

uses a combination of the presence of the 1661G>C, 2850C>T and 4180G>C SNPs and the absence of the -1584C>G SNP, while the GenoChip CYP2D6 calls the *41 allele solely based on the 2988G>A SNP. This SNP was first reported in 2004 (after the completion of the development of the AmpliChip) and is currently considered the most indicative SNP for the *41 allele by The Human Cytochrome P450 Allele Nomenclature Committee [<http://www.cypalleles.ki.se/>] (3, 7). The haplotypes which were previously genotyped as *41 and lacked the 2988G>A SNP were then reclassified as *2L and *2M which both share the -1584C, 2850C>T, 1661G>C and 4180G>C with the *41 haplotype (3, 15, 16). As observed in the Sanger sequencing results of the two discordant samples and the selected controls the 2988G>A SNP is not present in samples 3002 and 8008, which justified the genotyping result of the GenoChip CYP2D6 in these two samples (see Figure 3.2 and 3.3).

In a clinical setting the observed difference in genotyping result can affect drug therapy. Using the translation table provided in the package insert of the AmpliChip the difference in called diplotypes would lead to alternate gPhenotypes for patient 3002 (IM instead of EM) and 8008 (EM instead of UM) and as results these two patients would receive a different dose or drug (e.g. nortriptyline) based on the results of the AmpliChip or GenoChip CYP2D6 respectively (17, 18).

A limitation of this study was that a direct comparison of the genotyping results reported by both methods was not possible. The first reason hindering a direct comparison of the diplotypes is that the GenoChip CYP2D6 reports if a variant allele is present or not and does not provide a diplotype like the AmpliChip. In 1.0 % of the GenoChip results, the diplotype could not be unequivocally interpreted. For example, the result “heterozygous *4 variant detected” and “duplicated allele detected” can be interpreted as both the *1xN/*4 or the *1/*4xN diplotype (see Table 3.1). The *1xN/*4 diplotype can be translated into the gPhenotypes in EM in patients with a duplicate of *1 and UM in case of patients with 3 or more copies of *1. Depending on the applied translation table the *1/*4xN diplotype can also be interpreted in multiple gPhenotypes. Using the Dutch consensus on interpretation of CYP2D6 diplotypes the *1/*4xN would be interpreted as IM, while the translation table provided in the package insert of the AmpliChip would result in EM (18).

A second reason impeding a direct comparison of the genotyping results is the difference in the panel of alleles tested by both methods. The AmpliChip platform tests for the *2 and *35 alleles that have no effect on CYP2D6 enzyme activity and have no clinical consequences, whereas the GenoChip CYP2D6 does not test for these variants. Although the gPhenotype calls for individuals harboring the *2 or *35 allele are similar between the methods, a direct comparison of the diplotypes is not possible.

To perform the statistical analysis both obtained *CYP2D6* diplotypes were translated to gPhenotypes using the translation table provided in the package insert of the AmpliChip. This translation from diplotype to gPhenotypes could potentially mask differences between samples with a discordant diplotype. For example if a patient would be classified as $*1/*1$ by one method and as $*1/*10$ by the other the gPhenotype would still be EM in both cases. However, in the case of the miscalled $*41$ allele by the AmpliChip a difference in gPhenotype was observed for patients 3002 and 8008. If chosen for the *CYP2D6* genotype to phenotype translation table by Hicks et al., where patient carrying two decreased functional alleles (e.g. $*10/*10$ or $*29/*41$) would still be classified as EM, this would lead to the gPhenotype distribution of 160 EM's, 16 IM's, 17 PM's, 3 UM's and 2 unknown genotypes for both methods, masking the found difference in diplotype between patient 3002 and 8008 (17).

Although there are already many genotyping techniques available for *CYP2D6*, this study is the first report to show a direct comparison between a macroarray and the AmpliChip in a Caucasian population. The advantages of the GenoChip CYP2D6 are that the array does not require expensive equipment, has a relatively low cost per test and is easy to handle. The array tests for the most prevalent *CYP2D6* alleles with increased, decreased or absent function (6-8). However, it could be improved by adding a manner to specifically detect which allele is duplicated. In individuals who are carriers of a variant allele and a duplication of an allele, the interpretation of the results of the GenoChip CYP2D6 can lead to multiple diplotypes (e.g. $*1xN/*4$ or $*1/*4xN$).

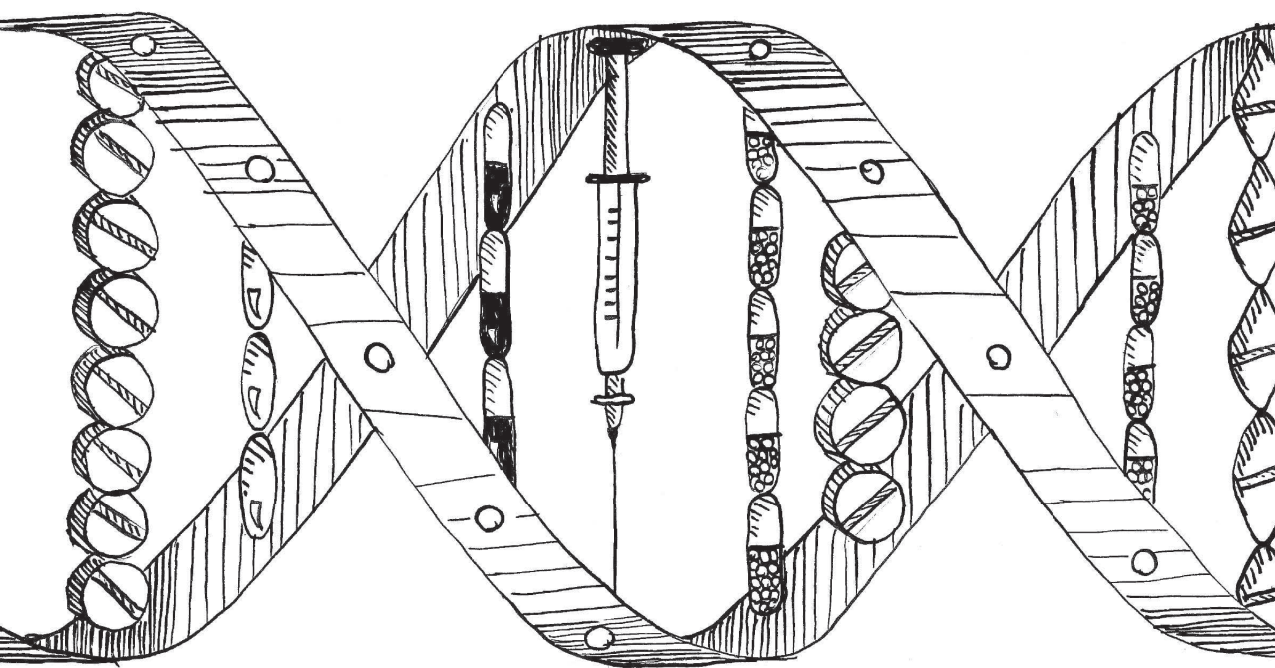
Conclusion

Based on this comparison it can be concluded that the GenoChip CYP2D6 is a valid method for detecting genetic variants of *CYP2D6* in a Caucasian population.

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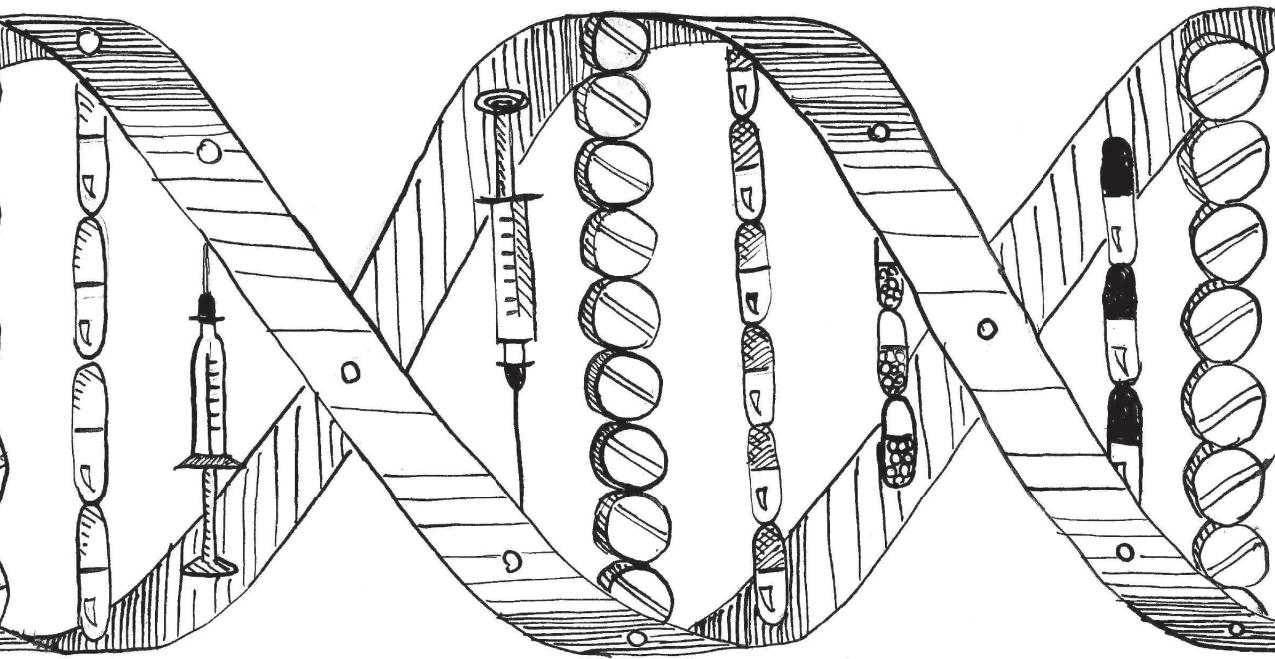
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Chapter 4

Implementation of pharmacist initiated pre-emptive pharmacogenetic testing in primary care

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Submitted for publication

Abstract

Despite the nationwide availability of pharmacogenetic (PGx) guidelines in electronic medication surveillance systems in The Netherlands, PGx guided prescribing is still uncommon in primary care. We set out to investigate the adoption of pharmacist initiated PGx testing in primary care. Community pharmacists were offered a free PGx test covering 40 variants in 8 genes to test patients receiving an incident prescription (iRx) of a selection of 10 drugs. Results of the PGx test along with predicted phenotypes and a therapeutic recommendation based on the Dutch Pharmacogenetics Working Group (DPWG) guidelines were transferred to the pharmacist and physician. Adoption was defined as the percentage of eligible patients that received genotyping. From November 2014 – July 2016, 200 patients were included with an adoption of 18.0%. Of the included patients 57.5% received an iRx for atorvastatin, 14.5% started with simvastatin and 28.0% received an incident prescription for amitriptyline, (es)citalopram, nortriptyline, or venlafaxine. 90% of the patients carried at least one actionable PGx test result in the selected PGx-panel. In 31.0% of the incident prescriptions a combination between a drug with a known gene-drug interaction and an actionable genotype was present and a therapeutic recommendation was provided. The provided recommendations were accepted by the clinicians in 88.7% of the patients. Pharmacist initiated implementation of PGx in primary care is feasible, and the frequency of actionable gene-drug interactions for the selected drugs is high.

Introduction

In recent years many of the hurdles hindering the successful application of pharmacogenetics (PGx) have been addressed (1, 2). The Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) both have published a range of guidelines on relevant gene-drug interactions providing PGx-test interpretation and therapeutic recommendations for patients with a known genotype (2-4).

Several pharmacogenetic implementation projects have been initiated in specialized and secondary care centers. Results have not only shown the feasibility of clinical pharmacogenetics but also highlighted the high frequency of clinically actionable genotypes in the general population underscoring the potential for the use PGx to improve pharmacotherapy (5-11). Currently, PGx testing is not routinely implemented in primary care (12). However, studies have shown that of the drugs with a known gene-drug interaction 25 are among the most frequently used medications in the USA by patients in a home setting. Examples of these drug include statins, TCA's, SSRI and SNRI's (13). Therefore, PGx-informed decision making has a high potential to lead to optimized pharmacotherapy in primary care. Indeed, preliminary studies in primary care setting have shown that PGx informed prescribing leads to a reduction in hospitalization, appears to be cost-effective and that gene-drug interactions may appear to be independent risk for hospitalization (14-17).

In the Netherlands the infrastructure for using PGx information to guide pharmacotherapy is well developed. The DPWG guidelines are fully integrated into available clinical decision support (CDS) nationwide through the G-standard (<https://www.knmp.nl/producten-en-diensten/gebruiksrecht-g-standaard/informatie-over-de-g-standaard/the-gstandaard-the-medicines-standard-in-healthcare>), and readily available as pop-up alerts (see Figure 4.1) during drug prescribing and dispensing (3, 4). With their dedication to safe and effective pharmacotherapy pharmacists are leading candidates to act on a patients' genotype. Patients also appear to be positive towards PGx-testing initiated within a community pharmacy setting (18). Based on the availability of PGx guidelines integrated in the workflow of healthcare professionals and the positive attitude to pharmacy delivered PGx we set out to investigate whether genotype guided dosing in primary care using a PGx test panel in the Netherlands is feasible. Additionally, we wanted to investigate the frequency of actionable genotypes in a panel of the genes *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* and *VKORC1* and to study the frequency of gene-drug interactions in primary care.

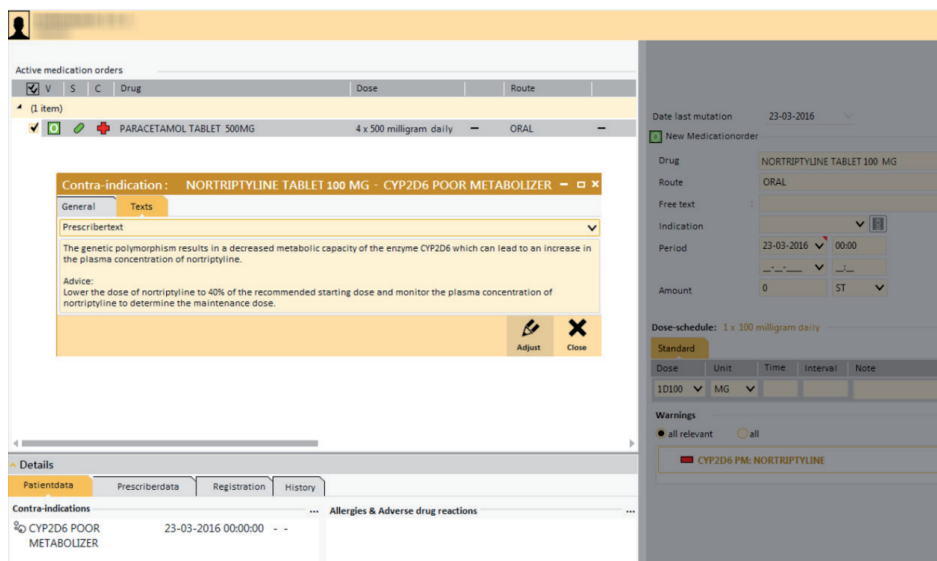


Figure 4.1: Example of an alert generated through Clinical Decision Support.

Methods

Study design and setting

The Implementation of Pharmacogenetics into Primary care Project (IP3) was designed as a prospective multicenter observational study of routine clinical practice with the objective to test the feasibility of pharmacist initiated pharmacogenetics testing in primary care. Secondary aims were to investigate the frequency of actionable phenotypes, the frequency of incident prescriptions with a combination between a drug with a known gene-drug interaction and the associated actionable genotype, and the effects of gene-drug interactions on pharmacotherapy in both the period of empirical treatment prior to inclusion (i.e. without the knowledge of the patients' genetic make-up) and during genotype-guided dosing in separate analyses. The central Medical Ethics Review board of the Leiden University Medical Center (LUMC) in Leiden, The Netherlands approved the protocol.

Endpoints

The primary endpoint was the adoption rate of PGx testing. This was defined as the number of genotyped patients divided by the total number of eligible patients in the timeframe of the study. Secondary endpoints included the frequency of actionable genotypes, the number of patients with a combination of an actionable genotype in combination with

a drug with a known gene-drug interaction, the number of drug related interventions (defined as stopping of the medication, switching to another drug, switching to a different dose), the number of visits to the pharmacy, and the drug dose. The medication history of the included patients was collected from the electronic patient records in the pharmacy. In order to compare drug dosages of different drugs defined daily dosages (DDD) were used. To account for changing dosages over time, a time-weighted average was calculated for each of the drugs of inclusion.

To study the effects of genotype guided dosing on the secondary endpoints follow-up on all secondary outcomes was prospectively collected from the point of inclusion until the end of the study in which general practitioner and pharmacist had knowledge of actionable PGx test results. In a similar analysis the effects of actionable genotypes on secondary outcomes during the period where individuals had been treated empirically by general practitioners and pharmacists without the knowledge of the patients' genotype was studied. The follow-up concerning the secondary endpoints was collected prospectively for the period in which general practitioner and pharmacist had knowledge of actionable PGx test results. For the retrospective analysis the DDD, number of visits to the pharmacy and drug related interventions were collected. Patients (see below) were identified through weekly searches in the electronic drug dispensing systems. Treating pharmacists informed eligible patients about the project. When the patient agreed to participate, written informed consent was obtained and a saliva sample for DNA extraction was collected.

Patients

Adult (18 years or older) patients with an incident prescription (defined as no prescription for the incident drug within the preceding 12 months) for at least 28 days for amitriptyline, atomoxetine, atorvastatin, (es)citalopram, clomipramine, doxepin, nortriptyline, simvastatin or venlafaxine in participating primary healthcare centers in the vicinity of Leiden were invited to participate in the study and offered free genetic testing. To investigate the impact of PGx informed prescribing historical data were used. Therefore, patients needed to have at least one prescription for one of the 10 selected drugs for a period of at least 28 days other than the incident prescription in the preceding year. After identification of the patients through automated queries the participating pharmacists manually checked whether patients fulfilled the in- and exclusion criteria. Finally, patients not recruited within 14 days after dispensing the incident prescription were excluded.

Genotyping

Saliva was collected using the Oragene DNA OG-250 (DNA Genotek Inc, Ottawa, Ontario, Canada) and sent to the department of Clinical Pharmacy & Toxicology at the LUMC. DNA was isolated according to the protocol of the manufacturer. Genotypes of *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* and *VKORC1* (see Supplementary Document S4.1) were determined using the Drug Metabolizing and Transporters (DMET) Plus Array (Affymetrix, Santa Clara, CA). *CYP2D6* copy-number variation was detected with qPCR (Thermo Fisher Scientific, Massachusetts, USA). The DMET test was further supplemented with the *DPYD* 1236G>A and 2846A>T variants which were routine tests in the LUMC at the time of study execution (19). The genotypes of the 8 genes in the pharmacogenetic profile were translated into genotype predicted phenotypes using the DPWG guidelines. Finally, a report for the general practitioner and pharmacists was generated which contained the genotypes, the genetic predicted phenotypes along with a therapeutic recommendation to continue current therapy in case of a wildtype genotype or to adjust pharmacotherapy in case of a combination between an actionable genotype and the prescribed drug. The report was transferred to the treating clinicians with the request to record the genetic profile in automated electronic drug prescribing and dispensing systems for active medication surveillance based on the patients' genotype.

Statistical analysis

Primary endpoint

The primary endpoint of this study was the extent of adoption of PGx-testing expressed as the percentage of eligible patients that received genotyping in a percentage.

Secondary endpoints

To explore whether gene-drug interactions resulted in a difference in the time-weighted DDD's a multinomial generalized estimating equation (GEE) was carried out using the categorized DDD's from the prescribed drug at inclusion (amitriptyline, atomoxetine, atorvastatin, (es)citalopram, clomipramine, doxepin, nortriptyline, simvastatin or venlafaxine) as dependent variable, and the occurrence of a gene-drug interaction as an independent variable. The GEE model was chosen because of the hierarchical structure in the data for the prospective collected DDD's of the drug prescribed at the time of inclusion with genotyped guided dosing. In addition, a multilevel log-linear Poisson analysis was carried out to determine whether a relationship was present between the actionable phenotype and number of switches in dose after inclusion. Furthermore, a multilevel log-

linear Poisson analysis was carried out to determine between actionable phenotype and the number of visits to the pharmacy in the prospective collected follow-up.

In three separate analyses, the retrospectively collected follow-up from the period before inclusion was analyzed in a similar manner. The categorized DDD's from one of the ten selected drugs used before inclusion were analyzed using a multinomial GEE with the categorized DDD's as dependent variable and presence of a gene-drug interaction as an independent variable. Multilevel log-linear Poisson analyses were carried out to analyze the effect gene-drug interactions on the number of switches in dose applied and the number of visits before inclusion. Sensitivity analyses for all the multilevel models were performed to investigate whether factors such as gender, age, weight, height and automated dose dispensing were confounding factors. Model specifications included an exchangeable correlation structure and a robust estimator. In all models the patient was used as the lowest level clustered within prescribing physician.

Results

Characterization of included patients & incident prescriptions

In total 200 patients were included in this study. The characteristics of the patients are presented in Table 4.1. The included study population contained 103 (51.5%) females and 97 (48.5%) males with mean age was 62 ± 11 years ranging from 27 up to 87. The majority of patients (92.5%) reported that both parents were of Caucasian descent (see Table 4.1). The majority of the patients received an incident prescription for a statin i.e. atorvastatin ($n = 115$) or simvastatin ($n = 29$). The remainder of the patients were included on amitriptyline ($n = 15$), citalopram ($n = 7$), escitalopram ($n = 3$), nortriptyline ($n = 17$) or venlafaxine ($n = 14$) (see Table 4.1).

Adoption of PGx

The adoption of PGx testing by healthcare professionals (primary endpoint) was 18.0% (see also Figure 4.2). In the 20 month recruitment period, 1,864 patients with an incident prescription for one of the 10 drugs and historical use of one of the other nine drugs were identified. Of those patients 483 were not eligible for inclusion. Reasons included duration of prescription < 28 days, use of the incident drug in the preceding 12 months, no use of one of the other nine selected drugs in the previous year, previously included in the study, or was too young (< 18 years). Furthermore 267 patients were not considered eligible by the pharmacists. Reasons included palliative treatment, no permanent address, not capable of

Table 4.1: An overview of the characteristics of the included patients

Gender (M / F)	97 / 103
Age (year)	
Mean (Std. error)	62 ± 11
Range	27–87
Length (cm)	
Mean (Std. error)	172 ± 10
Weight (kg)	
Mean (Std. error)	81.6 ± 17
Reported ethnicities of both parents (%)	
Both Caucasian descent	92.5
Both Asian descent	3.0
Both Arabic descent	1.5
Both Somali descent	0.5
Mixed descent	2.5
Medication prescribed on incident prescription (%)	
Amitriptyline	7.5
Atorvastatin	57.5
Citalopram	3.5
Escitalopram	1.5
Nortriptyline	8.5
Simvastatin	14.5
Venlafaxine	7.0
Medication in history (%)	
Amitriptyline	8.5
Atorvastatin	8.5
Citalopram	9.5
Clomipramine	1.5
Escitalopram	1.5
Nortriptyline	2.0
Simvastatin	63.0
Venlafaxine	5.5

providing written informed consent.. Of the 1,114 eligible patients 652 were not contacted by the pharmacist within two weeks after the patient picked up the incident prescription. Of the 462 patients that were contacted 207 consented to the study, whereas 255 patients did not agree to participate. Of the 207 patients that agreed to participate in the study 7 were excluded because they did not sign informed consent, failed to provide a saliva sample or the saliva contained too little DNA and the patient refused to provide a new sample.

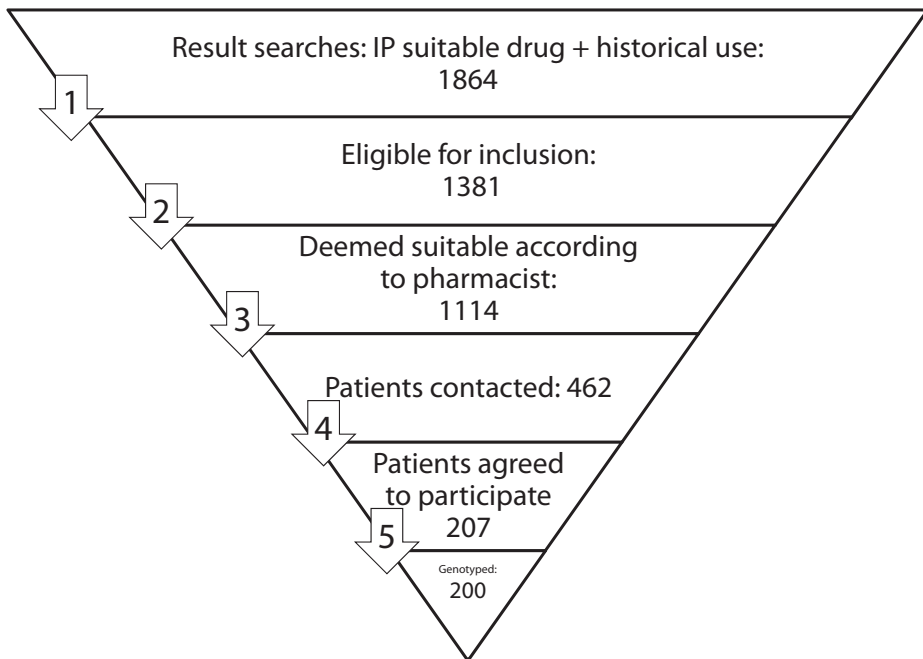


Figure 4.2: Overview of patient flow.

Step 1 – Not eligible: identified prescription too short (n = 5), incident prescription too short (n = 469), no historical use of other drug (n = 5), already included or asked (n = 3), < 18 years of age (n = 1).

Step 2 – Not deemed suitable by pharmacists: Mediol (automated dosing dispensing system) (n = 79), patient not capable of providing informed consent (n = 8), patient did not have a known home address (n = 2), patient received end-of-life care (n = 2), unknown (n = 176).

Step 3 – Not contacted: patient was missed (n = 429), pharmacists were too busy (n = 29), patient unreachable (n = 26), pharmacists on vacation (n = 16), unknown (n = 152).

Step 4 – Patient did not agree to participate (n = 255).

Step 5 – Not included: patient not show up on intake (n = 1), informed consent form not signed (n = 1), patient posted sample after six months (n = 1), index drug not visible anymore in CDS (n = 1), patient refused request for 2nd sample (n = 3).

Occurrence of actionable genotypes & gene-drug interactions

Ninety percent of the genotyped patients carried at least one actionable genotype with one and two actionable genotypes per patient being the most frequent with an occurrence of 28.5% and 32.5% respectively. The remainder of the patients carried up to five actionable genotypes out of the panel of eight tested genes (See Table 4.2). The frequencies of different genotype predicted phenotypes for each of the eight genes are presented in Table 4.3. For 31.0% of the patients a combination between a drug with a gene-drug interaction and actionable genotype was present requiring action from the healthcare professionals. The majority of the combinations of drugs with known gene-drug interactions and genotypes consisted of a gene-drug pair between the *SLCO1B1* gene with the drug atorvastatin (n =

Table 4.2: Overview of the number of actionable phenotypes found per patient (%)

0	10.0
1	28.5
2	32.5
3	19.5
4	7.5
5	2.0

Table 4.3: Overview of the frequencies of phenotypes sorted to gene (%)

<i>CYP2C9</i>	
Extensive metabolizer	62.5
Intermediate metabolizer	35.5
Poor metabolizer	2.0
<i>CYP2C19</i>	
Extensive metabolizer	71.5
Intermediate metabolizer	21.5
Poor metabolizer	3.0
Ultra-rapid metabolizer	4.0
<i>CYP2D6</i>	
Extensive metabolizer	53.0
Intermediate metabolizer	40.5
Poor metabolizer	5.0
Ultra-rapid metabolizer	1.5
<i>CYP3A5</i>	
Non-expressor	85.0
Heterozygous expressor	14.5
Homozygous expressor	0.5
<i>DPYD (GAS)</i>	
2.0	93.0
1.5	5.5
1.0	1.5
<i>SLCO1B1</i>	
521TT	74.5
521TC	23.5
521CC	2.0
<i>TPMT</i>	
Extensive metabolizer	84.5
Intermediate metabolizer	15.0
Poor metabolizer	0.5
<i>VKORC1</i>	
1173CC	33.0
1173CT	50.5
1173TT	16.5

35). The frequencies of other combinations were *CYP2C19* with (es)citalopram ($n = 3$), *CYP2D6* with amitriptyline ($n = 6$), *CYP2D6* with nortriptyline ($n = 7$), *CYP2D6* with venlafaxine ($n = 8$) and *SLCO1B1* with simvastatin ($n = 3$). Patients with combination of an actionable genotype and associated drug were not significantly different from patients without this gene-drug pair regarding gender, age, length and weight. In an exploratory analysis medication surveillance based on a pharmacogenetic profile did not result in a significant change in time weighed DDD's (OR 1.14, CI 0.68–1.92, $p = 0.614$), interventions performed on the medication (OR 1.33, CI 0.92–1.93, $p = 0.128$) or the number of visits to the pharmacy (OR = 0.93, CI 0.63–1.36, $p = 0.695$). Medication surveillance based on genomic information resulted in 32 additional alerts for drugs other than the drugs of inclusion in 25 patients during the follow-up period.

Medication use during genotype-guided dosing & guideline adherence

An average follow-up of 15.6 months (ranging from 6 to 25 months) of medication use with electronic medication surveillance using genetic predicted phenotypes from the determined panel was prospectively collected. The mean treatment period for the drug of inclusion was 357 days $\pm$ 221 (defined as the period between the projected end date of the last prescription and the start date). In 40.5% of the patients the medication of inclusion was stopped before the end of the follow-up period which was higher but not significantly different between patients with and without a gene-drug interaction combination between an actionable genotype and associated drug (48.4% vs. 37.0%, $p = 0.128$). Follow-up on the patients with such as gene-drug pair upon inclusion showed that in 55 (89%) of the cases with a relevant combination of an actionable genotype with a relevant drug the healthcare professionals adhered to the DPWG guidelines by switching the patient to another drug, adjusting the dose, guarded a maximum dose or performed additional monitoring as instructed per the DPWG guidelines. In 1 case (1.6%) the healthcare professionals did not adhere to the guidelines which was motivated by the fact that patient had tolerated the treatment in the period between inclusion and the moment where the genetic profile in combination with the therapeutic recommendations was provided to the healthcare professionals. In 5 cases (7.8%) the gene-drug pair between *CYP2D6* and venlafaxine was present while treatment continued and 1 case (1.6%) the dose-maximum for escitalopram as recommended by the DPWG was surpassed.

Impact of gene-drug interactions on pharmacotherapy in empirical dosing

One of the goals of this study was to investigate the effect of gene-drug interactions on pharmacotherapy during the period where healthcare professionals did not have access to the patients' pharmacogenetic profile. To study this research question patients were

required to have received at least one prescription of one of the ten selected drugs in the year before inclusion (other than the drug of the incident prescription). The majority of patients (63.0%) had received a prescription for simvastatin before inclusion in the study which in 108 patients was stopped for a switch to atorvastatin. 57 patients received a prescription for an antidepressant in the year preceding inclusion, most frequently citalopram ($n = 19$). In 33 cases the drug was still prescribed during follow-up (for example a patient who was included because of an incident prescription for an antidepressant continued statin treatment for hypercholesterolemia). In the retrospective analyses of the medication use in the period during empirically dosing the occurrence of gene-drug interactions did not lead to a significant difference in time weighted DDD's (OR = 0.67, CI 0.35–1.29, $p = 0.235$), interventions (OR = 1.27, CI 0.93–1.75, $p = 0.133$) or visits to pharmacies (OR = 1.08, CI 0.64–1.83 $p = 0.764$).

Discussion

This study demonstrates that genotype guided dosing in primary care is feasible. The IP3 project allowed healthcare professionals to gain experience with individualizing pharmacotherapy based on genetic variation in the setting of their own practice. During this implementation study healthcare professionals adopted PGx testing in 18.0% of all eligible patients. The results of this study further show that actionable genotypes in the tested panel of eight genes are quite common in our group of primary care patients. The majority of patient's carries at least one actionable genotype. Moreover, in 31% of the patients a combination of drug with a known gene-drug interaction with an actionable genotype was present at the time of inclusion.

Overall, the IP3 study is the first studies outside the US to evaluate a PGx panel approach to guide individualized pharmacotherapy using CDS in a primary care setting. Similar initiatives implementing PGx panel testing in a primary care setting in the US have been reported by research groups including Brixner et al., Elliot et al., Finkelstein et al. and Perez et al. (14–17). In the study performed by Brixner et al. the effect of panel-based genomic screening with 6 genes on the health resource utilization after starting pharmacotherapy with a selection of 61 drugs was studied. The research team compared a cohort of 205 patients who received pharmacogenetic screening with 820 untested patients. Results showed that the screened cohort had a lower rate of hospitalizations (relative risk (RR) of 0.61, 95% confidence interval (CI) = 0.39–0.95, $p = 0.027$), a lower rate of visits to the emergency department (RR = 0.29, 95% CI = 0.15–0.55, $p < 0.001$) and a higher rate of visits to the outpatient clinic (RR = 1.97, 95% CI = 1.74–2.23, $p < 0.001$). With this

decrease in hospitalizations and visits to emergency departments the authors concluded that prescribing using genomic information could potentially lead to cost-savings (14). Elliot et al. showed in a prospective, open-label, randomized clinical trial in a hospital-based home health agency that a pharmacogenetic screening in patients aged 50 years or older results in a lower rate of re-hospitalizations per patient at 60 days (RR = 0.48, 95% CI = 0.27–0.82, $p = 0.007$) and a lower rate of emergency visits per patient at 60 days (RR = 0.58, 95% CI = 0.34–0.99, $p = 0.045$). In a pilot study using a nested case-control approach Finkelstein et al. showed that frequently hospitalized older adults have more major drug-gene interactions compared to adults who are rarely hospitalized ($p < 0.05$). Finally, Perez et al. showed in a double-blind, parallel, multi-center randomized controlled trial in which 316 adult patients diagnosed with major depressive disorder were included that genotyping using a PGx panel that the response rate to anti-depressant treatment in the PGx-guided group was significantly higher compared to conventional therapy (Odd Ratio (OR) = 1.62, 95% CI 1.00–2.61, $p = 0.0476$). Additionally, the side-effect burden measured by the Frequency, Intensity and Burden of Side Effects Rating Burden sub score was significantly higher in to the control group compared to the PGx-guided group (OR = 2.06, 95% CI 1.09–3.89, $p = 0.026$). These studies indicate that a panel approach can reduce adverse drug reactions, improve drug efficacy and is probably cost-effective. When the current study is compared to the frequencies obtained in the pilot of Elliot et al. similarities in the frequencies of actionable genotypes can be seen for *CYP2C9* (37.5% vs. 29.8%) and *CYP2D6* (47.0% vs. 42.9%) (15).

Unlike the other four studies no significant effects of gene-drug interactions on dosages, interventions and visits were found in this study. This can be explained by the fact that this study was not powered to detect effects of gene-drug interactions on the secondary endpoints. An additional explanation for the lack of an effect of genotyping on the secondary endpoints can be found in considerable number of patients with an incident for atorvastatin. According to the guidelines of the DPWG a patient should be switched to an alternate cholesterol lowering therapy in case the patient is carrier of the TC variant of the *SLCO1B1* and additionally uses co-medication that inhibits CYP3A4 (3, 4). In 35 of the 62 patients with a gene-drug interaction observed in this study the drug-gene interaction was a combination between the *SLCO1B1* 521 TC or CC genotype and the drug atorvastatin and a check on drug-drug interactions mediated through CYP3A4 was executed by the pharmacist. However, only in 7 cases inhibition of activity of CYP3A4 by another drug was found, whereas in the remaining 28 cases no action was required. This dilution by the *SLCO1B1* and atorvastatin gene-drug interaction could have been restricted by applying a cap on the number of individuals included for each drug which is currently applied in the U-PGx protocol (20).

Another limitation was that our study setting was aimed to resemble routine clinical practice, while for the study informed consent was required, and resulted in a higher time investment from the participating pharmacists compared to ordering a PGx test within routine care. This may have negatively affected the adoption rate. Additionally, the healthcare professionals did not receive any financial compensation for the time required for the inclusion of patients and the inclusion of patients had to be performed alongside their regular work in clinical practice. In some cases, eligible patients may not have been contacted as healthcare professionals could not include patients as their regular clinical tasks had to be performed. Similar to previous studies this project has shown that a significant portion of patients in primary care is open to pharmacogenetic testing (18, 21, 22). Additionally, this study has shown that individualizing medication based on genetic make-up is possible. However, the body of evidence supporting large scale implementation of a preemptive PGx panel approach in primary care is still lacking. Large implementation projects such as the U-PGx program are designed to provide an answer to this question (20).

Conclusion

A pharmacist initiated implementation of PGx in primary care is feasible, and the frequency of actionable gene-drug interactions is high.

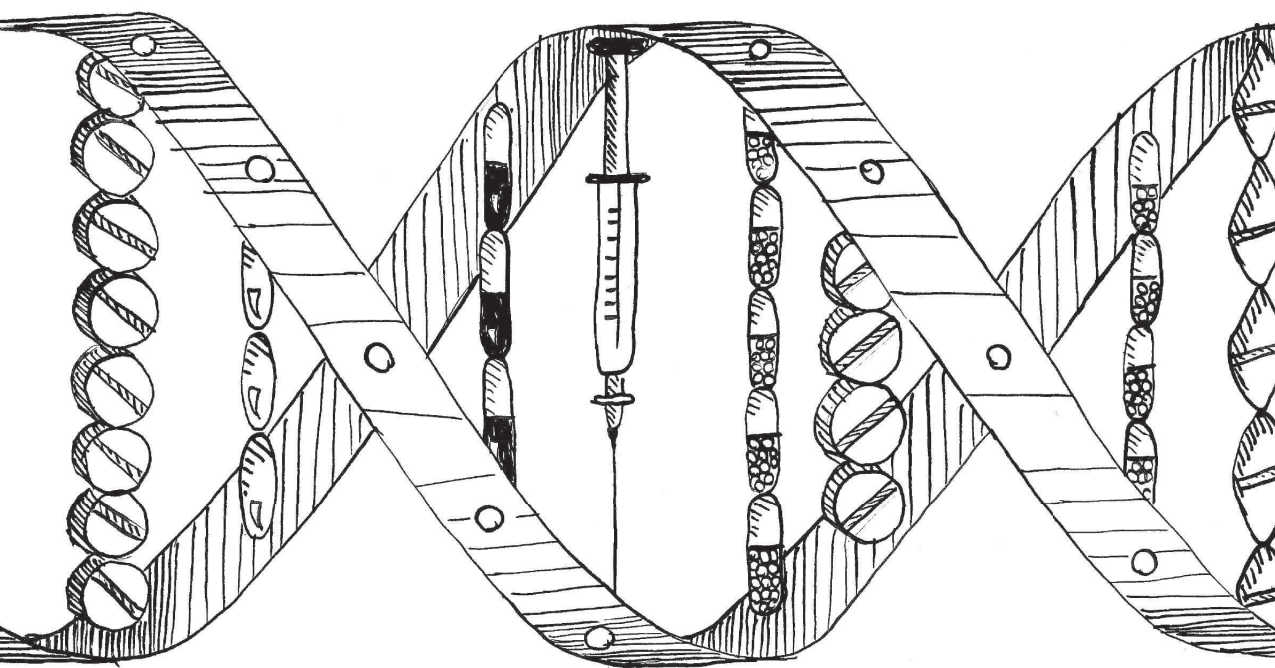
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Supplementary Document S4.1: Overview of tested PGx variants

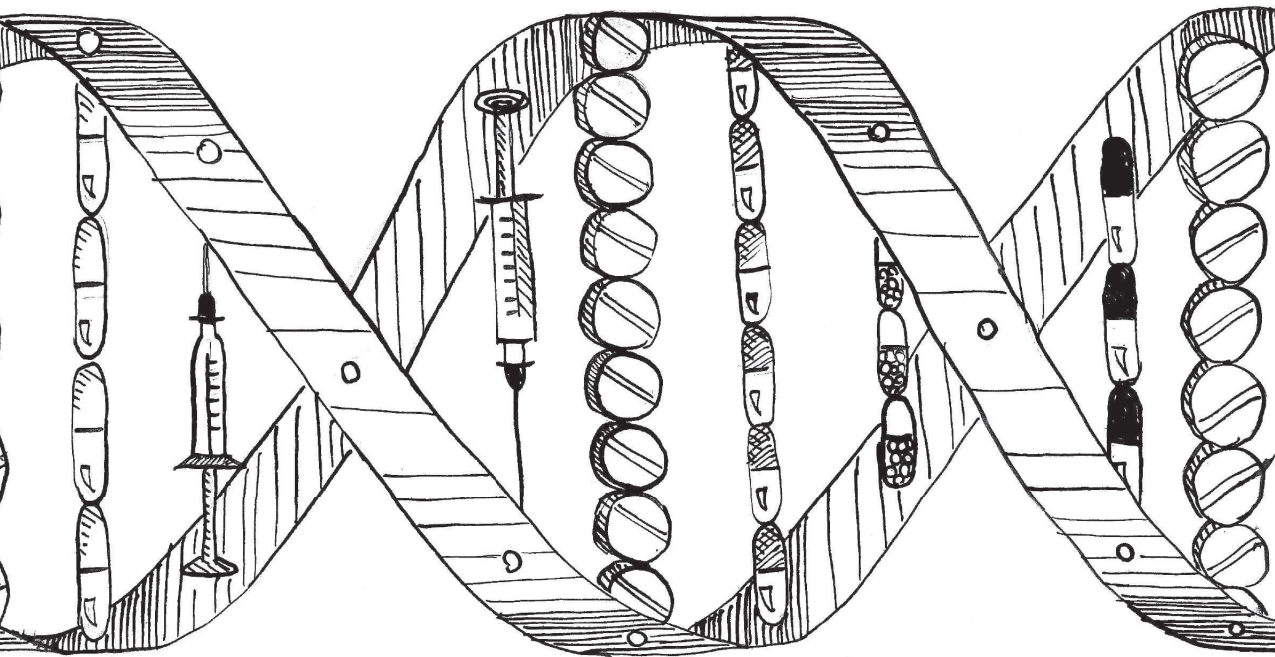
Gene	Allele	Reference sequence + variant	RS-number
<i>CYP2C9</i>	*2	NG_008385.1:g.3608C>T	rs1799853
<i>CYP2C9</i>	*3	NG_008385.1:g.42614A>C	rs1057910
<i>CYP2C19</i>	*2	NG_008384.3:g.19154G>A	rs4244285
<i>CYP2C19</i>	*3	NG_008384.3:g.17948G>A	rs4986893
<i>CYP2C19</i>	*17	NG_008384.3:g.-806C>T	rs12248560
<i>CYP2D6</i>	*2A	M33388:g.-1584C>G	rs1080985
<i>CYP2D6</i>	*10	M33388:g.100C>T	rs1065852
<i>CYP2D6</i>	*12	M33388:g.124G>A	rs5030862
<i>CYP2D6</i>	*11	M33388:g.883G>C	rs201377835
<i>CYP2D6</i>	*17	M33388:g.1023C>T	rs28371706
<i>CYP2D6</i>		M33388:g.1661G>C	rs1058164
<i>CYP2D6</i>	*6	M33388:g.1707delT	rs5030655
<i>CYP2D6</i>	*4	M33388:g.1846G>A	rs3892097
<i>CYP2D6</i>	*40	M33388:g.1863_1864insTTTCGCCCCCTTCGCCCC	rs72549356
<i>CYP2D6</i>	*20	M33388:g.1973_1974insG	rs72549354
<i>CYP2D6</i>	*19	M33388:g.2539delAACT	rs72549353
<i>CYP2D6</i>	*3	M33388:g.2549delA	rs35742686
<i>CYP2D6</i>	*38	M33388:g.2587delGACT	rs72549351
<i>CYP2D6</i>	*9	M33388:g.2615delAAG	rs5030656
<i>CYP2D6</i>		M33388:g.2850C>T	rs16947
<i>CYP2D6</i>	*7	M33388:g.2935A>C	rs5030867
<i>CYP2D6</i>	*44	M33388:g.2950G>C	rs72549349
<i>CYP2D6</i>	*41	M33388:g.2988G>A	rs28371725
<i>CYP2D6</i>	*29	M33388:g.3183G>A	rs59421388
<i>CYP2D6</i>	*42	M33388:g.3259_3260insGT	rs72549346
<i>CYP2D6</i>	*18	M33388:g.4132_4133insGTGCCCACT	rs1135836
<i>CYP2D6</i>		M33388:g.4180G>C	rs1135840
<i>CYP2D6</i>	*5	NC_000022.10:g.[0]	
<i>CYP2D6</i>	xN	Duplication	
<i>CYP3A5</i>	*3	NG_007938.1:g.12083G>A	rs776746
<i>CYP3A5</i>	*6	NG_007938.1:g.19787G>A	rs10264272
<i>DPYD</i>	*2A	NM_000110.3:c.1905+1G>A	rs3918290
<i>DPYD</i>	*13	NM_000110.3:c.1679T>G	rs55886062
<i>DPYD</i>		NM_000110.3:c.1236G>A	rs56038477
<i>DPYD</i>		NM_000110.3:c.2846A>T	rs67376798
<i>SLCO1B1</i>		NM_006446.4:c.521T>C	rs4149056
<i>TPMT</i>	*2	NM_000367.4:c.238G>C	rs1800462
<i>TPMT</i>	*3B	NM_000367.4:c.460G>A	rs1800460
<i>TPMT</i>	*3C	NM_000367.4:c.719A>G	rs1142345
<i>VKORC1</i>		NM_206824.2:c.173+1000C>T	rs9934438



Chapter 5

Nation-wide impact of implementing a preemptive pharmacogenetic panel approach to guide drug prescribing in primary care in The Netherlands

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Abstract

Pharmacogenetics (PGx) is currently implemented in hospitals to optimize therapy with high-risk drugs. However, many drugs investigated by Dutch Pharmacogenetics Working Group and the Clinical Pharmacogenetics Implementation Consortium are used in primary care and actionable phenotypes for the associated genes (*CYP2C9*, *CYP2C19*, *CYP2D6*, *SLCO1B1* and *VKORC1*) are common. The goal of this study was to estimate the clinical impact of implementation of a preemptive PGx-panel ($n = 8$) related to drugs used in primary care for 2016. In 23.6% of all incident prescriptions of 45 drugs ($n = 856002$ prescriptions/year) an actionable gene-drug interaction was present according to the guidelines of the DPWG. More importantly, these gene-drug interactions would result in a dose-adjustment or switch to another drug in 1 out of 19 ($= 5.4\%$) incident prescriptions. Consequently, with an anticipated near future where healthcare professionals will be regularly confronted with results of PGx panels, adjusting pharmacotherapy based on this information will become a routine task in drug prescribing and dispensing.

Introduction

The current use of prescription drugs is suboptimal. Many patients suffer from poor drug efficacy which in turn can lead to cessation of therapy or disease progression. Another significant portion of patients experience serious drug adverse events with possible hospitalization or even death as a result of the current one-size-fits-all approach (1, 2). In recent decades it has become clear that the interpatient variability in drug efficacy and -toxicity can be (partially) explained by genetic-variation between individuals (3). Therefore, in 2005, the Royal Dutch Pharmacists Association formed the Dutch Pharmacogenetics Working Group (DPWG) to aid in the personalization of pharmacotherapy based on an individual's genetic make-up. The DPWG has created a set of evidence based guidelines (n = 86) which has been fully integrated into the electronic drug prescribing and dispensing systems and are available through clinical decision support (4-6). Currently, in The Netherlands PGx testing is mostly performed for single gene-drug pairs. For example, testing for *DPYD* before starting therapy with capecitabine or 5-fluorouracil is routine care in the Netherlands and supported by a consistent body of evidence (7, 8). The impact of PGx in this drug-gene combination is considered high as DPD deficient patients receiving a normal dose of capecitabine have a high risk for severe toxicities (7). Nowadays, genotyping platforms allow for simultaneous characterization of multiple genes. This approach has been evaluated in multiple studies in secondary centers (9-14). Results indicate that > 95% of all individuals carry at least one actionable phenotype when tested for a panel of 12 genes (including e.g. *CYP2C9*, *CYP2C19*, *CYP2D6*, *SLCO1B1* and *VKORC1*) (15, 16). This panel-based approach has also been favorably evaluated in a number of small pilot studies in a primary care setting (17-19).

Most drugs frequently prescribed in primary care are not considered high risk. However, the combination of a high prescription volume and a high frequency of actionable phenotypes of the associated genes may also have result in a high impact (20, 21). In this study we set out to make a quantitative estimate of the potential impact of implementing PGx in primary care by calculating the number of action prescriptions in The Netherlands that comprise a gene-drug pair that is considered actionable by the DPWG. Additionally, we investigated the frequency of prescriptions where a change in choice of drug or dosage would have been required at the start of therapy as described in the guidelines of the DPWG based on a prediction of their genetic predicted phenotype.

Methods

Selection of drug-gene-interactions and classification of therapeutic recommendations

The methodology for guideline development of the DPWG has been described in detail previously (4-6). For this study, the DWPG guidelines were reviewed for drugs with an actionable therapeutic recommendation for a least one phenotype. Therapeutic recommendations were classified in no action, lower dose, higher dose, alternate drug, additional clinical monitoring of the patient, optional lower dose, optional higher dose and a maximum dose threshold. Additionally, the guidelines were checked to see whether the therapeutic recommendations were dependent on patient characteristics such as age and concomitant use of other medication. For citalopram and escitalopram the therapeutic recommendations are dependent on the age of the patient as a lower dose is recommended for patients ≥ 65 years of age (4, 5, 22). Secondly, for *SLCO1B1* and atorvastatin the therapeutic recommendation depends on concomitant use of a CYP3A4 inhibiting drug i.e. amiodaron, verapamil or diltiazem. Patients with a *SLCO1B1* 521TC or CC genotype and a CYP3A4 inhibitor are advised to switch to rosuvastatin or pravastatin, whereas in patients without a CYP3A4 inhibitor only increased monitoring for muscle pain is recommended.

Source of nationwide prescription data

The Foundation of Pharmaceutical Statistics (SFK) collects data on dispensed drugs from ~95% of all the community pharmacies in the Netherlands (23). To this end, patients are assigned an anonymous identification number that allows tracking within the participating community pharmacies (23). The SFK database also holds data on patient characteristics (gender and date of birth), dates on starting/stopping of medication by each patient, prescribed dosages, drug formulation, concomitant use of other medication which can be extracted for research (23). For this study dispensing data concerning incident prescription for the period January 1st – December 31st, 2016 were obtained. For citalopram, escitalopram and atorvastatin additional information concerning age of the patients and concomitant medication was also collected (4, 5, 22).

Frequencies of genetic predicted phenotypes

To estimate the potential clinical impact of implementation of preemptive testing for a panel consisting of 8 genes related to drugs used in primary care frequency data obtained in the “Implementation of Pharmacogenetics into Primary Care Project” (IP3) study were used as a representation of the national population (see Figure 5.1). In short, for

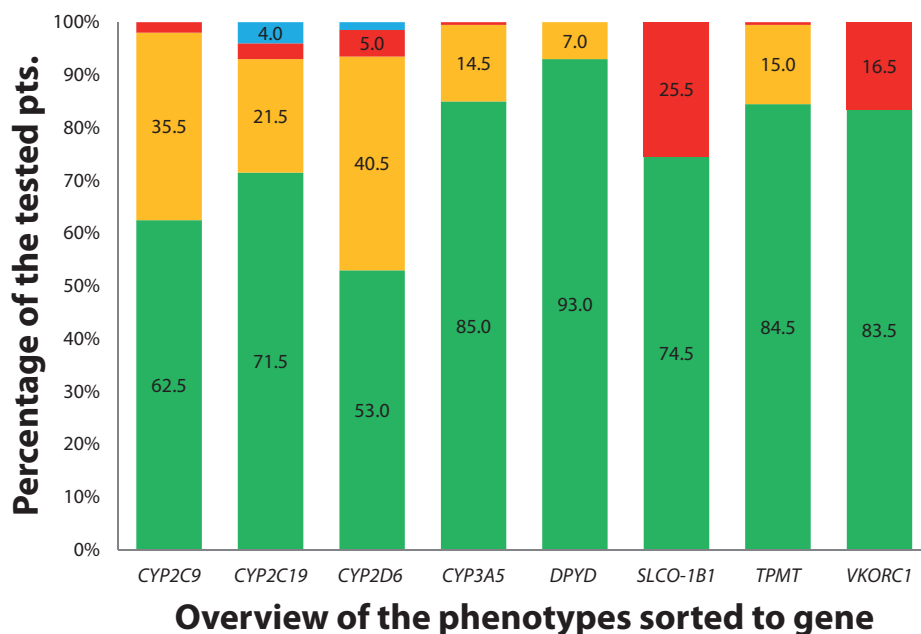


Figure 5.1: Overview of the frequencies of the phenotypes sorted to gene.

Green: extensive / normal metabolizer (*CYP's*, *DPYD*, *TPMT*) / normal transporter activity (*SLCO1B1*) / normal sensitivity (*VKORC1*). Orange: intermediate metabolizer (*CYP's*, *DPYD*, *TPMT*). Red: poor metabolizer (*CYP's*) / poor transporter activity (*SLCO1B1*) / high sensitivity (*VKORC1*). Blue: ultra-rapid metabolizer (*CYP's*).

the IP3 study 200 patients receiving an incident prescription for a selection of 10 drugs with a known gene-drug interaction were genotyped for a panel of 8 genes and 40 genetic variants using the Affymetrix DMET platform supplemented with a *CYP2D6* copy-number variation (CNV) assay. The genetic test results were translated to actionable phenotypes (e.g. extensive, intermediate, poor metabolizer or EM, IM and PM respectively) according to the interpretation tables provided by the DPWG guidelines, and communicated to the general practitioner and pharmacist to perform genotype guided dosing using clinical decision support (21).

Estimating the clinical impact of PGx in primary care

Frequencies of actionable phenotypes for the eight genes from the IP3 were inferred for the Dutch population and combined with the prescription data obtained from the SFK. After exclusion of drugs not registered in the Netherlands (warfarin) or primarily used in secondary care (5-fluorouracil, capecitabine and tegafur) a selection of 45 drugs remained (see Table 5.1). This final selection was used to estimate the occurrence of actionable

gene-drug pairs in daily practice in community pharmacies by multiplying the estimated frequency of actionable phenotypes for each gene with the observed incident prescriptions of the related drugs (see Table 5.2).

Results

For this analysis incident prescription data for the selection of 45 drugs were available from 1882 pharmacies (94.4% of total). In 2016, a total of 3338464 unique patients received a total of 3628597 incident prescriptions for the selected 45 drugs (see Table 5.1). The distribution of the phenotypes for the 8 genes is presented in Figure 5.1. Based on the frequencies of the actionable phenotypes of the eight genes and the amount of 3628597 incident prescriptions it can be estimated that in 856002 incident prescriptions (23.6%) a gene-drug pair is present (see Table 5.2). In ~195691 incident prescriptions (1:19; = 5.4%) the gene-pair requires immediate action by the prescribing physician or dispensing pharmacists by lowering the dose ($n = 43616$), increasing the dose ($n = 1315$) or switching to an alternate drug ($n = 150761$). For the remainder of the prescriptions where an actionable gene-drug pair is present ($n = 660311$) no immediate action is required, however more intensified monitoring for side effects is recommended ($n = 250980$), guarding a lower maximum threshold of the prescribed dose designated by the DWPG for specific phenotypes ($n = 26924$) or a potential decrease ($n = 90543$) or increase ($n = 291863$) of the dose in case of observed over- or undertreatment respectively (see Table 5.2). In case all patients treated in primary care received pre-emptive screening for the panel of eight genes ~12 per 1000 incident prescriptions would have required an intervention for the selected 45 drugs. Additionally, healthcare professionals would be required to monitor patients more intensively for side-effects or failure of treatment in ~42 per 1000 incident prescriptions of the drugs included in the selection.

CYP2C19 & (es)citalopram

For the selective serotonin reuptake inhibitors (SSRI) citalopram, escitalopram and sertraline the DPWG has provided a lower maximum dose for intermediate and poor metabolizers compared to extensive metabolizers (4, 5, 22). However, in contrast to sertraline the maximum dosages for citalopram and escitalopram have alternate thresholds for patients < 65 years of age vs. ≥ 65 years. In 41338 of the 56580 incident prescriptions for citalopram (= 73.1%) the patient was below the age of 65 whereas in 15424 cases the patient was 65 years or older (= 26.9%). From these data it can be inferred that in 8888 prescriptions a patient with an IM phenotype of age under 65 should have received a

Table 5.1: An overview of the total of incident prescriptions for drugs with an actionable DPWG recommendation dispensed in Dutch pharmacies that supply data to the SFK sorted to ATC-code

ATC	Name drug	No. incident prescriptions
A02BC01	OMEPRAZOLE	575353
A02BC02	PANTOPRAZOLE	361741
A02BC03	LANSOPRAZOLE	1536
A02BC05	ESOMEPRAZOLE	65370
A02BD04	PANTOPRAZOLE, AMOXICILLINE & CLARITROMYCINE	21768
B01AA04	FENPROCUMON	12621
B01AA07	ACENOCOUMAROL	49934
B01AC04	CLOPIDOGREL	98709
C01BC03	PROPAFENON	409
C01BC04	FLECAINIDE	13605
C07AB02	METOPROLOL	194724
C07BB02	METOPROLOL MET THIAZIDEN	1908
C10AA01	SIMVASTATIN	187362
C10AA05	ATORVASTATIN	111840
C10BA02	SIMVASTATINE & EZETIMIBE	4888
C10BA05	ATORVASTATINE & EZETIMIE	1909
J02AC03	VORICONAZOLE	891
L01BB02	MERCAPTOPURINE	2598
L01BB03	TIOGUANINE	1883
L02BA01	TAMOXIFEN	10807
L04AD02	TACROLIMUS	2722
L04AX01	AZATHIOPRINE	6943
M01AE52	NAPROXEN & ESOMEPRAZOL	673
N02AA05	OXYCODON	464799
N02AA55	OXYCODON & NALOXON	82
N02AJ06	CODEIN & PARACETAMOL	69300
N02AJ13	TRAMADOL & PARACETAMOL	124951
N02AX02	TRAMADOL	357389
N03AB02	FENYTOINE	828
N05AD01	HALOPERIDOLE	51217
N05AF05	ZUCLOPENTIXOLE	1873
N05AG02	PIMOZIDE	1060
N05AX12	ARIPIRAZOL	13869
N06AA02	IMIPRAMINE	988
N06AA04	CLOMIPRAMINE	7079
N06AA09	AMITRIPTYLINE	98750
N06AA10	NORTRIPTYLINE	20717
N06AA12	DOXEPIN	270
N06AB04	CITALOPRAM	56580
N06AB05	PAROXETINE	27018
N06AB06	SERTRALINE	28861
N06AB10	ESCITALOPRAM	24454
N06AX16	VENLAFAXINE	26603
N06BA09	ATOMOXETINE	1987
R05DA04	CODEIN	519728

Table 5.2: An overview of the estimates of the occurrences of gene-drug pairs among 45 drugs frequently prescribed in primary care

Drug	Count incident prescriptions (total)	Actionable phenotype	Count incident prescriptions	Type of therapeutic recommendation
CYP2C9				
PHENYTOIN	828	EM	518	No action
		IM	294	Lower dose required at start therapy
		PM	17	Lower dose required at start therapy
CYP2C19				
CITALOPRAM < 65 years	41338	EM	29557	No action
		IM	8888	Guard maximum daily dose
		PM	1240	Guard maximum daily dose
		UM	1654	No action
CITALOPRAM ≥ 65 years	15242	EM	10898	No action
		IM	3277	Guard maximum daily dose
		PM	457	Guard maximum daily dose
		UM	610	No action
CLOPIDOGREL				
CLOPIDOGREL	98709	EM	70577	No action
		IM	21222	Switch to alternate drug at start therapy
		PM	2961	Switch to alternate drug at start therapy
		UM	3948	Observe status of patient carefully
ESCITALOPRAM < 65 yrs.	21427	EM	15320	No action
		IM	4607	Guard maximum daily dose
		PM	643	Guard maximum daily dose
		UM	857	No action

ESCITALOPRAM ≥ 65 yrs.	3027	EM	2164	No action
		IM	651	Guard maximum daily dose
		PM	91	Guard maximum daily dose
		UM	121	No action
ESOMEPRAZOLE	65370	EM	46740	No action
		IM	14055	No action
		PM	1961	No action
		UM	2615	Optional increase of dose
ESOMEPRAZOLE & NAPROXEN	673	EM	481	No action
		IM	145	No action
		PM	20	No action
		UM	27	Optional increase of dose
LANSOPRAZOLE	1536	EM	1098	No action
		IM	330	No action
		PM	46	No action
		UM	61	Optional increase of dose
OMEPRAZOLE	575353	EM	411377	No action
		IM	123701	No action
		PM	17261	No action
		UM	23014	Optional increase of dose
PANTOPRAZOLE	361741	EM	258645	No action
		IM	77774	No action
		PM	10852	No action
		UM	14470	Optional increase of dose

Table 5.2 continues on next page

Table 5.2: Continued

Drug	Count incident prescriptions (total)	Actionable phenotype	Count incident prescriptions	Type of therapeutic recommendation
PANTOPAC	21768	EM	15564	No action
		IM	4680	No action
		PM	653	No action
		UM	871	Higher dose required at start therapy
SERTRALINE	28861	EM	20636	No action
		IM	6205	Guard maximum daily dose
		PM	866	Guard maximum daily dose
		UM	1154	No action
VORICONAZOLE	891	EM	637	No action
		IM	192	Observe status of patient carefully
		PM	27	Observe status of patient carefully
		UM	36	Higher dose required at start therapy
CYP2D6				
AMITRIPTYLINE	98750	EM	52338	No action
		IM	39994	Switch to alternate drug at start therapy
		PM	4938	Switch to alternate drug at start therapy
		UM	1481	Switch to alternate drug at start therapy
ARIPRAZOLE	13869	EM	7351	No action
		IM	5617	No action
		PM	693	Guard maximum daily dose
		UM	208	No action

ATOMOXETINE	1987	EM	1053	No action
		IM	805	Optional decrease of dose
		PM	99	Optional decrease of dose
		UM	30	Observe status of patient carefully
CLOMIPRAMINE	7079	EM	3752	No action
		IM	2867	Lower dose required at start therapy
		PM	354	Lower dose required at start therapy
		UM	106	Switch to alternate drug at start therapy
CODEINE	519728	EM	275456	No action
		IM	210490	No action
		PM	25986	No action
		UM	7796	Switch to alternate drug at start therapy
CODEINE & PARACETAMOL	69300	EM	36729	No action
		IM	28067	Optional increase of dose
		PM	3465	Switch to alternate drug at start therapy
		UM	1040	Switch to alternate drug at start therapy
DOXEPIN	270	EM	143	No action
		IM	109	Lower dose required at start therapy
		PM	14	Lower dose required at start therapy
		UM	4	Switch to alternate drug at start therapy
FLECAINIDE	13605	EM	7211	No action
		IM	5510	Lower dose required at start therapy
		PM	680	Lower dose required at start therapy
		UM	204	Observe status of patient carefully

Table 5.2 continues on next page

Table 5.2: Continued

Drug	Count incident prescriptions (total)	Actionable phenotype	Count incident prescriptions	Type of therapeutic recommendation
HALOPERIDOL	51217	EM	27145	No action
		IM	20743	No action
		PM	2561	Lower dose required at start therapy
		UM	768	Optional increase of dose
IMIPRAMINE (TOTAL)	988			
IMIPRAMINE (CYP2C19 EM, IM, UM)	958	EM	508	No action
		IM	388	Lower dose required at start therapy
		PM	48	Lower dose required at start therapy
		UM	14	Switch to alternate drug at start therapy
IMIPRAMINE (CYP2C19 PM)	30	EM	16	Lower dose required at start therapy
		IM	12	Lower dose required at start therapy
		PM	2	Lower dose required at start therapy
		UM	0	Switch to alternate drug at start therapy
METOPROLOL	194724	EM	103204	No action
		IM	78863	Optional decrease of dose
		PM	9736	Optional decrease of dose
		UM	2921	Optional increase of dose
METOPROLOL & THIAZIDS	1908	EM	1011	No action
		IM	773	Optional decrease of dose
		PM	95	Optional decrease of dose
		UM	29	Optional increase of dose

NORTRIPTLINE	20717	EM	10980	No action
		IM	8390	Lower dose required at start therapy
		PM	1036	Lower dose required at start therapy
		UM	311	Switch to alternate drug at start therapy
OXYCODON	464799	EM	246343	No action
		IM	188244	Observe status of patient carefully
		PM	23240	Observe status of patient carefully
		UM	6972	Observe status of patient carefully
OXYCODON & NALOXON	82	EM	43	No action
		IM	33	Observe status of patient carefully
		PM	4	Observe status of patient carefully
		UM	1	Observe status of patient carefully
PAROXETINE	27018	EM	14320	No action
		IM	10942	No action
		PM	1351	No action
		UM	405	Switch to alternate drug at start therapy
PIMOZIDE	1060	EM	562	No action
		IM	429	Lower dose required at start therapy
		PM	53	Lower dose required at start therapy
		UM	16	No action
PROPAFENON	409	EM	217	No action
		IM	166	Optional decrease of dose
		PM	20	Lower dose required at start therapy
		UM	6	Observe status of patient carefully

Table 5.2 continues on next page

Table 5.2: Continued

Drug	Count incident prescriptions (total)	Actionable phenotype	Count incident prescriptions	Type of therapeutic recommendation
TAMOXIFEN	10807	EM	5728	No action
		IM	4377	Switch to alternate drug at start therapy
		PM	540	Switch to alternate drug at start therapy
		UM	162	No action
TRAMADOL	357389	EM	189416	No action
		IM	144743	Optional increase of dose
		PM	17869	Optional increase of dose
		UM	5361	Lower dose required at start therapy
TRAMADOL & PARACETAMOL	124951	EM	66224	No action
		IM	50605	Optional increase of dose
		PM	6248	Optional increase of dose
		UM	1874	Lower dose required at start therapy
VENLAFAXINE	26603	EM	14100	No action
		IM	10774	Switch to alternate drug at start therapy
		PM	1330	Switch to alternate drug at start therapy
		UM	399	Optional increase of dose
ZUCLOPENTIXOL	1873	EM	993	No action
		IM	759	Lower dose required at start therapy
		PM	94	Lower dose required at start therapy
		UM	28	Optional increase of dose

CYP3A5				
TACROLIMUS	2722	Non-Ex	2314	No action
		Het-Ex	395	Higher dose required at start therapy
		Homo-Ex	14	Higher dose required at start therapy
SLCO1B1				
ATORVASTATIN (TOTAL)			111840	
ATORVASTATIN (WITHOUT CYP3A4 INHIBITOR)	108400	NT (521TT)	80758	No action
		PT (521TC)	25474	Observe status of patient carefully
		PT (521CC)	2168	Observe status of patient carefully
ATORVASTATIN (WITH CYP3A4 INHIBITOR)	3440	NT (521TT)	2563	No action
		PT (521TC)	808	Switch to alternate drug at start therapy
		PT (521CC)	69	Switch to alternate drug at start therapy
ATORVASTATIN & EZETIMIBE			1909	
ATORVASTATIN & EZETIMIBE (WITHOUT CYP3A4 INHIBITOR)	1739	NT (521TT)	1296	No action
		PT (521TC)	409	Observe status of patient carefully
		PT (521CC)	35	Observe status of patient carefully
ATORVASTATIN & EZETIMIBE (WITH CYP3A4 INHIBITOR)	170	NT (521TT)	127	No action
		PT (521TC)	40	Switch to alternate drug at start therapy
		PT (521CC)	3	Switch to alternate drug at start therapy
SIMVASTATIN	187362	NT (521TT)	139585	No action
		PT (521TC)	44030	Switch to alternate drug at start therapy
		PT (521CC)	3747	Switch to alternate drug at start therapy

Table 5.2 continues on next page

Table 5.2: Continued

Drug	Count incident prescriptions (total)	Actionable phenotype	Count incident prescriptions	Type of therapeutic recommendation
SIMVASTATIN & EZETIMIBE	4888	NT (521TT) PT (521TC) PT (521CC)	3642 1149 98	No action Switch to alternate drug at start therapy Switch to alternate drug at start therapy
TPMT				
AZATHIOPRINE	6943	EM IM PM	5867 1041 35	No action Lower dose required at start therapy Switch to alternate drug at start therapy
MERCAPTOPURINE	2598	EM IM PM	2195 390 13	No action Lower dose required at start therapy Switch to alternate drug at start therapy
TIOGUANINE	1888	EM IM PM	1591 282 9	No action Lower dose required at start therapy Switch to alternate drug at start therapy
VKORC1				
ACENOCUMAROL	49934	NS(1173CC) NS(1173CT) HS(1173TT)	16478 25217 8239	No action No action Lower dose required at start therapy
FENPROCUMON	12621	NS(1173CC) NS(1173CT) HS(1173TT)	4165 6374 2082	No action No action Lower dose required at start therapy

EM = extensive metabolizer, IM = intermediate metabolizer, PM = poor metabolizer, UM = ultra-metabolizer, Non ex = non expressor, Het = heterozygous expressor, HOM = homozygous expressor, NT = normal transport activity, PT = poor transport activity, NS = normal sensitivity, HS = high sensitivity.

dose restriction of 30 mg and in 3277 prescriptions a patient with an IM phenotype of age 65+ should have been restricted to a maximum dose of 15 mg. For PMs the dose should have been maximized to 20 mg in individuals of < 65 years old (n = 1240 prescriptions) and 10 mg for age 65+ (n = 457 prescriptions) respectively. In case of escitalopram the dose-restriction should have been 15 mg (n = 4607 prescriptions) and 7.5 mg (n = 651 prescriptions) for patient with the IM phenotype in the groups < 65 and ≥ 65 respectively. In 643 prescriptions of escitalopram the patient would likely have been PM and be younger than 65 years of age, thus requiring a dose restriction of 10 mg, whereas in 91 prescriptions the patient would likely have been PM and have been ≥ 65 years, thus requiring a dose restriction of 5 mg (see Table 5.2).

***SLCO1B1* & atorvastatin**

The frequency of patients with a reduced transport capacity mediated as a result of genetic variation in the *SLCO1B1* gene was found to be 25.5% of which 23.5% was the result of the 521TC genotype and 2.0% the result of a 521CC genotype. In 2016 a total of 113749 incident prescriptions for atorvastatin or atorvastatin combined with ezetimibe were dispensed. Based on genotype data from our previous study, in 29006 prescriptions the patient would likely carry the 521TC or the 521CC genotype. For these individuals, an additional check for concomitant use of inhibitors of CYP3A4 is required as per the DWPG guidelines and in case of presence of a CYP3A4 inhibitor healthcare professionals are recommended to switch to an alternate drug. Of the 29006 prescriptions where the patient carried a poor *SLCO1B1* phenotype a CYP3A4 inhibitor was not present in 28085 of the prescriptions and the patient should have been informed to monitor for signs of myopathy. Based on SFK data it could be observed that in 921 prescriptions the patient concomitantly used a drug that was a known inhibitor of CYP3A4 and the patient should have received an alternate therapy for management of their cholesterol or the inhibiting drug should have been stopped (see Table 5.2).

Discussion

This study provides an estimate of the potential nation-wide clinical impact of a pre-emptive pharmacogenetic panel for 8 genes approach in primary care in The Netherlands. Based on frequencies of actionable phenotypes gathered in a pilot study of 200 patients and nationwide prescription data (n = 3628597) we inferred that in 23.6% of the incident prescriptions of the selected 45 drugs a gene-drug pair is present. If genetic testing of the selected 8 genes had been performed in this population, we estimate that for 5.4% of the

incident prescriptions an intervention at the start of therapy would have been required and in 18.2% of the incident prescriptions more intense clinical monitoring of the patient would be required according to the guidelines of the DPWG.

A strength of this study is that the dataset used to calculate the frequency of gene-drug pairs compasses 1882 pharmacies (94.4% of total) and reflects all community pharmacies in the Netherlands (23). This allows for a detailed view of the medication use of the total population treated in primary care and provides accurate data of the number of incident prescriptions of one of the PGx drugs where patients have not yet reached their maintenance therapy as a result of empirical dosing by general practitioners. In a similar study by Samwald et al. prescription data from a combination of sources (private insurance, Medicaid or Medicare) were used. Whereas the dataset used in that study encompasses a considerable larger number of patients, it is at risk for selection bias as it is restricted to selected groups within the population of the U.S (24).

Although the data in this analysis encompasses 94.4% of the pharmacies in the Netherlands and provides a detailed view of drug use in primary care, other sections of pharmaceutical care are not represented in this dataset. A majority of the 5.6% of pharmacies that do not supply data to the database of the SFK are mainly outpatient pharmacies (community pharmacies as part of a hospital). In comparison with community pharmacies the outpatient pharmacies often dispense more specialized pharmacotherapy, for example HIV-therapy. This may result in a higher frequency of drug-drug interactions through CYP3A4 and higher frequency of relevant drug-drug-gene interactions for statins. The number of incident prescriptions where a patient uses a CYP3A4 inhibitor may likely be an underestimation of the reality.

As a result of the use of a PGx panel with a limited number of pharmacogenes this may lead to an underestimate of the potential impact of PGx in primary care. After the initiation of the IP3 pilot study the DPWG has published additional guidelines on gene-drug pairs other than the 8 genes existing on the DMET but not included in this panel (e.g. *CYP1A2* and *CYP2B6*). In addition, the chosen platform in the pilot study does not encompass all genes deemed relevant by the DPWG such as *FVL* and *HLA* genes. As a result, the estimation performed in this study is likely to be conservative. Similar to the U-PGx project current genotyping initiatives using a pre-emptive panel approach should use a platform which provides an extensive panel and is flexible to allow for adjusting if new guidelines are published by CPIC or the DPWG (25).

Currently, the implementation of PGx in primary care remains low in the Netherlands, despite the existence of guidelines containing therapeutic recommendations and their

integration into the workflow of healthcare professionals (4, 5, 26). In part, this may be explained by the fact that not all healthcare professionals are aware of the existence of clinical decision support in electronic medications surveillance systems. A recent survey among pharmacists showed that 65.4% was aware that clinical decision support contained therapeutic recommendations for genetic predicted phenotypes (26). The potential of PGx has grown, but probably other healthcare professionals (such as physicians) are not all aware of the state in which PGx is already available in their workflow. Finally, the clinical utility and cost-effectiveness of pre-emptive panel-based screening for pharmacogenetic variants has only been shown in small pilot studies, but large clinical trials providing the required evidence are still lacking (17-19). Initiatives such as the U-PGx (www.upgx.eu) which will try to provide an answer to these questions are currently ongoing (25).

Based on the data presented in this study it can be concluded that approximately one in every four incident prescriptions of a selection of the 45 drugs included in the DPWG guidelines present with a gene-drug interaction. If all patients with an incident prescription for one of the drugs of this selection would have been genotyped, 1 out of every 19 incident prescriptions would require a dose adjustment or switch at the start of therapy.

Conclusion

With an anticipated near future where healthcare professionals will be confronted with results of PGx panels adjusting pharmacotherapy as a result of relevant gene-drug interaction will likely become a routine task in drug prescribing.

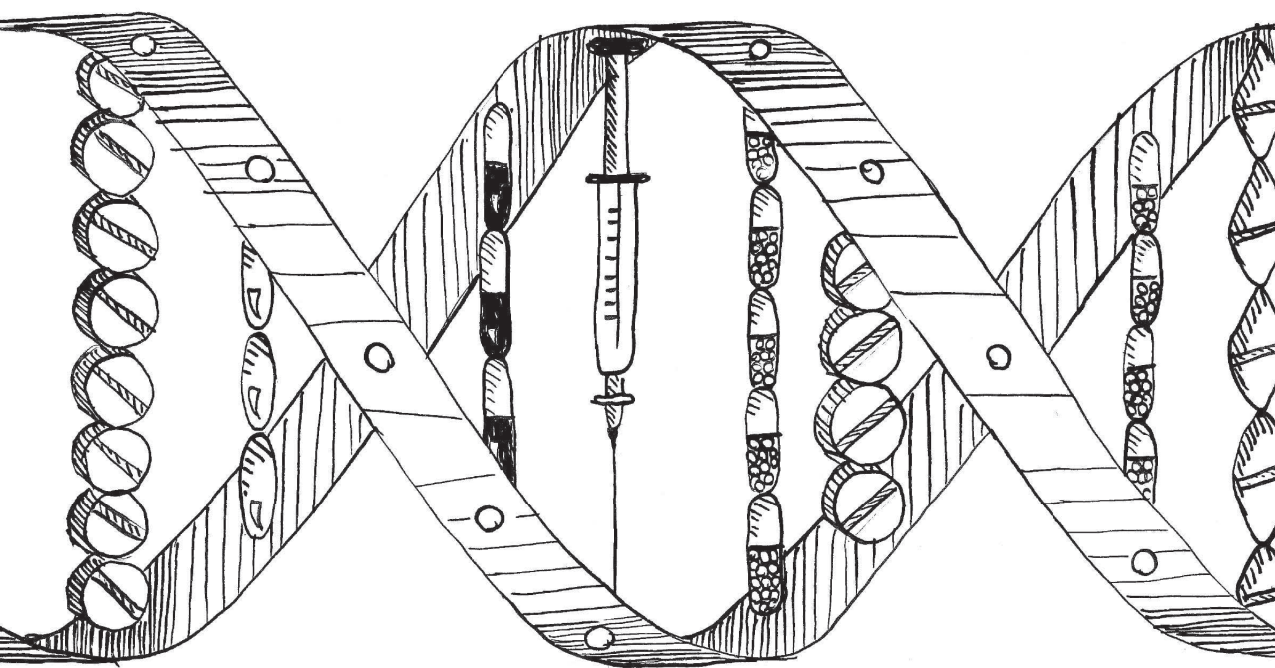
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Part III

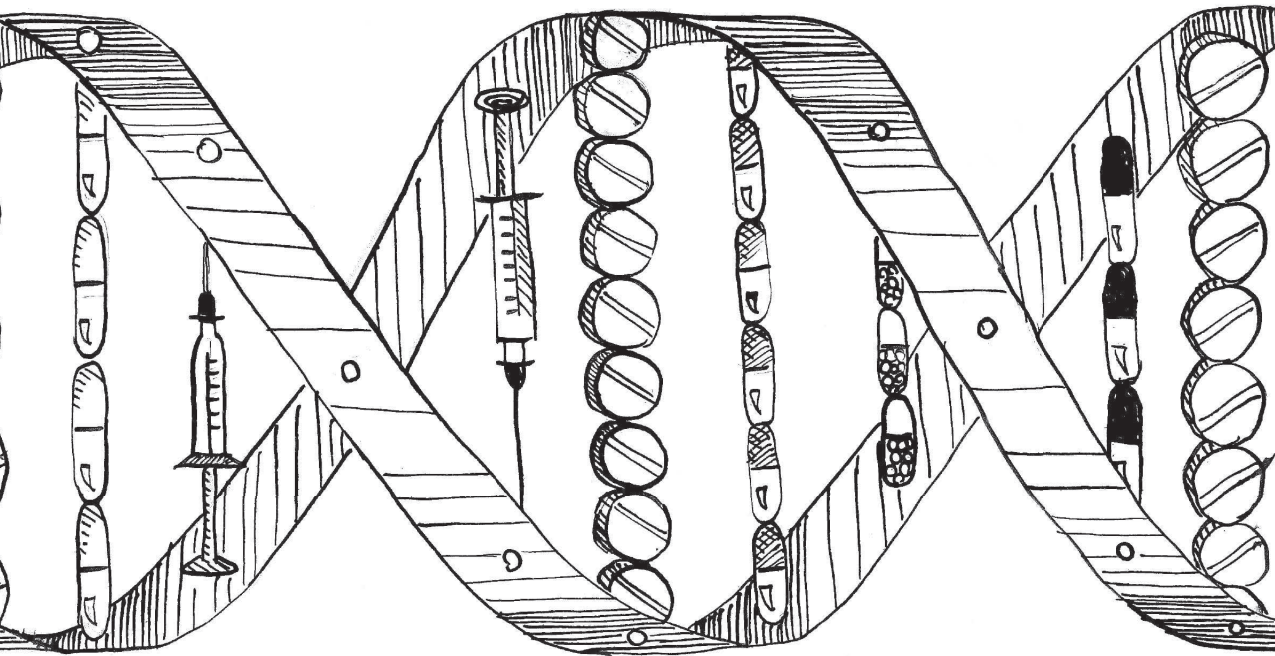
Harmonization of PGx-test
interpretation and therapeutic
recommendations



Chapter 6

Fluoropyrimidine toxicity in patients with a dihydropyrimidine dehydrogenase (DPD) splice site variant: the need for further revision of dose and schedule

Bank PCD, Guchelaar HJ, Swen JJ



Dear editor,

Magnani et al. report the results of a prospective study in which they screened fluoropyrimidine naive patients with gastrointestinal, breast and head and neck cancers who were about to receive a fluoropyrimidine containing regimen (XELOX, FOLFOX etc.) for the IVS14+1G>A variant in the *DPYD* gene (1). For heterozygous carriers of this allele the fluoropyrimidine dose was reduced by 50%, as recommended by the guidelines published by the Dutch Pharmacogenetics Working Group (DPWG) (2). Toxicities were recorded during the first two chemotherapy cycles. In 4 of the 180 screened patients a heterozygous IVS14+1G>A variant was found. Despite the 50% dose reduction, 3 of these 4 patients developed severe toxicities that required hospital admission and discontinuation of treatment during the first treatment cycle. Based on these data Magnani et al. suggest that even larger dose reductions are required for patients with the IVS14+1G>A variant than recommend in the DPWG guidelines. In this letter we would like to comment on some of the conclusions that Magnani and colleagues make in their article.

The DPWG recommendation to start fluoropyrimidine treatment with a 50% reduced dose followed by dose titration in response of toxicity in carriers of the IVS14+1G>A variant is based on systematic review of evidence from 3,773 fluoropyrimidine treated patients from 17 published studies. The data include prospective genotype guided dosing studies, prospective observational studies and case-control/case series with retrospective genotyping. Outcomes of these studies range from grade III or IV toxicity, DPD activity, dihydrouracil/uracil plasma ratio and 5-FU plasma clearance. The overall quality of evidence for this gene-drug interaction was at least scored as moderate (≥ 3 on a 5 point scale, with 0 being the lowest and 4 being the highest) and the clinical relevance was scored as an F on a 7 point scale (with AA having the lowest impact and F having the highest impact) (see Table 6.1) (2). Recently, the Clinical Pharmacogenetics Implementation Consortium (CPIC) published their guideline for *DPYD* genotypes and fluoropyrimidine dosing (3). They report that approximately 50% of the heterozygous carriers of a low activity allele develop severe toxicity compared to a general population risk of 10–40%. Based on a systematic literature review, CPIC recommends a dose reduction of at least 50% in heterozygous carriers of the IVS14+1G>A variant, in line with to the DPWG recommendation (3). Moreover, an interim analysis of the largest prospective study to date ($n = 1,700$) of *DPYD* adjusted fluoropyrimidine dosing showed that carriers of the IVS14+1G>A allele ($n = 17$) could safely be treated with a median dose of 48% (24–91%) of the recommended dose. By reducing the dose to 50% of the recommended dose for carriers of the IVS14+1G>A variant, the risk of grade ≥ 3 toxicity was reduced from 68% (in historical controls) to 15% which was comparable with the toxicity risk in the fully dosed non-carrier group (4). Also,

it was observed that the exposure to fluoropyrimidines in the genotype-guided heterozygote IVS14+1G>A variant group was comparable to fully dosed non-carriers (4). Therefore, we believe that a 50% dose reduction for patients with this genotype is supported by the best available evidence. A larger dose reduction, as suggested by Magnani et al., might further reduce the occurrence of toxicity in the heterozygous IVS14+1G>A population, but probably also at the cost of not fully benefiting from the fluoropyrimidine therapy (3).

We agree with Magnani et al. that for the prevention of all fluoropyrimidine induced toxicity genotype-guided dosing using only the IVS14+1G>A genotype (in addition to adjustments for clinical variables as bodyweight, renal function, etc.) might not be sufficient and screening for additional variants is warranted. Yet, the incidence of severe toxicity in patients with genotype-guided dosing reported in their paper is strikingly high compared to other studies (75% vs. 15%) (1, 4). A recent meta-analysis shows that the sensitivity and specificity of IVS14+1G>A genotyping for the prediction of overall grade ≥ 3 toxicity were 5.2% and 99.2% respectively, while the sensitivity and specificity of 2864A>T genotyping for the prediction of overall grade ≥ 3 toxicity were 5.4% and 99.2% respectively (5). Other SNPs in *DPYD* (such as 1236G>A), other genes including *ABCB1*, *MTHFR* and *TYMS*, environmental factors or co-administered drugs have all been implicated in fluoropyrimidine induced toxicity (3). Therefore, several other methods to detect DPD deficiency have been tested including measurement of DPD activity in peripheral blood mononuclear cells, a ^{13}C -uracil breath test, measurement of dihydrouracil (DHU) concentrations after an oral uracil loading dose, determination of the U/DHU ratio, and 5-FU therapeutic drug monitoring (TDM). However sensitivity, specificity and cost-effectiveness for all of these phenotyping methods remain to be elucidated.

As a final note we would like to add that the guidelines of the DPWG were developed to provide guidance to the treating physician and pharmacist when they are confronted with patients that have been genotyped at a previous occasion. At the time these guidelines were developed the evidence to justify prospective screening was limited. As seen in the research by Deenen et al. and Terrazino et al. there is now indeed sufficient evidence to prospectively screen patients who are about to receive fluoropyrimidines for the IVS14+1G>A variant in the *DPYD* gene and adjust the fluoropyrimidine dose based on the genotype. This genotype-guided dosing is now already implemented in a number of hospitals in the Netherlands including ours.

We conclude that the currently available evidence supports a 50% dose reduction followed by dose titration based upon toxicity for known heterozygous carriers of the *DPYD* IVS14+1G>A allele treated with a fluoropyrimidine.

Table 6.1: Overview of the included studies in the DPWG recommendation for fluoropyrimidines

Author and title study DPWG rating	Methodological design	No. patients and/or controls	Investigated genetic variants	Outcomes	Results	Conclusion authors on (DPYD) genotyping
1 Gross E et al. Strong association of a common dihydropyrimidine Dehydrogenase gene polymorphism with fluoropyrimidine-related toxicity in cancer patients. PLoS ONE 2008;3:e4003. DPWG rating: 3E/F	A retrospective single center case-control study	181 Caucasian patients with mixed tumor types treated with fluoropyrimidines (discovery cohort n = 128, independent cohort n = 53)	DPYD (sequencing): c.85T>C, IVS5+18GA, c.496A>G, IVS9-51T>G, c.1109delTA, IVS10-15T>C, c.1218G>A, c.1236G>A, IVS11-106T>A, c.1601G>A, c.1627A>G, IVS13+39C>T, IVS13+40G>A, c.1896T>C, IVS14+1G>A, IVS15+75A>G, c.2194G>A, IVS18-39G>A, c.2846A>T	Severe toxicity graded by NCI-CTC AE guidelines.	496A>G: The combined odds ratio calculated for 92 toxicity cases was 4.42 [95% CI 2.12-9.23]; p (trend) < 0.001; p (corrected) = 0.001; the attributable risk was 56.9%. Comparing tumor-type matched sets of samples, correlation of c.496A>G with toxicity was present in patients with gastro-esophageal and breast cancer.	Our results show compelling evidence that, at least in distinct tumor types, a common DPYD polymorphism strongly contributes to the occurrence of fluoropyrimidine-related drug adverse effects. Carriers of this variant could benefit from individual dose adjustment of the fluoropyrimidine drug or alternate therapies.
2 Captain O et al. The influence of fluorouracil outcome parameters on tolerance and efficacy in patients with advanced colorectal cancer. Pharmacogenomics J 2008;8:256-67. DPWG rating: 3E	A retrospective single center observational study	76 patients treated with fluoropyrimidines for advanced colorectal cancer	DPYD: IVS14+1G>A, 464T>A, 1679T>G, 2846A>A MTHFR: 677C>T, 1298A>C TYMS: - 2R - 3R	Severe toxicity graded by NCI-CTC AE guidelines Response assessed by the Response Evaluation Criteria in Solid Tumors Group Criteria 5-Fluorouracil clearance. UH₂/U plasma ratio Overall survival Progression Free Survival	Toxicity was linked to performance status >2 (p = 0.004), low UH₂/U ratio, 2846 A>T, IVS 14+1G>A for DPD (p = 0.031), and homozygous C/C for MTHFR 1298 A>C (p = 0.0018). The overall survival of the patients with a 3R/3R TS genotype associated with C/C for 677 C>T or A/A for 1298 A>C was statistically shorter (log-rank test p = 0.0065).	Genetic factors permit the tailoring of 5-FU treatment. They should occupy center stage in future clinical trials for specifically designing treatment for patients with a given biologic feature.

3	Sulzyc-Bielicka V et al. 5-Fluorouracil toxicity-attributable IVS14+1G>A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients. Pharmacol Rep 2008;60:238-442. DPWG rating: 3E	A retrospective single center observational study 252 Polish patients treated with 5-fluorouracil for colorectal cancer	DPYD: IVS14+1G>A	Severe toxicity graded by NCI-CTC AE guidelines	The DPYD IVS14+1G>A allele was identified only in one patient: a male who was one of 4 CRC patients suffering from grades 3-4 myelotoxicity upon 5-FU chemotherapy.	We conclude that DPYD IVS14+1G>A (DPYD *2A) variant occurs in the Polish population and is responsible for a significant proportion of life-threatening toxicity of 5-FU.
4	Schwab M et al. Role of genetic and non-genetic factors for fluorouracil treatment-related severe toxicity: a prospective clinical trial by the German 5-FU Toxicity Study Group. J Clin Oncol 2008;26:2131-8. DPWG rating: 3E	A prospective multicenter observational study 683 German patients treated with 5-fluoropyrimidine monotherapy with mixed tumor types	DPYD (re-sequencing): IVS14+1G>A, 623G>A, 1236G>A, 1601G>A, 1627A>G, 2194G>A, 2858G>C, 2846A>T, DPYD: promotor methylation MTHFR: 677C>T, 1298A>C TYMS: - 2R - 3R	Severe toxicity graded by CTC AE guidelines of the WHO DPD expression & activity defined by radio-enzymatic assay of DPD in liver samples	Grade 3 to 4 toxicity occurred in 16.1% of patients. The sensitivity of DPYD*2A genotyping for overall toxicity was 5.5% (95% CI, 0.02 to 0.11), with a positive predictive value of 0.46 (95% CI, 0.19 to 0.75; p = .01). Analysis according to toxicity type revealed significant association of DPYD variants with mucositis and leukopenia, whereas TYMS was associated with diarrhea.	DPYD, TYMS, and MTHFR play a limited role for FU related toxicity but a pronounced DPYD gene/sex-interaction increases prediction rate for male patients.

Table 6.1 continues on next page

Table 6.1: Continued

Author and title study DPWG rating	Methodological design	No. patients and/or controls	Investigated genetic variants	Outcomes	Results	Conclusion authors on (DPYD) genotyping
5 Mercier C et al. Prospective phenotypic screening for DPD deficiency prior to 5-FU administration: Decrease in toxicity, not in efficacy. J Clin Oncol 2008;26(May 20 suppl):abstr 14556. (meeting abstract) DPWG rating: 3	Prospective study with phenotype guided dosing	59 French patients treated with 5-fluoropyrimidine therapy for inoperable head and neck cancer	Not applicable	Severe toxicity graded by NCI-CTC AE guidelines. Response rate (method for grading response not reported) U/UH₂ ratio	DPD status evaluated from U/ UH₂ monitoring in plasma led to detecting 28 out of 59 (47%) pts. with mild (25%) to severe (22%) DPD deficiency (U/UH₂ ratios > 2 and > 3, respectively). 5-FU doses were subsequently reduced. Twenty five (88%) of the 28 patients with DPD deficiency and dose reduction did not display any severe toxicity after 5-FU administration. In first-line therapy, response rates (CR+PR) were respectively 64% (CT) and 81% (RTCT). In patients treated for relapse, response rates were 50% (CT) and 38% (RTCT).	5-FU dose tailoring based upon DPD status evaluation led to a 2 fold decrease in occurrence of severe toxicities without impairing efficacy.
6 Jatol A et al. Paclitaxel, carboplatin, 5-fluorouracil, and radiation for locally advanced esophageal cancer: phase II results of preliminary pharmacologic and molecular efforts to mitigate toxicity and predict outcomes: North Central Cancer Treatment Group (N0044). Am J Clin Oncol 2007;30:507-13. DPWG rating: 3AA	A phase II trial with prospective genotyping	50 patients with squamous cell or adenocarcinoma of the thoracic oesophagus or gastro-oesophageal junction	DPYD: IVS14+1G>A, 1627A>G, 1679T>G, 2194G>A, CYP3A4: -392A>G GSTM1: *0 deletion	Severe toxicity graded by NCI-CTC AE guidelines. Complete response rate (by computerized tomography) Median survival Progression free survival	Among the included patients, the pathologic complete response rate was 35% (95% confidence interval [CI], 23–49%). Median survival was 21.2 months (95% CI, 13.6–37.6 months), and median time to cancer recurrence/progression 19 months (95% CI, 11.4–44.6 months). Nearly all patients (94%) suffered at least one grade 3 or worse adverse event, including 3 treatment-related deaths.. Genotyping for polymorphisms of dihydropyrimidine dehydrogenase, cytochrome 3A4, and glutathione-S-transferase did not predict tumor response or severe adverse events.	This 3-drug, multimodality approach yielded a pathologic complete response rate of 35%, but the severe adverse event rate was high. Utilizing amifostine to reduce toxicity or employing molecular approaches to predict outcomes did not show promise.

7	Magne N et al. Dihydropyrimidine dehydrogenase activity and the IVS14+1G>A mutation in patients developing FU-related toxicity. Br J Clin Pharmacol 2007;64:237-40. DPWG rating: 3E	Retrospective case-series analysis supplemented with genotyping	131 Caucasian patients with mixed tumor types with well documented severe 5-fluoropyrimidine-related toxicity combined with 184 unselected patients	DPD: IVS14+1G>A	Severe toxicity graded by CTC AE guidelines of the WHO DPD activity defined by radio-enzymatic assay of DPD in PBMC	Lower DPD activity (10–504 pmol min ⁻¹ mg ⁻¹) was associated with higher scored toxicity grades (p < 0.01). Toxicity-related deaths occurred in nine patients (eight women) who significantly expressed lower DPD activity than other patients. Two of the deceased patients had normal DPD activity. The IVS14+1G>A mutation, analyzed in 93 patients, was detected in two patients (nonlethal toxicity).	Present data suggest that IVS14+1 mutation screening has limited effectiveness in identifying patients at risk for severe 5FU toxicity. Also, patients with normal PBMC-DPD activity may develop life-threatening toxicity.
8	Saif MW et al. Dihydropyrimidine dehydrogenase deficiency (GPD) in GI malignancies: experience of 4-years. Pak J Med Sci Q 2007;23:832-9. DPWG rating: 2E	Retrospective case-series analysis supplemented with genotyping and breath analysis.	23 patients of mixed ethnicity who experienced severe toxicities following 5-fluoropyrimidine or capecitabine treatment for mixed tumor types	DPD: IVS14+1G>A	Severe toxicity graded by NCI-CTC AE guidelines. DPD activity defined by radio-enzymatic assay of DPD in PBMC's ¹³ C uracil breath test	Of the included patients 7 (30%) were DPD deficient. Genotypic analysis of the <i>DPYD</i> gene in one patient with severe leucopenia demonstrated a heterozygous mutation (IVS14+1 G>A, <i>DPVD</i>). The UraBT was performed in two patients and revealed 1 to be DPD-deficient (DOB ₅₀ of 130.9; PDR of 52.5%). There were 2 toxicity-related deaths among DPD-deficient patients (28%).	DPD deficiency was observed in several ethnicities. Akin to 5-FU, capecitabine can also lead to severe toxicities in DPD-deficient patients. Screening patients for DPD deficiency prior to administration of 5-FU or capecitabine using UraBT could potentially lower risk of toxicity. Future studies should validate this technique.

Table 6.1 continues on next page

Table 6.1: Continued

Author and title study DPWG rating	Methodological design	No. patients and/or controls	Investigated genetic variants	Outcomes	Results	Conclusion authors on (DPYD) genotyping
9 Boisron - Celle M et al. 5-Fluorouracil-related severe toxicity: a comparison of different methods for the pretherapeutic detection of dihydropyrimidine dehydrogenase deficiency. Cancer Lett 2007;249:271-82. DPWG rating: 3E/F	A prospective clinical study	252 French patients of Caucasian descent receiving a 5-fluoropyrimidine regimen for advanced colorectal carcinoma.	DPYD: IVS14+1G>A, 2846A>T, DelTCAT295-298, 1156G>T, 2657G>A, G2983, -1590T>C	Severe toxicity graded by NCI-CTC AE guidelines. DPD activity expressed by uracil plasma levels DPD activity expressed by dihydrouracil / uracil ratio DPD activity expressed by DPD mRNA expression 5-FU plasma clearance	Mean clearances were $100.9 \pm 42.9 \text{ L h}^{-1} \text{ m}^{-2}$ and $133.4 \pm 48.9 \text{ L h}^{-1} \text{ m}^{-2}$ for 5-FU/4 h (n = 81) and LV5FU2 (n = 153), respectively. The mean uracil concentration was $14.9 \pm 7.7 \mu\text{g/L}$ excluding the two exceptional high concentrations (365 & 3097 $\mu\text{g/L}$), and $28.9 \pm 196.3 \mu\text{g/L}$ when they were included. The mean pre-therapeutic UH ⁺ /U plasma ratio was 7.6 ± 2.7 . The mean DPD expression (weighed by G3PDH) was 0.22 ± 0.13 . A strategy of genotyping and uracil plasma measurement, followed by dihydrouracil/uracil ratio to confirm DPD deficiency, appeared the best approach, with 83% and 82% sensitivity and specificity.	Heterozygosity of IVS14 + 1G > A or 2846A > T is not a strict contra-indication to 5-FU treatment when an initial 5-FU dosage reduction and an individual dose adjustment based on close clinical and pharmacokinetic follow-up is applied. In the case of a homozygous status for a relevant SNP, with a uracil plasma level higher than 100 $\mu\text{g/L}$ or a UH2/U ratio below 1, then 5FU administration must be discussed and an alternative treatment proposed.
10 Cho et al. Thymidylate synthase (TYMS) and dihydropyrimidine dehydrogenase (DPYD) polymorphisms in the Korean population for prediction of 5-fluorouracil-associated toxicity. Ther Drug Monit 2007;29:190-6. DPWG rating: 3AA	A retrospective single center case-control study	100 healthy Korean individuals & 21 Korean patients with colorectal cancer who experienced severe toxicity in response to receiving 5-fluoropyrimidine therapy	DPYD (sequencing): 58T>C, 1525-11G>A, 1525-9A>G, 1627A>G, (1740+39C>T), 1737T>C, 1896T>C, (1305+1G>A), (1305+19C>A), 2194G>A, 2194G>T TYMS: -2R -3RC -3RG	Severe toxicity graded by NCI-CTC AE guidelines	For the TYMS gene, the 3RC-del 6bp was significantly associated with severe toxicity with 5-FU treatment. For DPYD, the most common polymorphism was 1627A>G, observed in 20.5% of all alleles. The 496A>G allele and a novel 1774C>T allele were identified in two patients. The DPYD*2A allele, causing exon 14 skipping, was not identified in the study group.	These findings suggest that polymorphisms of the DPYD gene are not associated with an increased risk for toxic response to 5-FU toxicity and that there may be an important relationship between the TYMS haplotypes examined and 5-FU toxicity. The novel variant in the DPYD gene, identified in this study, should be further investigated to confirm its functional significance.

11	Salgado J et al. Polymorphisms in the thymidylate synthase and dihydropyrimidine dehydrogenase genes predict response and toxicity to capecitabine raltitrexed in colorectal cancer. <i>Oncol Rep.</i> 2007;17:325-8. DPWG rating: 3E	A prospective single center observational study	58 patients receiving a 5-fluoropyrimidine / raltitrexed combination therapy for metastatic colorectal cancer	DPD: IVS14+1G>A TYMS: - 2R - 3R	Severe toxicity graded by CTC AE guidelines of the WHO Response graded by the WHO criteria for reporting results of cancer treatment	Only one heterozygous subject for the IVS14+1G>A mutation was detected. The patient showed 5-FU high toxicity after the first cycle of treatment. The statistical analysis indicated that the 3R/3R genotype was preferentially found within the poor-response group of patients ($p < 0.01$) while the 2R/2R genotype was more abundant in the good-response group.	These polymorphisms could represent an accurate, rapid and effective determination panel, indicative of resistance and toxicity for patients undergoing fluoropyrimidine based treatment.
12	Morel A et al. Clinical relevance of different dihydropyrimidine dehydrogenase gene single nucleotide polymorphisms on 5-fluorouracil tolerance. <i>Mol Cancer Ther</i> 2006;5:2895-904. DPWG rating: 3E/F	A prospective single center observational study	487 French patients of Caucasian descent treated with 5-fluoropyrimidine based therapies for mixed tumor types in adjuvant setting or for advanced carcinomas	DPD: (n = 487 patients) -1590T>C, 85T>C, 295-298 del TCAT, 1156G>T, 1679T>G, IVS14+1G>A, 2657G>A, 2846A>T, 2983G>T (n = 171 patients) 61C>T, 62G>A, 257C>T, 601A>C, 623A>G, 703C>T, 812 del T, 1039-1042 del TG, 1003G>T, 1475C>T, 1601G>A, 1897delC, 2933A>G	Severe toxicity graded by NCI-CTC AE guidelines 5-FU clearance	Five different SNPs were found in 187 patients (IVS14 + 1G>A, 2846A>T, 1679T>G, 85T>C, -1590T>C). Sixty percent of patients with either IVS14 + 1G>A or 2846A>T SNPs and the only patient with 1679T>G SNP experienced early grade 3 to 4 toxicity. Sensitivity, specificity, and positive and negative predictive values of the detection of these three major SNPs as toxicity predictive factors were 0.31, 0.98, and 0.62 and 0.94, respectively.	Pretreatment detection of three DPD SNPs could help to avoid severe toxic side effects. This approach is suitable for clinical practice and should be compared or combined with pharmacologic approaches. In the case of DPD deficiency, 5-FU administration often can be safely continued with an individual dose adjustment

Table 6.1 continues on next page

Table 6.1: Continued

Author and title study DPWG rating	Methodological design	No. patients and/or controls	Investigated genetic variants	Outcomes	Results	Conclusion authors on (DPYD) genotyping
13 Largillier R et al. Pharmacogenetics of capecitabine in advanced breast cancer patients. Clin Cancer Res 2006;12:5496-502. DPWG rating: 3F	A prospective single center observational study	105 French patients receiving oral capecitabine for advanced breast cancer.	DPYD: IVS14+1G>A MTHFR: 677C>T TYMS: - 2R - 3RC - 3RG	Severe toxicity graded by NCI- CTC AE guidelines Clinical response according to the Response Evaluation in Solid Tumors Criteria	A higher global toxicity grade 3 and 4 was observed in patients homozygous for the TS 3RG allele (vs. pat. heterozygous for the 3RG allele or not carrying the 3RG allele, 50% versus 19% versus 13% respectively, $p =$ 0.064). The sole patient bearing the DPD IVS14 + 1G>A mutation (heterozygous) deceased from hematologic toxicity. The median response duration was 5.8 months (95% CI, 4.3–7.2). Duration of response was significantly shortened in patients homozygous for the 3RG allele compared with others ($p = 0.037$).	The present data suggest that breast cancer patients with the 3RG/3RG are not good candidates for capecitabine therapy. In addition, attention should be paid to DPD deficiency in breast cancer patients receiving capecitabine. These preliminary data require further confirma- tion on a larger number of patients.
14 Salgueiro N et al. Mutations in exon 14 of dihydropyrimidine dehydrogenase and 5-Fluorouracil toxicity in Portuguese colorectal cancer patients. Genet Med 2004;6:102-7. DPWG rating: 3E	A retrospective single center observational study	73 Portuguese patients receiving a 5-fluoropyrimidine regimen in adjuvant setting for the treatment of colorectal carcinoma	DPYD (sequencing of exon 14); IVS14+1G>A, 1845G>T	Severe toxicity graded by NCI-CTC AE guidelines	Of the 73 patients one patient was heterozygous for the splice- site mutation IVS14_1G>A and one patient was heterozygous for a novel missense mutation 1845G>T (E615D) in exon 14 of DPYD. The two mutations described above were found in two of the eight patients (25%) who developed grade 3 or 4 toxicity. A statistically significant association between exon 14 DPYD gene mutations and grade 3–4 5-FU toxicity was found ($p = 0.01$). The sensitivity and specificity for both mutations were 25% and 100%, respectively.	In conclusion, the common use of 5-FU in cancer therapy and the relatively high frequency of mutations in exon 14 of DPYD in patients developing grade 3–4 toxicity, indicate that genetic screening for the presence of DPYD mutations in cancer patients before the administration of 5-FU is appropriate, in order to prevent serious and sometimes lethal 5-FU- related toxicities.

15	Van Kuilenburg AB et al. High prevalence of the IVS14 + 1G>A mutation in the dihydropyrimidine dehydrogenase gene of patients with severe 5-fluorouracil-associated toxicity. Pharmacogenetics 2002;12:555-8. DPWG rating: 3E	A retrospective single center case-control study	60 patients with mixed tumor types who experienced grade 3 or 4 toxicity after administration of a 5-fluoropyrimidine regimen. The control group comprised 19 healthy volunteers and 35 tumour patients.	DPVD: IVS14+1G>A	Severe toxicity graded by NCI-CTC AE guidelines DPD activity defined by radio-enzymatic assay of DPD in PBMC's	A large range of DPD activities (0.09–17.9 nmol/mg/h) was observed in PBM cells of patients suffering from unexpected severe toxicity after the administration of 5FU. In 60% of the cases the DPD activity was lower than 70% of the mean DPD activity (9.9 ± 2.8 nmol/mg/h, n = 54) The observed allele frequency of the IVS14 + 1G>A mutation (15%) was significantly higher compared to the 0.91% observed in the normal population (p < 0.001).	Considering the common use of 5FU in the treatment of cancer patients and the increasing percentage of patients receiving high doses of 5FU in adjuvant therapy, it would be preferable to identify those patients at risk before the start of treatment with 5FU. The apparently high prevalence of the IVS14 + 1G>A mutation in cancer patients suffering from severe 5FU-associated toxicity warrants genetic screening for this mutation in cancer patients before the administration of 5FU.
16	Raida M et al. Prevalence of a common point mutation in the dihydropyrimidine Dehydrogenase (DPD) gene within the 5'-splice donor site of intron 14 in patients with severe 5-fluorouracil (5-FU)-related toxicity compared with controls. Clin Cancer Res 2001;7:2832-9. DPWG rating: 3F	A retrospective single center case-control study	25 Caucasian patients with mixed tumor types who experienced grade 3 or 4 toxicity after administration of a 5-fluoropyrimidine regimen. For the control group a cohort of 851 randomly selected Caucasian individuals (incl. 51 healthy volunteers) was used.	DPVD: IVS14+1G>A	Severe toxicity graded by CTC AE guidelines of the WHO	Within the control cohort eight heterozygote carriers of the IVS14+1G>A variant were detected (0.94%); one heterozygote in 51 healthy donors, (1.96%); five heterozygotes in 572 hospital patients (0.87%); and two heterozygotes in 228 colorectal tumor patients (0.88%). Among the 25 patients with severe 5-FU-related toxicity, 5 were heterozygous and 1 was homozygous for the exon 14-skipping mutation. All six patients had experienced WHO grade 4 myelosuppression. Lethal outcome was seen in the homozygous and two of the heterozygous cases.	We conclude that carriers of the DPD exon 14-skipping mutation are at significantly increased risk to experience life-threatening myelosuppression upon 5-FU treatment, even when the allelic status is heterozygous. These data lead us to suggest routine testing for the exon 14-skipping mutation before 5-FU treatment.

Table 6.1 continues on next page

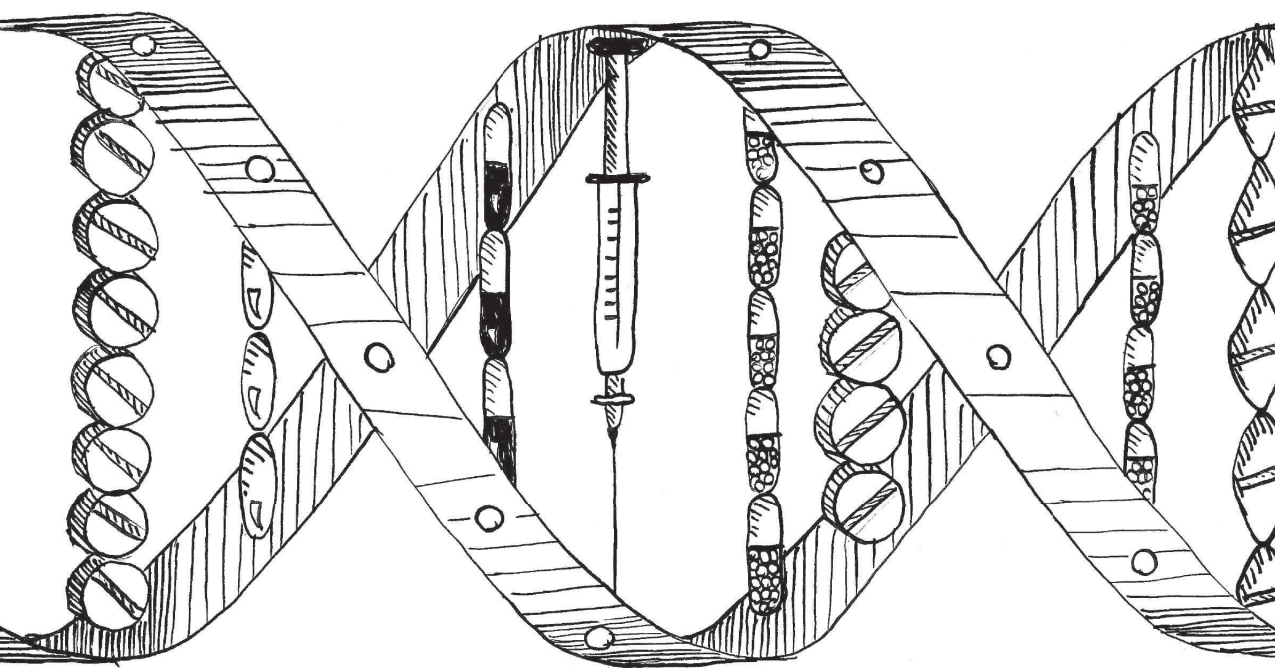
Table 6.1: Continued

	Author and title study DPWG rating	Methodological design	No. patients and/or controls	Investigated genetic variants	Outcomes	Results	Conclusion authors on (DPYD) genotyping
17	Yamaguchi K et al. Germline mutation of dihydropyrimidine- dehydrogenase gene among a Japanese population in relation to toxicity to 5-fluorouracil. Jpn J Cancer Res2001;92:337-42. DPWG rating: 3AA	A retrospective single center case-control study	104 Japanese cancer patients with mixed tumor types (of whom 69 had received a 5-fluoropyrimidine based therapy) and 3 healthy controls.	DPYD (sequencing): 74A>G, 85T>C, 812 del T, 1627A>G, 1714C>G, 1896T>C	Severe toxicity graded by CTC AE guidelines of the WHO	The silent mutation of 1896T>C in codon 632 was found in homozygous alleles of one subject (TTC/TTT) and in heterozygous alleles (TTC/TTT) of 19 subjects. The mutations of 74A>G in codon 25 (H25R), 812delT in codon 271 and 1714C>G in codon 572 (L572V) were found in one subject, the mutation of T85C in codon 29 (C29R) found in 8 patients. Of 12 patient who were heterozygous carriers of a DPYD variant, 8 had received 5FU- based chemotherapy. None of the eight patients had shown a toxic response over grade 2.	Our observations of Japanese patients implied that heterozygosity of the found variants is not associated with increased toxic response to 5FU, although a further investigation with a larger number of patients will be necessary to confirm our findings.

18	van Kuilenburg AB et al. Clinical implications of dihydropyrimidine dehydrogenase (DPD) deficiency in patients with severe 5-fluorouracil-associated toxicity: identification of new mutations in the DPD gene. Clin Cancer Res 2000;6:4705-12. DPWG rating: 3E	Retrospective case-series analysis supplemented with DPVD genotyping and DPD activity analysis	37 Caucasian patients with mixed tumor types who experienced grade 3 or 4 toxicity after administration of a 5-fluoropyrimidine regimen.	DPVD (exonsequencing): 85T>C, 496A>G, 1627A>G, IVS14+1G>A, 2194G>A 2846A>T	Severe toxicity graded by NCI-CTC AE guidelines DPD activity defined by radio-enzymatic assay of DPD in PBMC's	This study demonstrated that in 59% of the cases, a decreased DPD activity could be detected in PBMC's. It was observed that 55% of patients with a decreased DPD activity suffered from grade IV neutropenia compared with 13% of patients with a normal DPD activity ($p < 0.01$). Analysis of the DPD gene of 14 patients with a reduced DPD activity revealed the presence of mutations in 11 of 14 patients, with the splice site mutation *2A being the most abundant one (43%). Two novel missense mutations 496A>G (M166V) and 2846A>T (D949V) were detected in exon 6 and exon 22.	In conclusion, a partial DPD deficiency appears to play an important role in the etiology of 5FU-associated toxicity. Considering the common use of 5FU in the treatment of cancer patients, the severe 5FU-related toxicities in patients with a low activity of DPD, and the high frequency of the IVS14+1G3A mutation in DPD-deficient patients, it would be preferable for the analysis of DPD activity in PBM cells or screening for the IVS14+1G3A mutation to be routinely carried out before the start of treatment with 5FU.
19	Johnson MR et al. Life-threatening toxicity in a dihydropyrimidine dehydrogenase deficient patient after treatment with topical 5-fluorouracil. Clin Cancer Res 1999;5:2006-11. DPWG rating: 2D	A case-report supplemented with RT-PCR, genomic PCR, immunoprecipitation and Western blot analysis.	A 76 Caucasian male with basal cell carcinoma treated with a 2-week course of topical 5-fluoropyrimidine	DPVD: IVS14+1G>A	Severe toxicity graded by NCI-CTC AE guidelines DPD activity defined by radio-enzymatic assay of DPD in PBMC's Uracil plasma levels	Three separate determinations (over a 6-month period) demonstrated no detectable DPD enzyme activity in the patient's PBMC cytosol. In addition, uracil levels were elevated in the patient's plasma. Resolution of PCR products on a 1% agarose gel revealed that the 1688-bp DPD cDNA amplicon was slightly smaller than the normal amplicon from individuals with normal DPD activity. Sequence analysis showed that this 1688-bp amplicon contained a 165-bp deletion. Sequence analysis showed that there was a G to A mutation in the 59 donor splice consensus sequence of intron 14.	We conclude that the presence of this metabolic defect combined with topical 5-FU (a drug demonstrating a narrow therapeutic index) results in the unusual presentation of life-threatening toxicity after treatment with a topical drug.

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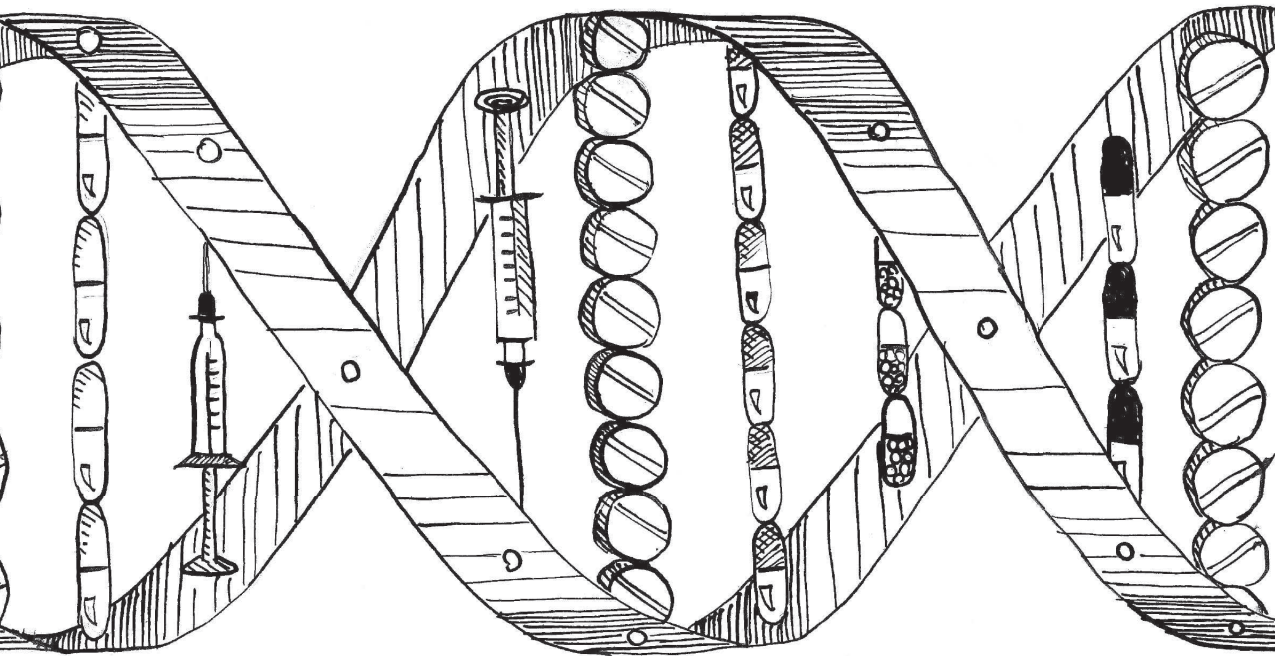
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Chapter 7

Comparison of the Guidelines of the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group

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Abstract

Both the Clinical Pharmacogenetics Implementation Consortium (CPIC®) and Dutch Pharmacogenetics Working Group provide therapeutic recommendations for well-known gene-drug pairs. Published recommendations show a high rate of concordance. However, as a result of different guideline development methods used by these two consortia, differences between the published guidelines exist. The aim of this paper is to compare both initiatives and explore these differences, with the objective to achieve harmonization.

Introduction

An important barrier for the implementation of pharmacogenetics in clinical practice is the translation of the results of a genetic test into clinical action (1-3). Kirchheiner et al. were among the first to extract dosing recommendations based on pharmacokinetic (PK) data of patients with known *CYP2D6* and *CYP2C19* genotypes (4). Anticipating a proximate future in which both pharmacists and physicians would be confronted with patients with a known genotype, two consortia, the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC®), provide widely recognized therapeutic recommendations for specific gene-drug pairs (5-32).

The DPWG was founded by the Royal Dutch Pharmacists Association (KNMP) in 2005 and in the last decade has reviewed 86 potential gene-drug pairs of which 47 guidelines provide therapeutic recommendations for one or more aberrant phenotypes (see Table 7.1 and Box 7.1 for additional information)

Table 7.1: Characteristics of the two consortia

	CPIC	DPWG
Founded	2009	2005
Type of membership	Open for application of new members with a clinical interest in pharmacogenetics, N=206 as of March 2017	By invitation, N = 14
Composition	Multidisciplinary	Multidisciplinary
Objectives	1. To address the barriers to implementation of pharmacogenetic tests into clinical practice 2. To provide guidelines that enable the translation of genetic laboratory test results into actionable prescribing decisions for specific drugs	1. To develop pharmacogenetics-based therapeutic (dose) recommendations 2. To assist drug prescribers and pharmacists by integrating the recommendations into computerized systems for drug prescription and automated medication surveillance
Number of gene-drug pairs covered	40	86
Number of gene-drug pairs with therapeutic recommendation	40	47
Frequency of scheduled updates	As needed, reviewed at least every 2 years	If needed, max 4 years
Funding	National Institutes of Health	Royal Dutch Pharmacist's Association and H2020 contract number 668353-I

Integration of therapeutic recommendations of the DPWG into clinical care

The DPWG guidelines are available at point of care in the Netherlands through all electronic prescribing and medication surveillance systems and continuously updated and distributed through the G-standard. The G-standard is the Dutch national drug database which contains information used in medication surveillance. The information of the G-standard supports the prescribing, dispensing, ordering and reimbursement of drugs and is used by physicians, pharmacists, health insurers, government and drug wholesalers in the Netherlands (<https://www.knmp.nl/producten-en-diensten/gebruiksrecht-g-standaard/informatie-over-de-g-standaard/the-g-standaard-the-medicines-standard-in-healthcare>). English versions of the DPWG guidelines have been published in 2008 and 2011 in the international literature, and a subset is currently available at the PharmGKB website: <https://www.pharmgkb.org/>.

The CPIC, established in 2009 as a joint project between the Pharmacogenomics Research Network (PGRN) and the Pharmacogenomics Knowledgebase (PharmGKB), has a similar goal to provide actionable, genotype-based prescribing recommendations for known gene-drug pairs (see Table 7.1) (3). To date, CPIC has published 19 guidelines (eight that have been updated since the original publication) covering 40 gene-drug pairs which are publicly available through both the PharmGKB (<https://www.pharmgkb.org/>) and CPIC websites (<https://cpicpgx.org/>) (8-33). The aim of this paper is to compare both initiatives and explore differences in the methodology and therapeutic recommendations of both consortia.

Methodology of comparison

The process of guideline synthesis was compared based on the information provided in papers describing the two initiatives (5, 34). A list of gene-drug pairs evaluated by both DPWG and CPIC was created. CPIC guidelines published up to March 2017 were extracted from <https://cpicpgx.org/guidelines/>. Information on DPWG guidelines was extracted from the G-standard on the 1st of March 2017. For each gene-drug pair, guidelines were systematically compared for used terminology (used for the determination of allele function and phenotypes), allele classification / genotype, dose recommendations, evidence, and clinical relevance scores. Differences in allele classification were labeled as a category I

difference and differences in genotype to phenotype translation were labeled as a category II difference. Relevant differences in therapeutic recommendations were defined as different therapeutic strategies (e.g., no adjustment vs. changes in dose vs. alternate therapy) or a $\geq 20\%$ difference in the recommended dose between the two guidelines for a specific genotype. The found relevant differences were then subdivided based on the attributed explanation for the differences. Discordances in therapeutic recommendations presumably explained by differences in the methodologies of the two consortia were allocated to category III, discordances presumably explained by a time-effect were classified as category IV, and discordances in recommendations which were presumably explained by differences in clinical practice between nations were allocated to category V.

Methodology of CPIC

To select relevant gene-drug pairs, the CPIC uses a survey-based approach supplemented with nominations from members and external experts and is informed by actions such as the U.S. Food and Drug Administration labeling (3). The CPIC also takes into account the actionability of a gene-drug pair (i.e., genetic information should/could be used to change prescribing of affected drug) and the degree of testing for variations in the gene. For each new gene-drug pair, the CPIC coordinator forms a multidisciplinary writing committee consisting of experts with a relevant track record of related publications and/or other expertise. To assist in the literature search, compiling and evaluation of identified evidence, a scientific curator from PharmGKB is added to the team. The curator and coordinator are responsible for drafting the gene background information, phenotype assignments, and compilation of the tabular materials necessary for clinical implementation of the guideline (34, 35). Clinical studies, case studies, pre-clinical studies and *in vitro* information of the drug(s) of interest with a genetic variant are evaluated and are systematically rated as weak, moderate or high. Based on this body of evidence, as well as the evidence for the alternative therapy being recommended, the writing committee derives clinical recommendations stratified by phenotype. Each recommendation is scored using a system based on that by Valdes et al. as Strong, Moderate, Optional, or No Recommendation (36). A draft of the guideline is written by the committee and reviewed by other CPIC members. Feedback from this process is incorporated into the guideline before it is subjected to external peer review (34, 35). The guideline is considered for update whenever new evidence impacts prescribing recommendations (see Figure 7.1).

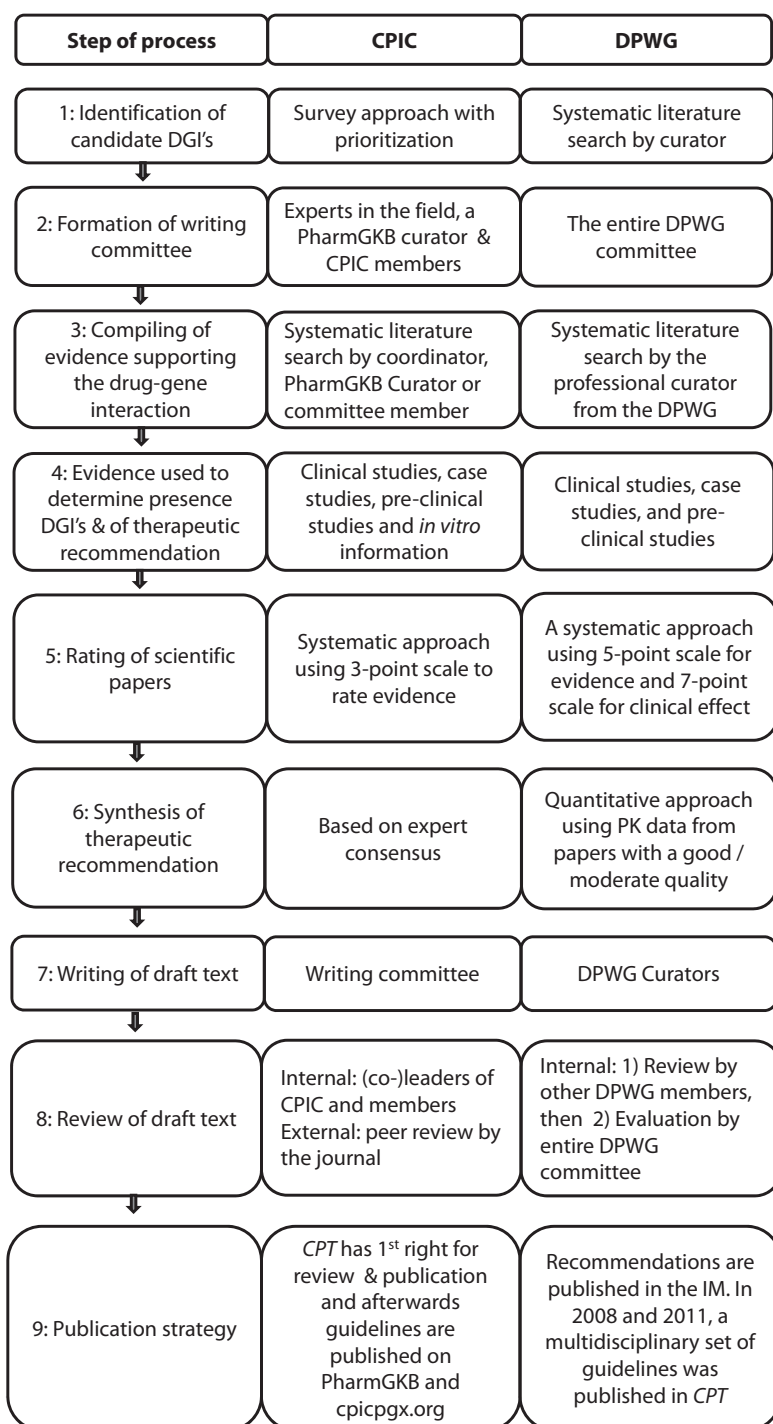


Figure 7.1: A comparison of the methodology for guideline synthesis of the two consortia.
DGI = Drug-Gene Interaction.

Methodology of DPWG

To select relevant gene-drug pairs, curators from the DPWG perform systematic searches in PubMed on known gene variants that affect drug pharmacokinetics and pharmacodynamics. For each gene-drug pair identified papers are rated by two independent DPWG members based on a scoring system (37). Based on the scores the DPWG assesses whether a gene-drug pair is indeed present and whether a therapeutic (dose) recommendation is required. Recommendations can include a dose adjustment or a therapeutic strategy (i.e., therapeutic drug monitoring or stricter clinical monitoring of patients). Dose-adjustments are calculated using PK-data from available papers with evidence rated 3 or 4 on a 0–4 point scale. All evidence is condensed into a final report containing the DPWG conclusion whether a gene-drug pair is indeed present, whether action is required and if so, the therapeutic recommendation. These reports are then integrated into a database for electronic medication surveillance, the G-standard, which feeds all available electronic drug prescribing and dispensing systems in the Netherlands (5, 6). Gene-drug pairs are updated if needed but at least every four years (see Figure 7.1).

Differences in methodology

An overview of the characteristics and objectives of both consortia is presented in Table 7.1, and the methodology of both consortia in selection of relevant gene-drug interactions, literature review and guideline synthesis are in Figure 7.1. Although the initial selection of the relevant gene-drug pairs was different, the general process of guideline synthesis by the DPWG and the CPIC is highly similar. Both consortia use professional curators to systematically search and evaluate scientific evidence (36, 37). Yet, there are some minor differences. DPWG reviews the level of evidence and the level of clinical relevance on separate scales using a five-point (0–4) and seven-point (AA-F) scale, respectively, while the CPIC rates the level of evidence on a three-point (weak-moderate-high) scale (36, 37).

A second difference is the sources of information considered for guideline development. The DPWG only provides a recommendation if data from at least one clinical study of good or moderate quality are available, whereas the CPIC also considers data from preclinical studies and case-reports. A third difference is the process used to synthesize a dose recommendation. The DPWG applies a quantitative method, whereas CPIC applies an approach based on expert consensus.

Differences in terminology for allele function and phenotype assignment

Differences in the terminology used to describe allele function also exist. An example is the difference in the word used to describe an allele that has a “greater than normal function”. The CPIC uses the term “increased function”, whereas the DPWG uses “gain-of-function”. A similar difference in terminology is seen for alleles with decreased function (see Table 7.2). With the publication of the first therapeutic guideline of *CYP2D6*, CPIC opted for the historical term “extensive metabolizer” to describe individuals who carry one or two alleles which encode for a fully functional enzyme (8, 15). However, based on the results from the recent CPIC term standardization project, normal metabolizer will replace extensive metabolizer in all new and updated CPIC guidelines (38). The term extensive metabolizer is also used by the DPWG in the published guidelines and is currently still used in clinical practice (5-7). In this comparison the term normal metabolizer will be used to describe individuals who were previously (CPIC) or are currently (DPWG) categorised as extensive metabolizers (see Table 7.2). The CPIC term standardization project also resulted in the addition of phenotypes such as *CYP2C19* rapid metabolizer with a functional definition of “increased enzyme activity compared to normal metabolizers, but less than ultra-rapid metabolizers” and the *SLCO1B1* increased function with a functional definition of “increased transporter function compared to normal function” (38).

Table 7.2: Discordances in terminology for allele function and phenotypes

Category	Functional definition	Consortium	Term
Allele	Greater than normal function	CPIC DPWG	Increased function Gain-of-function*
Allele	Less than normal function	CPIC DPWG	Decreased function Decreased activity*
Phenotype	Individuals who carry two alleles which encode for a fully functional enzyme or individuals with a combination of an allele which encodes for a fully functional enzyme and an allele which encodes for an enzyme with decreased function	CPIC DPWG	Normal metabolizer Extensive metabolizer*
Phenotype	An individual carrying one normal function allele and one increased function allele	CPIC DPWG	Rapid metabolizer -

*In this article the terminology of the CPIC will be used.

Differences in allele classification and genotype to phenotype conversion

Both CPIC and DPWG provide therapeutic recommendations at the phenotype level. As a result, a genotype-predicted phenotype (gPhenotype) needs to be inferred from the results of the genetic test. This process requires “translation tables” provided by both consortia. For the genes *CYP2C9*, *CYP2C19*, *CYP2D6* and *DPYD*, differences in both the classification of alleles (category I) and the translation of genotype to phenotype (category II) can be observed.

CYP2C9

Based on a publication from 2004, the DPWG guideline categorizes the *CYP2C9**8 allele as a “gain-of-function” allele (39). CPIC categorizes the same allele as a “possible decreased function” allele based on 2 more recent reports by Liu et al. and Allabi et al. (40, 41). The allele frequency of *CYP2C9**8 is ~0% in Caucasians and 4.70% in African-Americans (42, 43). As a result of the low allele frequency, this difference in allele classification does not appear to have clinical consequences for Caucasians. However, in African-American patients this difference could result in different therapeutic recommendations.

CYP2C19

Both consortia recognize the *CYP2C19**17 allele as an allele with a function greater than normal and categorize the genotypes *17/*17 as “ultra-rapid metabolizer” and *2/*17 and *3/*17 as intermediate metabolizers, respectively. However, a difference exists between the genotype to phenotype translation of the *1/*17 genotype. In guidelines of CPIC published before July 2016 the diplotype *1/*17 is categorized as the phenotype ultra-rapid metabolizer (24, 26, 44-50), while the DPWG classifies this diplotype as normal metabolizer based on the same literature (5, 6, 44-50) (see Table 7.3). As of July 2016 CPIC introduced the additional phenotype “rapid metabolizer” to fill the need to distinguish between individuals with a *1/*17 and *17/*17 on a phenotype level. This new phenotype was introduced in the CPIC guideline providing information and therapeutic recommendations on the gene-drug interaction of *CYP2C19* and voriconazole (32, 38).

Based on the *17 allele frequency of 18% among African populations and 18–24% among Caucasian populations, this difference in genotype to phenotype translation can result in a difference in treatment recommendations for many individuals (6, 24). For example, a prescription with amitriptyline for a patient with a *CYP2C19**1/*17 genotype results in a

recommendation to switch to an alternate therapy based on the CPIC guideline, while the DPWG guidelines advise the normal starting dose for the same genotype (6, 24).

DPYD

CPIC provides fluoropyrimidine dosing recommendations for normal/high, intermediate, and deficient dihydropyrimidine dehydrogenase (DPD) activity phenotypes based on *DPYD* genotypes (14). In contrast the DPWG uses an activity-score (AS) to accommodate the increasing number of *DPYD* allelic variants and their difference in function (see Table 7.3) (7, 51). Further differences can be seen in the amount of variants that are discussed in the guidelines. For example, the 496A>G, 1156G>T, 1651G>A, and 1845G>T variants are not mentioned in the CPIC guideline, while the IVS10-15TC variant is mentioned without a classification of the status. In contrast, the DPWG indicates that the variant alleles 496A>G and IVS10-15TC are only associated with toxicity in a single study and the 1156G>T, 1651G>A and 1845G>T variant alleles are mentioned as cause of toxicity in case reports. Inversely, the 1129-5923C>G is mentioned by the CPIC, but not by the DPWG.

Finally, a difference between the two guidelines exists in the evidence supporting the allele classification of the *13 and the 1236G>A/haplotype B3 variant. CPIC reports that there is a clear association of the *13 allele with reduced clearance of capecitabine and 5-FU in addition to evidence from case-reports (14). In contrast, DPWG concludes that the

Table 7.3: Discordances in genotype to phenotype translation

Gene	Genotype / activity score	Classification*	
<i>CYP2C19</i>	*1/*17	CPIC: Rapid metabolizer DPWG: Normal metabolizer	(24;26;44-50) (5;6;44-50)
<i>CYP2D6</i>	AS 1.0	CPIC: Normal metabolizer DPWG: Intermediate metabolizer	(15;16) (5-7)
<i>CYP2D6</i>	AS 2.5	CPIC: Ultra-rapid metabolizer DPWG: Normal metabolizer	(15;16) (5-7)
<i>DPYD</i>	2846AT / 1236GA	CPIC: Normal metabolizer DPWG: AS 1.5 / Intermediate metabolizer	(14) (7;14;51)
<i>DPYD</i>	e.g. (*2A + (2846AT or 1236GA))	CPIC: Intermediate metabolizer DPWG: AS 0.5 / Intermediate metabolizer	(14) (7;14;51)

* As a result of a consensus in a CPIC project to standardize terms for PGx test results the CPIC has adopted the term normal metabolizer to replace the historical term extensive metabolizer as experts participating in the CPIC project found it less confusing for clinicians. In this comparison the term normal metabolizer is used (See Table 7.2) (38).

evidence supporting a decreased activity of the *13 allele is limited and only described in case-reports (7).

Inversely, the DPWG categorizes the 1236G>A/haplotype B3 as a reduced function variant with a body of evidence similar to the 2846A>T variant (7). In contrast, CPIC mentions this variant without assigned status, similar to the IVS10-15TC variant (14).

CYP2D6

Both consortia classify the *CYP2D6**36 allele as a variant allele; however, there is a difference in the interpretation of the functionality of this allele between the two consortia. The DPWG classifies the activity of the *36 allele as reduced (52-57), while the CPIC classifies the allele as non-functional based on four articles published after 2002 (58-61). The difference in allele classification of functionality can potentially be explained by the distinction between the single variant which has no residual *CYP2D6* activity and the *36 + *10 tandem allele with residual activity (of the *10 allele). In contrast, the DPWG still uses the old classification of the *36 and has not yet made a difference between the single and tandem variants. Due to the low allele frequency of the *36 and the *36+*10 tandem in African Americans and Europeans (0.00–0.98%) this difference in allele status will not have much clinical consequences for these populations. However, in patients from Asian descent the frequency of these two variant alleles of *CYP2D6* is much higher, 1.52% (0.00–16.40) and 26.41% (22.45–32.65) for the single and tandem variant of the *36, respectively, and this could have implications for the therapy of Asian individuals carrying the single variant of *CYP2D6* (6, 26).

A second more important difference between the DPWG and the CPIC guidelines concerns the translation of the genotype to the *CYP2D6* phenotype. Both consortia use the AS of *CYP2D6* proposed by Gaedigk et al. to attribute the scores of 0, 0.5, 1.0, and N to alleles with no function, decreased function, normal function, and alleles with normal function and a duplication of N times, respectively, to calculate a gene-activity score for *CYP2D6*. Although DPWG and CPIC agree on the diplotype score of 0 for poor metabolizers, they use a different conversion of the AS to the intermediate metabolizer gPhenotype (62, 63). The DPWG has assigned the scores of 1.0 (combination of a functional and a non-functional allele or a combination of two alleles with reduced function) and 0.5 to intermediate metabolizer phenotype and the scores of 1.5 and 2.0 to the normal metabolizer phenotype (57), whereas CPIC assigned the scores of 1.0–2.0 to the normal metabolizer phenotype and the score of 0.5 to the intermediate metabolizer phenotype, respectively (see Table 7.3 and Figure 7.2) (15, 16).

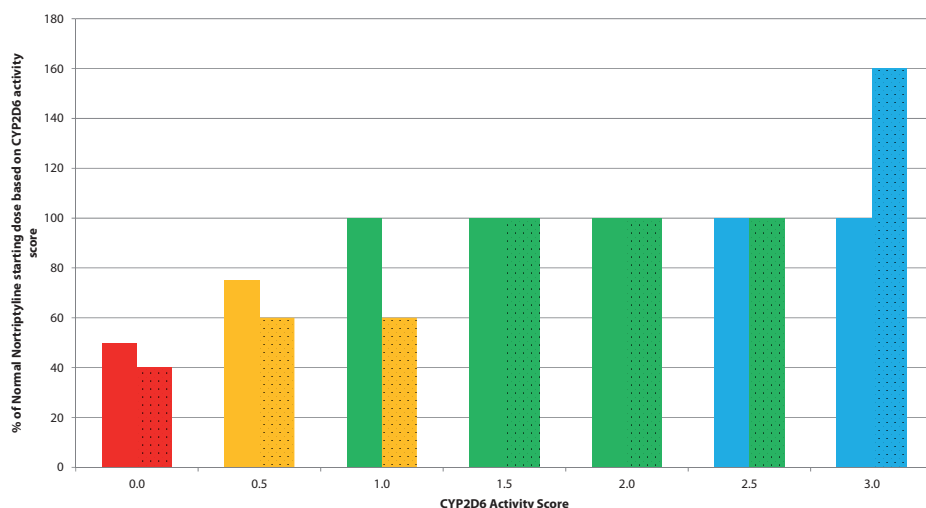


Figure 7.2: Phenotype translation and nortriptyline dose recommendations of the CPIC and DPWG based on *CYP2D6* activity scores.

Solid bars: CPIC interpretation of phenotype and dosing recommendation. Dotted bars: DPWG interpretation of phenotype and dosing recommendation. Red: poor metabolizer, Orange: intermediate metabolizer, Green: normal metabolizer, Blue: ultra-rapid metabolizer. Note: CPIC provides no specific dose adjustment for amitriptyline but recommends to consider increasing the dose and using therapeutic drug monitoring to guide dose adjustments.

Due to the relatively high frequency of null alleles as the *3, *4 and *5 among African, American and European populations, and the high occurrence of the *10 allele among Asian individuals, a large group of patients will have an AS of 1.0. These patients will be classified as either normal metabolizers or intermediate metabolizers and may receive different treatment recommendations. The different translation of *CYP2D6* genotype to phenotype between the DPWG and CPIC guidelines has a potentially significant impact on the treatment with drugs of individuals with an AS of 1.0 (see Figure 7.2).

Therapeutic recommendations

A total of 40 and 86 gene-drug pairs were reviewed by CPIC and the DPWG, respectively. For 27 gene-drug pairs both CPIC and DPWG provide guidelines which were included in the comparison. For 5 gene-drug pairs the rating of the evidence and the therapeutic recommendations were equal. For 8 gene-drug pairs, differences in the rating of the body of evidence supporting the same therapeutic recommendation were observed, but no clinically relevant differences in the therapeutic recommendations were identified (see Table 7.4). An example of this difference is the therapeutic recommendation for the

CYP2C19 intermediate metabolizer and clopidogrel. Both consortia recommend to switch to a different platelet inhibitor for the CYP2C19 intermediate metabolizer phenotype but the rating of the available evidence by CPIC and the DPWG are moderate and strong, respectively (6, 20). For 16 of the 27 gene-drug pairs with a total of 31 individual gene-drug-phenotype combinations, relevant differences (see definition) were observed in the therapeutic recommendations. In the case of 6 gene-drug pairs relevant differences in therapeutic recommendations were seen for only one aberrant phenotype, whereas for 8 gene-drug pairs differences were seen for two aberrant phenotypes and for 2 gene-drug pairs differences were seen in > 2 aberrant phenotypes. All discordant therapeutic recommendations can be found in Table 7.4. Some of the discordances will be highlighted below.

CYP2C19 & CYP2D6 + Tricyclic antidepressants

The DPWG has provided individual therapeutic recommendations for *CYP2D6* and the tricyclic antidepressants (TCAs) amitriptyline, clomipramine, doxepin, nortriptyline, and imipramine, as well as *CYP2C19* and imipramine based on AUC and steady-state concentrations (7). CPIC additionally provides recommendations for *CYP2C19* and amitriptyline, clomipramine, doxepin, and trimipramine as well as *CYP2D6* and desipramine, while the DPWG has not (yet) provided therapeutic recommendations because the consortium has categorized these gene-drug pairs as low clinical impact based on the scientific literature (7). As a result of the used methodology, the CPIC has provided equal therapeutic recommendations for the TCAs amitriptyline, clomipramine, doxepin, imipramine and trimipramine to avoid these drugs in CYP2C19 ultra-rapid, rapid and poor metabolizers. In addition, the same therapeutic recommendation of a 25% dose reduction is provided for CYP2D6 intermediate metabolizers and a recommendation to avoid TCAs in CYP2D6 ultra-rapid and poor metabolizers (Table 7.4).

Other differences in the therapeutic recommendations for TCAs can be seen in the dosing recommendations for CYP2D6 ultra-rapid metabolizers. In this case, CPIC recommends to avoid using a TCA due to the potential lack of efficacy and to consider an alternative drug not metabolized by CYP2D6. If a TCA is warranted, CPIC recommends to consider titrating to a higher target dose and using therapeutic drug monitoring to guide dose adjustments. In contrast, the DPWG provides specific, PK-based dosing advice. If a TCA is warranted, the DPWG recommends starting dosages for amitriptyline, clomipramine, doxepin, imipramine, and nortriptyline (see Figure 7.2) of 125%, 150%, 200%, 170% and 160% of the normal starting doses, respectively, followed by a recommendation to utilize therapeutic drug monitoring (6, 7, 24) (Table 7.4).

Table 7 4: Gene-drug pairs and dose recommendations by CPIC and the DPWG (differences in dosing recommendations of ≥ 20% are marked bold)

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP2C9	Phenytoin	CPIC: Yes	IM	Yes	Consider 25% reduction of recommended starting maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring and response.	M	(19)
			PM	Yes	Consider 50% reduction of recommended starting maintenance dose. Subsequent doses should be adjusted according to therapeutic drug monitoring and response.	S	(19)
			IM	Yes	Standard loading dose. Reduce maintenance dose by 25%. Evaluate response and serum concentration after 7–10 days. Be alert to ADEs (e.g., ataxia, nystagmus, dysarthria, sedation).	4D	(5–7)
			PM	Yes	Standard loading dose. Reduce maintenance dose by 50–60%. Evaluate response and serum concentration after 7–10 days. Be alert to ADEs (e.g., ataxia, nystagmus, dysarthria, sedation).	4D	(5–7)
CYP2C9	Warfarin	CPIC: Yes	*1/*2	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	(17;18)
			*1/*3	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	(17;18)
			*2/*2	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	(17;18)
			*2/*3	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	(17;18)
			*3/*3	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	(17;18)
		DPWG: Yes	*1/*2	Yes	Initiate therapy with recommended starting dose	4A	(7)
			*1/*3	Yes	Consider a reduction to 65% of the normal starting dose	4D	(7)
			*2/*2	Yes	Consider a reduction to 65% of the normal starting dose	4A	(7)
			*2/*3	Yes	Consider a reduction to 45% of the normal starting dose	4A	(7)
			*3/*3	Yes	Consider a reduction to 45% of the normal starting dose	4C	(7)

CYP2C19	Amitriptyline	CPIC: Yes	IM PM	No Yes	Initiate therapy with recommended starting dose 1) Avoid amitriptyline use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) Consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments. 1) Avoid amitriptyline use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) If amitriptyline is warranted, utilize therapeutic drug monitoring to guide dose adjustments.	S M O IN IN IN S M M 4A 4A 3AA	III III III III III III III III III III III III	(24;25) (24;25) (24;25) (7) (7) (7) (26) (26) (26) (6;7) (6;7) (6;7)
CYP2C19	Citalopram / Escitalopram	CPIC: Yes	IM PM UM	No Yes Yes	Initiate therapy with recommended starting dose. Initiate therapy with recommended starting dose. Initiate therapy with recommended starting dose. Initiate therapy with recommended starting dose. Initiate therapy with recommended starting dose. 1) Consider a 50% reduction of recommended starting dose and titrate to response. 2) Select alternative drug not predominantly metabolized by CYP2C19. Consider an alternative drug not predominantly metabolized by CYP2C19. 1) Consider a maximum daily dose of 20 mg for age < 65 or 10 mg for ≥ 65 years. 2) Consider a 50% reduction in starting dose and raise to normal dose under monitoring of ECG to 40 mg for age < 65 or 20 mg for ≥ 65 years. Consider a maximum daily dose of 20 mg for age < 65 or 10 mg for ≥ 65 years. Initiate therapy with recommended starting dose.	S M M 4A 4A 3AA	III III III III III III III III	(26) (26) (26) (6;7) (6;7) (6;7)

Table 7.4 continues on next page

Table 7.4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP2C19	Clopidogrel	CYPIC: Yes	IM	Yes	Consider alternative drug not metabolized by CYP2C19.	M	(20:21)
			PM	Yes	Consider alternative drug not metabolized by CYP2C19.	S	(20:21)
			UM	No	Initiate therapy with recommended starting dose.	S	(20:21)
		DPWG: Yes	IM	Yes	Consider alternative drug not metabolized by CYP2C19.	4F	(6:7)
			PM	Yes	Consider alternative drug not metabolized by CYP2C19.	4F	(6:7)
			UM	No	Initiate therapy with recommended starting dose.	4A	(6:7)
CYP2C19	Clomipramine	CYPIC: Yes	IM	No	Initiate therapy with recommended starting dose.	O	(24:25)
			PM	Yes	1) Avoid domipramine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine.	O	(24:25)
		DPWG: Yes	UM or RM	Yes	2) Consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24:25)
					1) Avoid domipramine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine.		
					2) If domipramine is warranted, utilize therapeutic drug monitoring to guide dose adjustments.		
					Initiate therapy with recommended starting dose.		
					Initiate therapy with recommended starting dose.		
					Initiate therapy with recommended starting dose.		

CYP2C19	Doxepin	CPIC: Yes	IM	No	Initiate therapy with recommended starting dose	O		(24;25)
			PM	Yes	1) Avoid doxepin use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) Consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	III	(24;25)
		DPWG: No	UM or RM	Yes	1) Avoid doxepin use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) If doxepin is warranted, utilize therapeutic drug monitoring to guide dose adjustments.	O	III	(24;25)
			IM	No	Initiate therapy with recommended starting dose.	IN		(7)
			PM	No	Initiate therapy with recommended starting dose.	IN	III	(7)
	Imipramine	CPIC: Yes	UM	No	Initiate therapy with recommended starting dose.	IN	III	(7)
			IM	No	Initiate therapy with recommended starting dose.	O		(24;25)
		DPWG: Yes	PM	Yes	1) Avoid imipramine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) Consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	III	(24;25)
			UM or RM	Yes	1) Avoid imipramine use due to potential for sub-optimal response. Consider alternative drug not metabolized by CYP2C19. TCAs without major CYP2C19 metabolism include nortriptyline and desipramine. 2) If imipramine is warranted, utilize therapeutic drug monitoring to guide dose adjustments.	O	III	(24;25)
			IM	No	Initiate therapy with recommended starting dose.	4A		(6;7)

CYP2C19	Imipramine	DPWG: Yes	PM	Yes	1) Consider a 30% reduction of recommended starting dose and utilize therapeutic drug monitoring of imipramine and desipramine. 2) Consider alternative drug not metabolized by CYP2C19.	4A	III	(6;7)
			UM	No	Initiate therapy with recommended starting dose	4A	III	(6;7)

Table 7.4 continues on next page

Table 7.4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP2C19	Sertraline	CPI/C: Yes	IM	No	Initiate therapy with recommended starting dose.	S	(26)
			PM	Yes	1) Consider a 50% reduction of recommended starting dose and titrate to response.	O	(26)
					2) Select alternative drug not predominantly metabolized by CYP2C19.		
			UM	Yes	Initiate therapy with recommended starting dose. If patient does not respond to recommended maintenance dosing, consider alternative drug not predominantly metabolized by CYP2C19.	O	(26)
		DPWG: Yes	IM	Yes	Consider a maximum daily dose of 100 mg and utilize clinical monitoring on response/side effects or therapeutic drug monitoring of sertraline + desmethylsertraline to guide dose adjustments.	4A	(6:7)
			PM	Yes	Consider a maximum daily dose of 50 mg and utilize clinical monitoring on response/side effects or therapeutic drug monitoring of sertraline + desmethylsertraline to guide dose adjustments.	4C	(6:7)
			UM	No	Initiate therapy with recommended starting dose.	4AA	(6:7)
			IM	No	Initiate therapy with recommended starting dose.	M	(32)
			PM	Yes	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole	M	(32)
			RM	Yes	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole	M	(32)
CYP2C19	Voriconazole	CPI/C: Yes	UM	Yes	Choose an alternative agent that is not dependent on CYP2C19 metabolism as primary therapy in lieu of voriconazole	M	(32)
			IM	Yes	Monitor serum concentration	4A	(5-7)
			PM	Yes	Monitor serum concentration	4A	(5-7)
			UM	Yes	Consider a 50% increase of the recommended starting dose & monitor serum concentration.	4A	(5-7)

CYP2D6	Amitriptyline	CPIC: Yes	IM	Yes	Consider 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments. 1) Avoid amitriptyline use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If amitriptyline is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	M	III	(24;25)
	PM			Yes	1) Avoid amitriptyline use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If amitriptyline is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	S		(24;25)
	UM			Yes	1) Avoid amitriptyline use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6. 2) If amitriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments	S		(24;25)
	IM	DPWG: Yes		Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider a decrease of up to 60% of the recommended dose under therapeutic drug monitoring of amitriptyline and nortriptyline.	3C	III	(6;7)
	PM			Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider a decrease of up to 50% of the recommended dose under therapeutic drug monitoring of amitriptyline and nortriptyline.	3A		(6;7)
	UM			Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider an increase of up to 125% of the recommended dose under therapeutic drug monitoring of amitriptyline and nortriptyline. Be alert of a possible decrease in therapeutic levels and an increase of active cardiotoxic hydroxymetabolites.	3C		(6;7)

Table 7.4 continues on next page

Table 7 4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP2D6	Clomipramine	CPI(C): Yes	IM	Yes	Consider 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			PM	Yes	1) Avoid clomipramine use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If clomipramine is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			UM	Yes	1) Avoid clomipramine use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6. 2) If clomipramine is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
		DPWG: Yes	IM	Yes	1) Consider 30% reduction of recommended starting dose. 2) Utilize therapeutic drug monitoring of clomipramine and desmethylclomipramine.	4C	(5-7)
			PM	Yes	Depression: 1) Consider 60% reduction of recommended starting dose. 2) Utilize therapeutic drug monitoring of clomipramine and desmethylclomipramine. Anxiety: 1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider a decrease of up to 50% of the recommended dose under therapeutic drug monitoring of clomipramine and desmethylclomipramine.	4C	(5-7)
			UM	Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider an increase of up to 150% of the recommended dose under therapeutic drug monitoring of amitriptyline and nortriptyline. Be alert of a possible decrease in therapeutic levels and an increase of cardiotoxic active hydroxymetabolites.	3C	(5-7)

CYP2D6	Codeine	CPIC: Yes	IM	Yes	Use label-recommended age- or weight-specific dosing. If no response, consider alternative analgesics such as morphine or a non-opioid.	M	(15;16)
			PM	Yes	Avoid codeine use due to lack of efficacy.	S	(15;16)
			UM	Yes	Avoid codeine use due to potential for toxicity.	S	(15;16)
			IM	Yes	Cough: no action required Pain: Be on alert for a lack of clinical effect. In case of a lack of clinical effect consider a raise in daily dose or consider an alternative drug	3A	(5-7)
		DPWG: Yes	PM	Yes	Cough: no action required / Pain: Consider an alternative drug	4B	(5-7)
UM	Yes		Contraindicated	3F	(5-7)		
CYP2D6	Doxepin	CPIC: Yes	IM	Yes	Consider 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			PM	Yes	1) Avoid doxepin use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If doxepin is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			UM	Yes	1) Avoid doxepin use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6. 2) If doxepin is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			IM	Yes	Consider a 20% reduction of recommended starting dose. Utilize therapeutic drug monitoring to monitor doxepin and nortoxepin to guide dose adjustments.	3A	(6;7)
			PM	Yes	Consider a 60% reduction of recommended starting dose. Utilize therapeutic drug monitoring to monitor doxepin and nortoxepin to guide dose adjustments.	3F	(6;7)
		DPWG: Yes	UM	Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider an increase of up to 200% of the recommended dose under therapeutic drug monitoring of doxepin and nortoxepin.	3A	(6;7)

Table 7.4 continues on next page

Table 7.4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP2D6	Fluvoxamine	CYPIC: Yes	IM	No	Initiate therapy with recommended starting dose.	M	(26)
			PM	No	1) Consider a 25–50% reduction of recommended starting dose and titrate to response. 2) Use an alternative drug not metabolized by CYP2D6.	O	(26)
			UM	No	No recommendation due to lack of evidence.	O	(26)
		DPWG: No	IM	No	Initiate therapy with recommended starting dose	IN	(7)
			PM	No	Initiate therapy with recommended starting dose	3AA	(7)
			UM	No	Initiate therapy with recommended starting dose	IN	(7)
CYP2D6	Imipramine	CYPIC: Yes	IM	Yes	Consider 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			PM	Yes	1) Avoid imipramine use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If imipramine is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
		DPWG: Yes	UM	Yes	1) Avoid imipramine use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6. 2) If imipramine is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments.	O	(24;25)
			IM	Yes	Consider 30% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	4A	(5-7)
			PM	Yes	Consider 70% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	4C	(5-7)
			UM	Yes	1) Consider alternative drug not metabolized by CYP2D6. 2) If an alternative is not possible consider an increase of up to 170% of the recommended dose under therapeutic drug monitoring of imipramine and desipramine.	4A	(5-7)

CYP2D6	Nortriptyline	CPIC: Yes	IM	Yes	Consider 25% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	M	(24;25)
			PM	Yes	1) Avoid nortriptyline use due to potential for side effects. Consider alternative drug not metabolized by CYP2D6. 2) If nortriptyline is warranted, consider 50% reduction of recommended starting dose. Utilize therapeutic drug monitoring to guide dose adjustments.	S	(24;25)
			UM	Yes	1) Avoid nortriptyline use due to potential lack of efficacy. Consider alternative drug not metabolized by CYP2D6. 2) If nortriptyline is warranted, consider titrating to a higher target dose (compared to normal metabolizers). Utilize therapeutic drug monitoring to guide dose adjustments.	S	(24;25)
		DPWG: Yes	IM	Yes	Consider a 40% reduction of recommended starting dose. Utilize therapeutic drug monitoring of nortriptyline and 10-hydroxytriptyline to guide dose adjustments	4C	(5-7)
			PM	Yes	Consider a 60% reduction of recommended starting dose. Utilize therapeutic drug monitoring of nortriptyline and 10-hydroxytriptyline to guide dose adjustments	3C	(5-7)
			UM	Yes	1) Consider alternative drug not metabolized by CYP2D6 2) If an alternative is not possible consider an increase of up to 60% of the recommended dose. Utilize therapeutic drug monitoring of nortriptyline and 10-hydroxytriptyline to guide dose adjustments.	3C	(5-7)
CYP2D6	Paroxetine	CPIC: Yes	IM	No	Initiate therapy with recommended starting dose.	M	(26)
			PM	Yes	1) Consider alternative drug not predominantly metabolized by CYP2D6. 2) If paroxetine use warranted, consider a 50% reduction of recommended starting dose and titrate to response.	O	(26)
			UM	Yes	Consider alternative drug not predominantly metabolized by CYP2D6.	S	(26)
		DPWG: Yes	IM	No	Initiate therapy with recommended starting dose	4A	(5-7)
			PM	No	Initiate therapy with recommended starting dose	4A	(5-7)
			UM	Yes	Consider alternative drug not metabolized by CYP2D6	4C	(5-7)

Table 7.4 continues on next page

Table 7.4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
CYP3A5	Tacrolimus	CPI/C: Yes	IM (heterozygous expressor)	Yes	Increase starting dose 1.5 to 2 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	S	(30)
			NM (homozygous expressor)	Yes	Increase starting dose 1.5 to 2 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	S	(30)
			Heterozygous expressor	Yes	Increase starting dose 1.75 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	4E	(6;7)
			Homozygous expressor	Yes	Increase starting dose 2.5 times recommended starting dose. Total starting dose should not exceed 0.3 mg/kg/day. Use therapeutic drug monitoring to guide dose adjustments.	4E	(6;7)
DPYD	Capecitabine/ 5-Fluorouracil	CPI/C: Yes	IM	Yes	Start with at least a 50% reduction in starting dose followed by titration of dose based on toxicity or pharmacokinetic test (if available).	M	(14)
			PM	Yes	Select alternate drug.	S	(14)
			1.5	Yes	Start with at least a 25% reduction in starting dose followed by titration of dose based on toxicity and efficacy.	4F	(6;7;51)
			1.0	Yes	Start with at least a 50% reduction in starting dose followed by titration of dose based on toxicity and efficacy.	4F	(6;7;51)
			0.5	Yes	Start with at least a 75% reduction in starting dose followed by titration of dose based on toxicity and efficacy.	4F	(6;7;51)
			0.0	Yes	Select alternate drug.	4F	(6;7;51)
DPYD	Tegafur	CPI/C: Yes	IM	Yes	Start with at least a 50% reduction in starting dose followed by titration of dose based on toxicity or pharmacokinetic test (if available).	M	(14)
			PM	Yes	Select alternate drug.	S	(14)
			1.5	Yes	Select alternate drug.	2E	(6;7;51)
			1.0	Yes	Select alternate drug.	2D	(6;7;51)
			0.5	Yes	Select alternate drug.	0E	(6;7;51)
			0.0	Yes	Select alternate drug.	0E	(6;7;51)

HLA-B	Abacavir	CPIC: Yes DPWG: Yes	*57:01/*X; *57:01/ *57:01 *57:01/*X; *57:01/ *57:01	Yes Yes	Abacavir is not recommended. Abacavir is contraindicated.	S 4E	(12;13) (6;7)
HLA-B	Carbamazepine	CPIC: Yes DPWG: Yes	*15:02/*X; *15:02/*15:02 *15:02/*X; *15:02/*15:02 *15:11/*X; *15:11/*15:11 *31:01/*X; *31:01/*31:01	Yes Yes	A. If patient is carbamazepine-naïve, do not use carbamazepine B. If patient has previously used carbamazepine for longer than 3 months without incidence of cutaneous adverse reactions, cautiously consider use of carbamazepine For HLA-B*15:02, the recommendation is to choose an alternative. If an alternative is possible, choosing an alternative is also recommended for HLA-A*31:01 and HLA-B*15:11	S(A) O(B) 4E (ALL)	(11) (7)
SLCO1B1	Simvastatin	CPIC: Yes DPWG: Yes	Decreased Function (521TC) Poor Function (521CC) 521TC 521CC	Yes Yes Yes Yes	1) Prescribe a lower dose. 2) Consider an alternative statin (e.g., pravastatin or rosuvastatin). 3) Consider routine CK surveillance. 1) Prescribe a lower dose. 2) Consider an alternative statin (e.g., pravastatin or rosuvastatin). 3) Consider routine CK surveillance. 1) Consider alternative drug. 2) If simvastatin is warranted, prescribe a maximum dose of 40 mg/day. Select alternative drug.	S S 4D 4D	(22;23) (22;23) (7) (7)

Table 7.4 continues on next page

Table 7.4: Continued

Gene	Drug	Gene-drug interaction	Phenotype	Action required?	Therapeutic recommendations + classification of evidence	Cat.	Ref.
TPMT	Azathioprine/ mercaptopurine	CPIC: Yes	IM	Yes	If disease treatment normally starts at the "full dose", consider starting at 30–70% of target dose (e.g., 1–1.5 mg/kg/d), and titrate based on tolerance. Allow 2–4 weeks to reach steady state after each dose adjustment.	S	(8:9)
			PM	Yes	1) Consider alternative agents. 2) If using azathioprine start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of azathioprine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. Azathioprine is the likely cause of myelosuppression.	S	(8:9)
			IM	Yes	1) Select alternative drug. 2) Reduce dose by 50%. Increase dose in response of hematologic monitoring and efficacy.	4E	(6:7)
			PM	Yes	1) Select alternative drug. 2) Reduce dose by 90%. Increase dose in response of hematologic monitoring and efficacy.	4F	(6:7)
TPMT	Thioguanine	CPIC: Yes	IM	Yes	Start with reduced doses (reduce by 30–50%) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 2–4 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, and depending on other therapy, emphasis should be on reducing thioguanine over other agents.	M	(8:9)
			PM	Yes	Start with drastically reduced doses (reduce daily dose by 10-fold and dose thrice weekly instead of daily) and adjust doses of thioguanine based on degree of myelosuppression and disease-specific guidelines. Allow 4–6 weeks to reach steady state after each dose adjustment. In setting of myelosuppression, emphasis should be on reducing thioguanine over other agents. For nonmalignant conditions, consider alternative nonthiopurine immunosuppressant therapy.	S	(8:9)
			IM	Yes	1) Select alternative drug. 2) Reduce dose by 25%. Increase dose in response of hematologic monitoring and efficacy.	3E	(6:7)
			PM	Yes	1) Select alternative drug. 2) Reduce dose to 6–7% of starting dose. Increase dose in response of hematologic monitoring and efficacy.	2F	(6:7)

VKORC1	Warfarin	CPIC: Yes	-1639GA	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	V	(17;18)
			-1639AA	Yes	Calculate dose based on validated published pharmacogenetic algorithm	S	V	(17;18)
		DPWG: Yes	-1639GA	Yes	Initiate therapy with recommended starting dose	4A	V	(7)
			-1639AA	Yes	Consider a reduction to 60% of the normal starting dose	4A	V	(7)

IM = intermediate metabolizer; NM = normal metabolizer; PM = poor metabolizer; RM = rapid metabolizer; UM = ultra-rapid metabolizer; ADE = adverse drug event; M = moderate; S = strong; O = Optional; IN = Insufficient evidence; 0 = data on file; 1 = published incomplete case reports; 2 = well documented case reports / case series; 3 = published controlled studies of moderate quality; 4 = published controlled studies of good quality; A = minor clinical effect; B = clinical effect: short-lived discomfort (< 48 h) without permanent injury; C = clinical effect: long-standing discomfort (48–168 h) without permanent injury; D = clinical effect: long-standing effect (> 168) and permanent symptom or invalidating injury; E = Increased risk of failure of lifesaving therapy / expected bone marrow depression; F = death, arrhythmia, unexpected bone marrow depression.

*Category of discordance: I = discordance in allele classification, II = discordance in genotype to phenotype translation, III = discordance in therapeutic recommendation attributed to a difference in methodology of the two consortia, IV = discordance in therapeutic recommendation attributed to a difference in clinical practice.

***DPYD* + fluoropyrimidines**

As mentioned previously, the DPWG uses an AS for *DPYD* while the CPIC uses phenotypes of normal/high, intermediate and deficient activity (soon to be changed to normal, intermediate, and poor metabolizers, respectively, in the next *DPYD* guideline update based on the results of the CPIC term standardization project) (14, 51). The gene activity model scores alleles with a reduced function as 0.5, while fully dysfunctional alleles are classified as 0. The AS model allows scores of 1.5 and 0.5 in addition to the scores of 2.0 (EM), 1.0 (IM) and 0.0 (PM) (see Table 7.3). Both guidelines include an initial 50% dose-reduction for intermediate metabolizers (AS = 1) and a recommendation to switch to an alternative drug for poor metabolizers (AS = 0). The DPWG also contains a recommendation for 25% and 75% dose reduction of the starting dose for the AS of 1.5 and 0.5, respectively (7, 14) (Table 7.4).

TPMT + thiopurines

In the guidelines of the DPWG the dosing advice for azathioprine and 6-mercaptopurine are a 50% dose-reduction and 90% dose-reduction for TPMT intermediate metabolizers and poor metabolizers, respectively. The CPIC recommends a mean starting dose of 50% (range 30–70%) and 10% of the conventional dose for TPMT intermediate metabolizers and poor metabolizers, respectively. Thrice weekly dosing instead of the normal daily dosing is also recommended for poor metabolizers. In the case of thioguanine the DPWG recommends a slightly (15%) smaller dose reduction for intermediate metabolizers compared to the 30–50% dose reduction and thrice weekly dosing advised in the CPIC guideline (Table 7.4).

Analysis of differences

From the 19 guidelines published by CPIC (covering 40 gene-drug pairs) and 86 guidelines by the DPWG, 27 guidelines cover the same gene-drug pairs. Based upon the comparison of the guidelines, there is substantial agreement between the recommendations given by the two consortia. However, for 13 gene-drug pairs there are differences ($\geq 20\%$) in therapeutic recommendations for one or more aberrant phenotypes. Most of the observed differences in therapeutic recommendations probably result from differences in applied methodologies. In some cases this results in a situation where CPIC provides a recommendation for a gene-drug combination based on an expert consensus formed for a group of drugs (e.g. TCAs), whereas the DPWG finds the evidence for certain individual gene-drug combinations insufficient and is unable to calculate a dosing recommendation (e.g. *CYP2C19* and amitriptyline). In other cases both consortia recognize a gene-drug

interaction, but come to a different dosing recommendation for a certain phenotype as a result of the used methodology (e.g. the CYP2C19 poor metabolizer and imipramine). A second explanation of the differences between the CPIC and DPWG are the result of a “time effect,” as literature searches are performed at different time points by the two consortia and new articles are published continuously (category IV, see Table 7.4). For example, a difference exists in the therapeutic recommendation for fluvoxamine between the guideline for *CYP2D6* and *CYP2C19* genotypes and dosing of SSRIs of CPIC from 2015 and the DPWG guideline for fluvoxamine. This difference can partially be explained by the article of Suzuki et al. which was not included in the DPWG guidelines because it was published after the literature search of the DPWG (64). This article showed a significant effect of *CYP2D6* genotype on fluvoxamine steady-state concentration. This example underscores the need to update existing recommendations.

Additionally, differences in therapeutic recommendations are sometimes the result of differences in clinical practices between countries (category V, see Table 7.4). An example of this difference can be seen in the recommendations for the gene-drug combination of *CYP2C19* and voriconazole (6, 7, 32). The CPIC recommends to choose an alternative agent that is not dependent on CYP2C19 metabolism in poor and ultra-rapid metabolizers and a standard regimen in intermediate metabolizers. Therapeutic drug monitoring is mentioned as a factor that can warrant a change in dosing regimen or choice of drug similar to other clinical factors (e.g. drug-drug interactions or impaired renal/hepatic function) that can lead to change in selection of therapy or dose adjustments. Therapeutic drug monitoring is only mentioned specifically in the therapeutic recommendation for a poor metabolizer in the event that voriconazole is considered more appropriate than alternative agents. In contrast, the DPWG provides the recommendation to start with a standard of care dosing and always follow-up with therapeutic drug monitoring in case of poor and intermediate metabolizers. In case ultra-rapid metabolizers a 50% increase of the starting dose is recommended followed by therapeutic drug monitoring. These differences between the guidelines clearly show a difference in the place of therapeutic drug monitoring within voriconazole therapy between the different practice settings.

Another example of differences as a result of clinical practice can be seen in the recommendations for coumarins. The DPWG and CPIC both provide recommendations for the gene-drug pairs of *CYP2C9* and *VKORC1* and warfarin. In addition the DPWG also provides therapeutic recommendations for acenocoumarol & phenprocoumon which are mainly used in the Netherlands (6, 7, 17, 18). The CPIC provides recommended daily maintenance dosing regimen for warfarin in mg/day based on specific algorithms, while the DPWG guidelines only provides a decrease in the loading dose. In the Netherlands,

patients using coumarins are strictly monitored by anticoagulation clinics using the international normalized ratio (INR). With this strict control of the INR, the need for a predicted daily coumarin dose based on pharmacogenetic information is limited. Due to the large difference in the monitoring of patients the added clinical value of pharmacogenetics is different in both settings, resulting in other therapeutic recommendations of the DPWG and CPIC, respectively.

Specifically for *CYP2D6*, differences in the translation of genotypes to phenotypes that exist between the guidelines of the two consortia can be explained by the different interpretations of certain genotypes throughout literature. For the gene *CYP2D6*, some consider an AS of 1.0 an intermediate metabolizer, while the package insert of the AmpliChip categorizes this score as a normal metabolizer (56). In part this is due to variability in how AS is translated into phenotype for different probe drugs. Studies using tramadol, dextromethorphan and sparteine as the probe drug show no difference in the kinetic profile between individuals with and AS of 1.0 and individuals with an AS of 2.0 (54, 55, 65-69). In contrast, using trimipramine, doxepine, haloperidol and debrisoquine as the probe drug shows a significant difference between individuals with an AS 1.0 and 2.0 (54, 70-75). Specifically for *CYP2D6*, it can be concluded that the use of different model substrates have led to mixed interpretations of genotypes which in turn have led to different interpretations of the AS of 1.0 by the two consortia. In fact, an international team of CPIC and DPWG members has recently agreed to try to impose standards on how AS are interpreted into phenotypes for major *CYP2D6* substrates.

As previously mentioned, the differences between guidelines can potentially lead to differences in dosages or choice of drugs for patients with the same genotype. In case of some discordances a minor update (e.g., an update of the status of the *CYP2C9*8* and *CYP2D6*36* alleles in the DPWG guidelines) (category I, IV) can solve discrepancies, while in some cases harmonization is warranted to create uniform interpretations of genotypes into phenotypes (category II).

Finally, the difference in publication strategy should also be addressed. A current disadvantage of the DPWG guidelines is the limited availability in English. In addition, the available English versions date back to 2011 and do not contain the most recent information. Currently, as a part of the EU granted Ubiquitous Pharmacogenomics (U-PGx) project, the DPWG is working on the English translation of their therapeutic recommendations and it is anticipated that in the near future these documents will be made available to clinicians of other nations in the form of European guidelines which further strengthens the need for harmonization with CPIC (<http://upgx.eu/>) (76).

Conclusion

In conclusion, this comparison shows that the CPIC and the DPWG guidelines are generally similar in terms of allele classification, genotype to phenotype translations and therapeutic recommendations for most gene-drug pairs. However, some differences between the guidelines of the two consortia exist and should be harmonized where possible, especially in the case of different allele classifications and genotype to phenotype translations.

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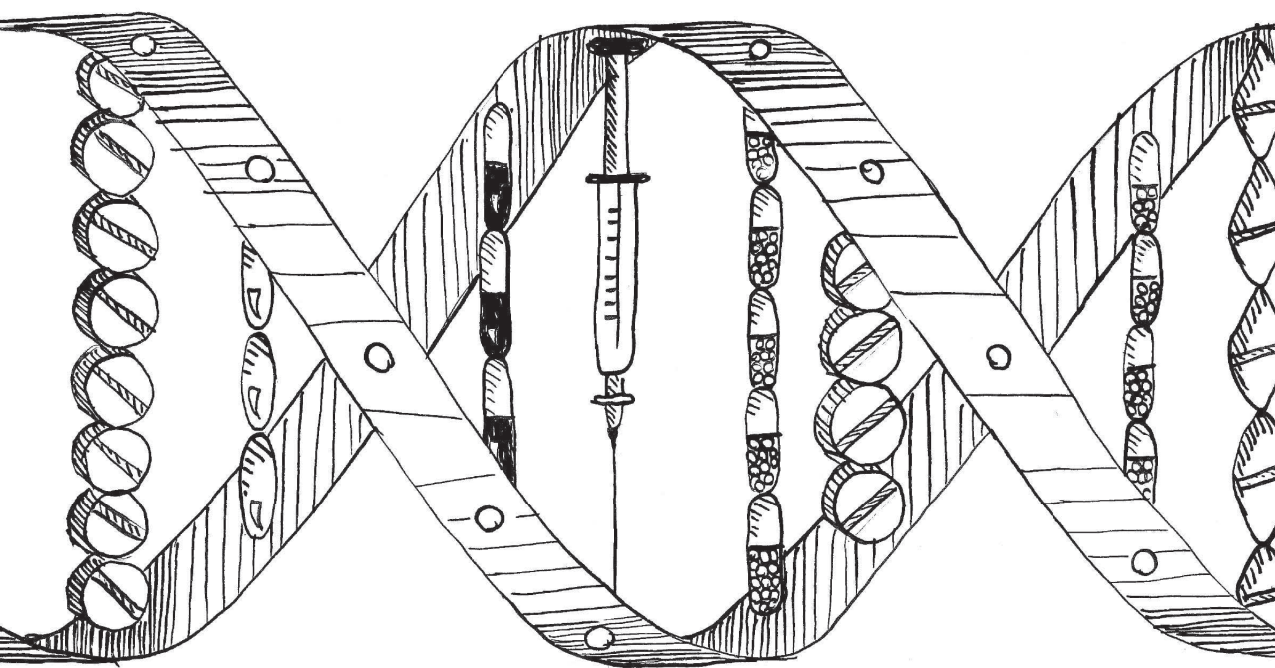
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Part IV

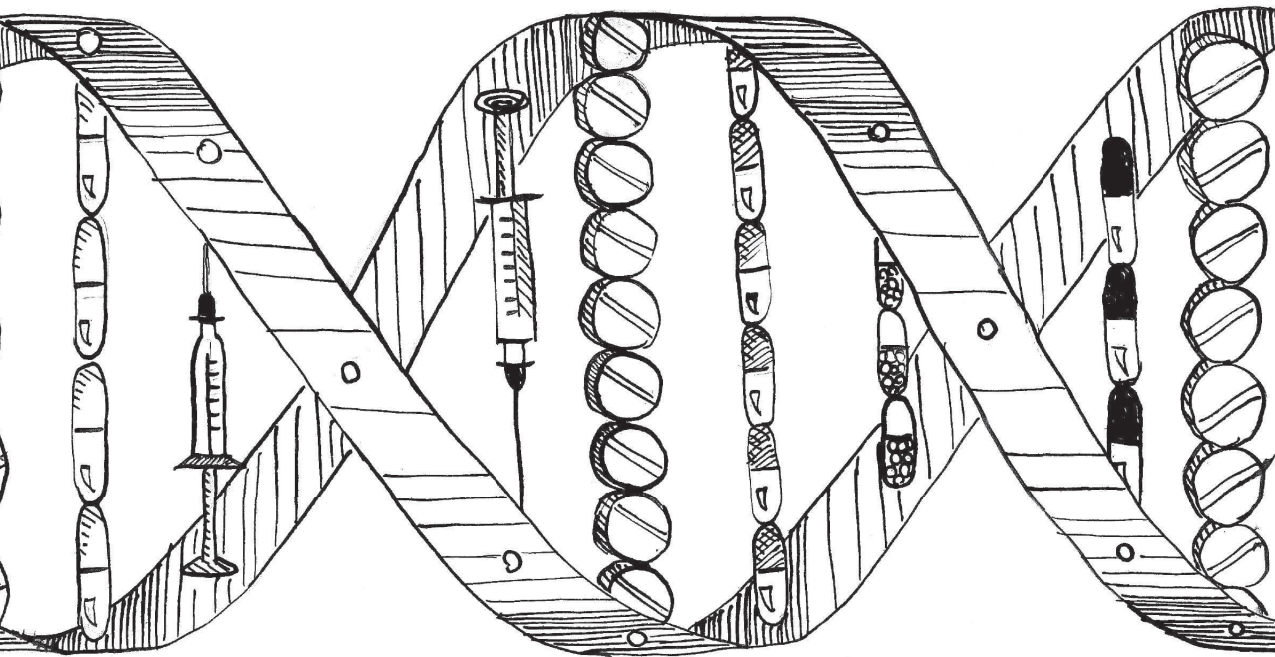
Knowledge, experience and
attitudes towards PGx
of pharmacists and
pharmacy students



Chapter 8

A nationwide survey of pharmacists' perception of pharmacogenetics in the context of a clinical decision support system containing pharmacogenetics dosing recommendations

Bank PCD, Swen JJ, Guchelaar HJ



Abstract

To benchmark Dutch pharmacists knowledge, experience, and attitudes towards PGx with a specific focus on the effects of awareness of the Dutch Pharmacogenetics Working Group guidelines. A web-based survey containing 41 questions was sent to all certified Dutch pharmacists. 667 pharmacists completed the survey (18.8%). Virtually all responders believed in the concept of PGx (99.7%). However, only 14.7% recently ordered a PGx test (≤ 6 months), 14.1% felt adequately informed and 88.8% would like to receive additional training on PGx. Being aware of the DPWG guidelines did not have any significant effect on knowledge or adoption of PGx. Dutch pharmacists are very positive towards PGx. However, test adoption is low and additional training is warranted.

Introduction

The field of pharmacogenetics (PGx) has progressed significantly with a large number of studies showing the relation between heritability and drug-response. In the United States and European Union currently 137 and 77 labels of registered drugs contain information on PGx respectively (1, 2). Moreover, initiatives by the Dutch Pharmacogenetics Working Group (DPWG) and Clinical Pharmacogenetics Implementation Consortium (CPIC) have provided guidelines containing drug/dose recommendations for a significant number of drug-gene-interactions (DGI's) (3-5).

With their knowledge on pharmacology, reputation as medication experts, and overview of drug use by their patient's, pharmacists are alleged to play a key role in the implementation of PGx into clinical care (6). However, despite their excellent position, pharmacists may not yet feel fully prepared for this task. A survey among Canadian pharmacists indicates that although they have high expectations towards PGx, only 7.7% felt comfortable interpreting and advising patients based on PGx test results (7). Similar results were found in a survey among US physicians where 10.3% of the responders felt adequately informed about PGx testing (8). Both these results stress the existence of a knowledge gap hindering the clinical uptake of PGx.

In The Netherlands, PGx guidelines developed by the DPWG providing clear cut recommendations for patients with a known genotype are available at point-of-care by incorporation into computerized systems for drug prescription, dispensing, and automated medication surveillance (Figure 8.1) (3). These computerized systems are used by all pharmacists working in a clinical setting in the Netherlands (in both primary and secondary care). The availability of DPWG guidelines in the routine workflow of healthcare professionals through interruptive clinical decision support (CDS) may reduce the perceived knowledge-gap and result in a higher clinical uptake of PGx (9, 10). The aim of this study was to benchmark Dutch pharmacists about knowledge, experience, and attitudes towards PGx. We specifically focused on investigating if the incorporation of DPWG recommendations on DGI's into CDS leads to reduction in the perceived knowledge gap, a reduction in the need of additional training on the subject and higher adoption of PGx testing.

Methods

Study design

A nationwide web-based cross-sectional survey was performed using the survey tool NetQ (11). Community, hospital and outpatient pharmacists in the Netherlands were

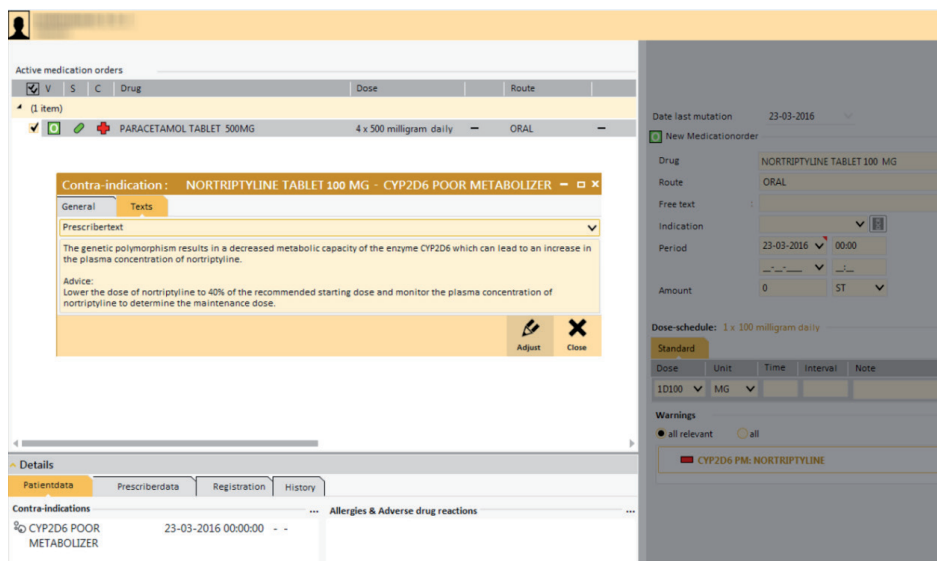


Figure 8.1: An example of an pop-up generated through clinical decision support.

A typical alert generated by automated medication surveillance after prescription of nortriptyline to a patient known to be a poor metabolizer of *CYP2D6* (condensed & translated from Dutch).

invited to participate by an email sent via their professional societies, with a reminder after two weeks. Community pharmacists were defined as pharmacists working in a primary healthcare setting outside of a hospital. Hospital pharmacists were defined as pharmacists working in a secondary healthcare setting within a hospital. Outpatient pharmacists were defined as pharmacists dispensing to outpatients from a pharmacy located in a hospital. Participants had the opportunity to complete the survey in the period from 15 November 2014 until 1st of January 2015. Responding to the invitation was completely voluntary and results were processed anonymously.

Questionnaire

A questionnaire was constructed by adapting two published surveys and translating the questions to Dutch (7, 8, 12, 13). A brief introduction explaining the terms pharmacogenetics & pharmacogenomics and the topics that would be surveyed preceded the questionnaire. The questionnaire consisted of a total of 41 questions (Supplementary Document S8.1).

Survey analysis

The survey responses of the participants were automatically tabulated and stored within NetQ [101]. Incomplete surveys were excluded from the analysis. Age was recoded into a six-level categorical variable (≤ 29 , 30–39, 40–49, 50–59, and ≥ 60 years). Practice setting was recoded into a three-level variable: community pharmacy + other, outpatient pharmacy, hospital pharmacy.

Due to the low rate of responses in some answer categories the results of the questions 7–13 and 38 were condensed for the univariate and multivariate analysis into a two-level (Q 7–13) and three-level (Q38) scale respectively (8).

The univariate analyses were performed using a χ^2 test (excl. Q36). Variables that showed a significant association in the univariate analysis were included in the multivariate analyses. For the univariate and multivariate analysis of past adoption the variable future adoption was not included and for the analyses of future adoption the group of past adopters were excluded (8). Data was analysed with SPSS version 20 (SPSS, Inc., Illinois, USA) and $p < 0.05$ was considered statistically significant.

Results

Characterization of responders

In total 3,550 pharmacists were invited to complete the questionnaire (596 hospital pharmacists, 171 outpatient, 2,780 community pharmacists and other). Of the invited pharmacists, 727 participants responded to the link and 667 (18.8%) completed the survey. Of the 667 responders 54.3% was female. Age was distributed bimodally with a median age of 41. The practice setting of the majority of the responders was community-based (69.6%), while 24.6% and 4.8% worked in a hospital or outpatient pharmacy respectively (Table 8.1). Of note, in the Netherlands pharmaceutical care in a secondary healthcare setting is delivered by hospital pharmacists. Unlike other countries, specialization does not focus on areas of medicine but on the different task within hospital pharmacy i.e. drug manufacturing, quality control, therapeutic drug monitoring, drug dispensing etc. In the primary healthcare setting drugs are dispensed to patients either by community pharmacies or outpatient pharmacies. Examples of “other” practice settings consisted of regulatory bodies, industry, and (temporarily) non-practising pharmacists.

The response rate among hospital pharmacists was significantly higher than the response rate among pharmacists working in the community + other or outpatient setting (27.5% vs. 16.9% and 18.4% respectively, $p < 0.001$). In the Netherlands all pharmacists receive

Table 8.1: Characteristics of responders

Characteristic	N	%
Gender		
Male	305	45.7
Female	362	54.3
Age		
20–29	105	15.7
30–39	209	31.3
40–49	144	21.6
50–59	158	23.7
≥ 60	51	7.6
Possession of specialty		
Yes	549	82.3
In residency	85	12.7
No	33	4.9
Practice setting		
Outpatient	32	4.8
Hospital	164	24.6
Community + other (*)	471	70.6

* For statistical purposes the group of responders working in a community setting (n = 464) and the group of responders working in a setting other than community, outpatient or hospital (n = 7) were combined.

six year university training resulting in a Pharm D degree. Afterwards pharmacists can enroll in a two or four year post-graduate specialty training to obtain a registration as community or hospital pharmacist respectively. Of the responders 12.7% were in a post-graduate residency programme to obtain a license. During their PharmD program or their postgraduate training 39.7% and 24.4% of the responders received any form of PGx training respectively (Supplementary Document S8.1, S8.2).

Belief in concept of PGx and expectations towards PGx testing

Virtually all responders (99.7%) believed that the genetic profile of a patient can influence the response to medication (Figure 8.2). To assess their expectations towards PGx testing pharmacists were asked three questions. On the question whether a PGx test could prevent their patients from taking the wrong dose or drug 80.8% of the responders answered at least 2 on a scale from 0 (no expectation) to 3 (high expectation). Using the same 4 point scale 84.9% responders answered ≥ 2 on the question whether PGx could detect which drug or dose will be more efficacious in their patient and 81.3% answered ≥ 2 on the question whether a PGx test will allow detection which drug or dose will cause less side effects (Figure 8.3 and Supplementary Document S8.1).

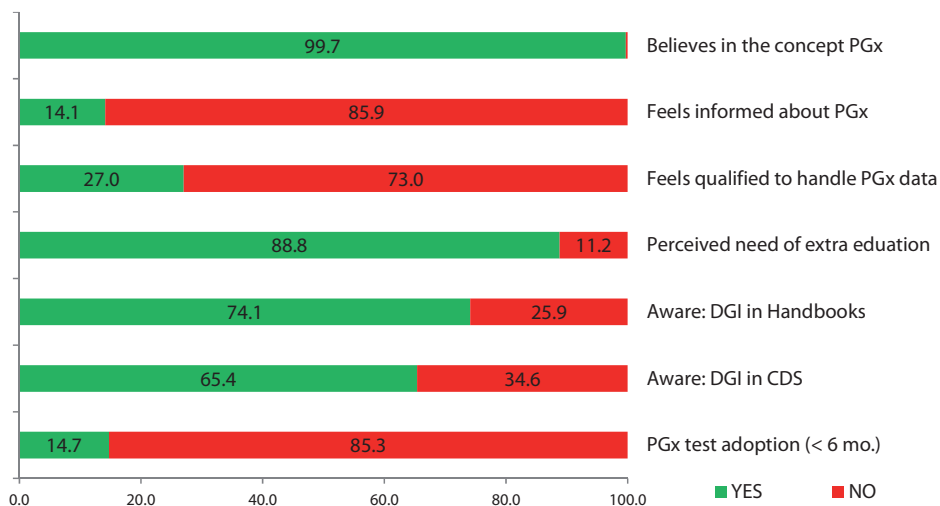


Figure 8.2: Key findings of this study to benchmark the knowledge of -, experience with - and attitude of Dutch pharmacists towards PGx testing in %.

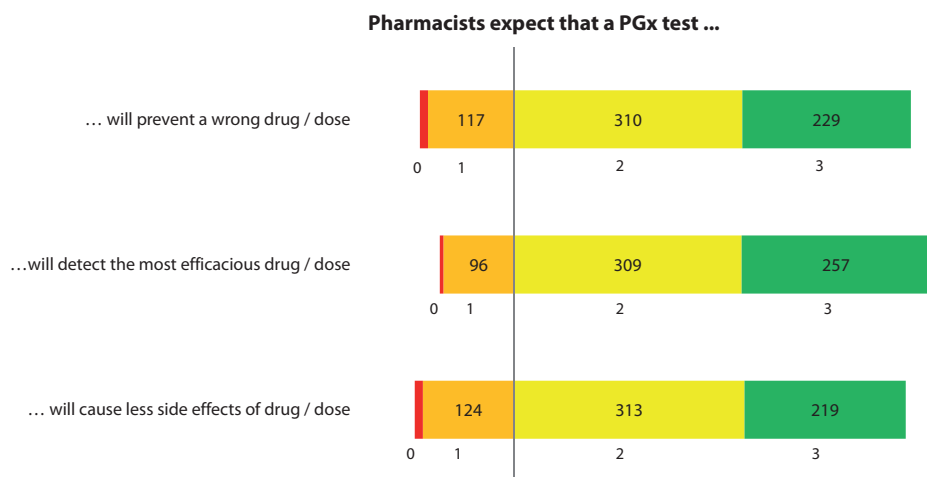


Figure 8.3: Expectations of pharmacists towards PGx testing.

Red = I have no expectations that PGx ..., orange = I have low expectations that PGx ..., yellow = I have high expectations that PGx ..., green = I have very high expectations that PGx ... (the size of the bar is proportional to the number of responders).

Adoption of PGx

In the Netherlands all pharmacists in clinical practice (community, outpatient and hospital setting) are allowed to order PGx tests directly or recommend PGx testing to the prescribing physicians. In this survey only 98 responders (14.7%) reported ordering or recommending

a PGx test in the last six months (Figure 8.2). The majority did so to improve the drug therapy of a patient (92.9%) and stated that the PGx test improved drug efficacy (52.0%), reduced toxicity (74.5%), improved the patients' understanding of their disease (18.4%), or improved the adherence of the patient to the treatment (6.1%). Of these past adopters of PGx testing 79.6% expected to order or recommend equal or more PGx test in the next six months, while 12.2% stated that they expect to order less PGx test in the near future. Of the 569 responders (85.3%) who did not order or recommend a PGx test in the last six months 71.7% stated that they did not expect to do so in the next six months. Overall, 27.1% of the surveyed pharmacists expected to order or recommend a PGx test in the next six months. Of the responders who had not adopted PGx testing or did not anticipate ordering or recommending a PGx test in the coming six months, the majority (60.3%) indicated a lack of knowledge and information on PGx testing as primary reason. Other main reasons for not ordering or recommending a PGx test consisted of "not dispensing drugs where PGx is relevant" (25.6%) or uncertainty regarding reimbursement of PGx (14.0%). From the PGx test adopters 8 stated that they did not expect to order a PGx in the next six months. Reasons given by these past adopters to not order a PGx test in the near future consisted of a lack of knowledge, resistance of patients towards PGx testing, alternate methods for monitoring of patients and limited clinical relevance.

Working in a hospital setting (OR 9.44, CI 4.13–21.57, $p < 0.001$) was the only variable independently associated with past use of PGx testing. Feeling informed about PGx testing (OR 1.99, CI 0.99–3.99, $p = 0.052$), the use of genetic laboratories as sources of information for availability of PGx tests and application in treatment (OR 2.16, CI 0.99–4.71, $p = 0.054$) and the use of other sources of information to guide drug treatment (OR 2.92, CI 0.98–8.71, $p = 0.054$) showed a trend towards significance with past adoption of PGx testing. Being aware of the existence of the DPWG guidelines (OR 1.00, CI 0.38–2.67, $p = 0.999$) and their integration in the electronic prescription and dispensing systems (OR 1.23, CI 0.57–2.69, $p = 0.596$) did not show an independent associations with past use of adoption (Supplementary Document S8.3). The multivariate analysis for future adoption indicated that working in a hospital setting (OR 5.85, CI 2.67–12.82, $p < 0.001$) or outpatient setting (OR 3.01, CI 1.05–8.57, $p = 0.039$), feeling comfortable to recommend a PGx test to a patient to predict whether a drug is effective in their case (OR 2.18, CI 1.03–4.64, $p = 0.043$), using post-academic education as source of information for availability of pharmacogenetic tests and how to apply them in pharmacotherapy (OR 2.91, CI 1.36–6.23, $p = 0.006$) and having high worries that there is no suitable drug for their patients (OR 1.31, CI 1.06–1.611, $p = 0.011$) were independently associated with future adoption of PGx testing (Supplementary Document S8.3). Differences between adopters, future adopters and non-adopters can be seen in Supplementary Document S8.4.

Attitude towards own ability to interpret PGx test results

Currently, 48.4% of the participants would feel comfortable to recommend a PGx test to predict drug efficacy. However, if the PGx test could also reveal information about disease risk only 7.8% would feel comfortable to do so. Of the surveyed pharmacists, 27.0% would feel qualified to receive PGx test results of a patient, interpret them and advise a patient or treating healthcare professional on the choice of drug or dose based on the results (Figure 8.2). Reflecting the questions informing about education and training, the majority of pharmacists (66.3%) would see themselves qualified after receiving training on the subject. Participants were also asked if they would recommend a drug treatment despite a PGx test result indicating non-response or severe side effects for their patient. Approximately half of the responders (49.0%) would not recommend the treatment, while a slightly smaller group (47.5%) would recommend the drug treatment provided it concerned a life-threatening disease. Only 3.4% would recommend the drug irrespectively of the results of the PGx test or condition of the patient.

Access to and use of PGx information

Although approximately half of the pharmacists received some sort of PGx training (see above) only 14.1% felt adequately informed about the availability of PGx tests and how to apply PGx in relation to drug therapy. Furthermore, almost all responders (88.8%) indicated they would like additional training on PGx (Figure 8.2). Responders indicated using the summary of product characteristics (SmPC), the European drug package insert (78.7%), internet (63.2%), colleagues (38.8%), post-academic courses (30.6%), genetic laboratories (24.0%) or other such as the Informatorium Medicamentorum (IM), a handbook published by the The Royal Dutch Pharmacist's Association (KNMP) containing the DPWG recommendations in addition to information on drug dosages, (contra) indications, drug-drug-interactions, (25.6%) as sources of information for availability of PGx tests and application in treatment. Concerning the evidence for adoption of PGx testing recommendation by a guideline (93.0%), scientific publication (81.3%), approval or recommendation by regulatory authorities (80.4%) were seen as high value by responders, while less responders indicated recommendation by opinion leaders as high value (46.7%).

The majority of the participants (88.0%) would use the IM to make a choice about the drug and dose in case of a patient with genotype results. Other sources indicated to guide drug treatment included the SmPC (61.0%), scientific literature (58.3%), Farmacotherapeutisch Kompas (FK), a handbook containing information on drug dosages, (contra)indications, drug-drug-interactions published by The National Health Care Institute in the Netherlands (34.2%), regulatory authorities (25.9%) or a colleague (22.9%). Only 10 pharmacists (1.5%)

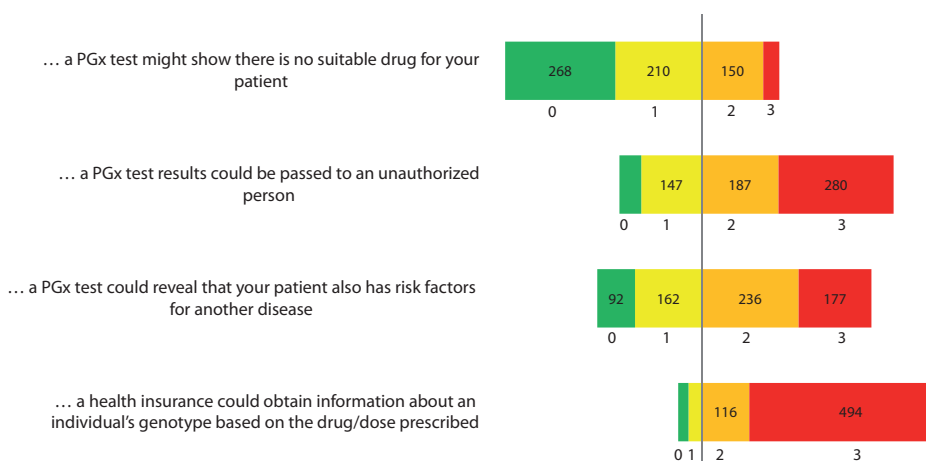
had encountered a patient who had ordered a genetic test on their own account in the preceding six months (Supplementary Document S8.1).

74.1% of the responders were aware of the existence of the DPWG dosing guidelines and 65.4% was aware that these guidelines were incorporated in CDS systems (Figure 8.2). Responders who were aware of the guidelines and their integration in the CDS systems, were more likely to be early adopter ($p < 0.001$) and more likely to feel informed about PGx testing ($p < 0.001$). A subgroup analysis of pharmacy specialties showed that only hospital pharmacists were more likely to feel informed about PGx testing if they were aware of the DPWG guidelines ($p < 0.05$). In the multivariate analysis of “feeling informed about PGx testing” working in a hospital setting (OR 3.67, CI 1.57–8.55, $p = 0.003$), not being in need for additional training (OR 2.96, CI 1.38–6.33, $p = 0.005$), scoring “undecided” or “(very) important” for approval or recommendation of a PGx test by regulatory authorities (OR 42.28, CI 3.59–498.48, $p = 0.003$ and OR 14.38, CI 1.33–155.55, $p = 0.028$), still advising the only available drug to treat your patient’s disease if a pharmacogenetic test revealed ineffective or leads to severe side effects (OR 8.54, CI 2.25–32.42, $p = 0.002$) and still advising the only available drug to treat your patient’s life-threatening disease if a pharmacogenetic test revealed ineffective or leads to severe side effects (OR 0.46, CI 0.24–0.86, $p = 0.015$) were independently associated with feeling informed about PGx testing. No independent association between being aware of the DPWG guidelines (OR 1.36, CI 0.51–3.65, $p = 0.537$) and their incorporation in the electronic medication systems (OR 1.84, CI 0.82–4.13, $p = 0.139$) with feeling informed about PGx testing was observed (Supplementary Document S8.3).

In a similar multivariate analysis factors associated with the perceived need of extra PGx training were studied. The analysis revealed that feeling qualified to receive PGx test results of a patient, interpret them and advise upon PGx test results after training (OR 3.70, CI 1.41–9.76, $p = 0.008$) and feeling not informed about PGx testing (OR 2.40, CI 1.17–4.90, $p = 0.016$), using the SmPC as source of information to guide drug treatment (OR 1.85, CI 1.05–3.26, $p = 0.032$) were independently associated with the need for additional training on PGx related subjects. Being aware of the existence of the DPWG guidelines (OR 0.89, CI 0.41–1.95, $p = 0.770$) and the incorporation in CDS (OR 1.03, CI 0.51–2.08, $p = 0.927$) showed no significant association with a reduced need for training (Supplementary Document S8.3).

Worries related towards PGx testing, privacy and coverage

Participants were asked a total of eight questions concerning worries related to consequences of PGx test results, privacy of PGx data and coverage of PGx tests by insurance

Pharmacists are worried that ...**Figure 8.4: Worries of pharmacists towards PGx testing.**

Green = I have no worries that ..., yellow = I have low worries that ..., orange = I have high worries that PGx ..., red = have very high worries that PGx ... (the size of the bar is proportional to the number of responders).

companies. Four questions assessed potential concerns related to PGx using a 4-point scale ranging from 0 (no worries) to 3 (very worried). To the question inquiring to whether a PGx test might reveal that there is no suitable drug available for their patients 28.3% of the participants answered that they were at least moderately worried (score ≥ 2). Of the responders 52.9% was at least moderately worried that a PGx test might reveal that a patient has risk factors for another disease. Furthermore, 70.0% of the responders was at least moderately worried that results of a PGx test could come in hands of an unauthorized individual and the majority of the responders (91.4%) was at least moderately concerned that a health-insurance company could infer a patients' genotype based on the prescribed drug or dose (Figure 8.4).

In line with the four previous questions, 76.9% of the responders are more concerned about the loss of privacy of patients' PGx test results than the results from any other laboratory or diagnostic test. With regard to privacy of the data responders agreed that the treating physician (99.3%) and the pharmacist (97.3%) should have access to the PGx test results, while nurses, psychologists and dieticians should not. The opinion whether other health-care professionals should be able to access PGx data was mixed (Supplementary Document S8.2). With regard to unfavourable results almost two-thirds of the responders (63.7%) thought that a PGx test with negative test result could have a possible adverse psychological effect on a patient and/or the patients' family. The final question of this

section related to coverage of PGx by health-insurers. Virtually all responders (99.7%) stated that health-insurance companies should provide coverage for PGx tests, however there is a mixed opinion whether PGx should always be covered (69.1%) or only in specific occasions (30.6%).

Differences between pharmacy specialties

Survey results of the different practice settings were compared and significant differences were observed between the three groups. The group of responders working in a community + other setting contained relatively more individuals of age ≥ 50 ($p = 0.003$). Hospital pharmacists had received post-graduate training on PGx during their specialization more often compared to the community + other and outpatient setting (60.4% vs. 12.7% vs. 12.5%, $p < 0.001$), were more often aware of the DPWG guidelines (91.5% vs. 67.3% vs. 84.4%, $p < 0.001$) and their integration in the CDS systems (79.9% vs. 60.3% vs. 65.6%, $p < 0.001$) compared to the other two groups. Furthermore, hospital pharmacist felt comfortable to recommend PGx testing more often (74.4% vs. 40.1% vs. 37.5%, $p < 0.001$), felt qualified to receive PGx testing results, interpret them and advise a patient based on PGx test results more often (59.1% vs. 16.3% vs. 18.8%, $p < 0.001$) and felt less need for training on the subject (24.4% vs. 7.0% vs. 6.2%, $p < 0.001$) compared to the community pharmacy + other and outpatient setting. Other differences between specialties can be seen in Supplementary Document S8.2.

Discussion

This survey shows that Dutch pharmacists are generally very positive towards PGx. Virtually all responders believe that indeed drug response can be at least partially explained by genetic variation and the majority of the participants have high expectations of PGx. We hypothesized that incorporation of the DPWG guidelines in automated CDS systems would lead to higher adoption of PGx, while simultaneously reducing the need for additional PGx training (9). However, the results from the current study show that being aware of the availability of the DPWG guidelines was not independently associated with feeling informed about PGx testing or past adoption of PGx testing. Furthermore, the percentage of PGx test adoption is comparable to the findings of a survey among physicians in the US (14.7% vs. 12.9%), where mostly information on DGI's is not readily available in CDS systems. Similar to the US, there appears to be a knowledge gap present among the Dutch pharmacists, as only 14.1% of the responders in this survey feels adequately informed about PGx testing. Similar to the association between feeling informed and adoption of PGx testing found

in the study by Stanek et al. ($p < 0.001$) our survey shows a trend towards an association between feeling informed and PGx test adoption (8). The combined data from both these studies indicate that a lack of knowledge about availability of PGx tests and their application in drug therapy is one of the remaining barriers for clinical implementation of PGx into clinical practice. The current study confirms that a large amount of responders would like additional training on the subject and that being aware of the availability of the DPWG guidelines was not associated with a reduced need for additional PGx training. A similar need for training (96.6%) is seen in a survey performed among 284 pharmacists in Canada, with a similar electronic drug prescribing and dispensing system as used in the US (7).

It appears that PGx implementation programmes should be accompanied by extensive training programmes as currently implemented in the 1200 patient project and the programmes PG4KDS, eMERGE-PGx, PREDICT and U-PGx (14-18). In all, the results from this study combined with findings from previous studies refute our hypothesis that nationwide availability of interruptive CDS containing dosing advices on DGI's leads to higher adoption of PGx and reduces the need for additional training.

Our results indicate a need of more education about PGx. Only 39.7% and 24.4% of the responders received training on PGx-related subjects in their PharmD program or their postgraduate training respectively. A stronger embedding of PGx in the curricula of pharmacists in training could be a manner to prevent a potential knowledge gap in future generations of pharmacists. An example could be by integrating PGx into courses that train PharmD students in medication surveillance as in our opinion PGx should be an integral part of this area within pharmacy practice. Post-academic education is also considered important by the responders for disseminating PGx knowledge, and was associated with adoption of PGx in the near future (OR 2.91, CI 1.36–6.23, $p = 0.006$). However an ideal setting for a PGx training for (community & outpatient) pharmacists remains to be established. Additional research in the form of targeted surveys, structured interviews and / or focus groups could further provide a more detailed answer how specific demands for PGx related information and education can be met.

The current cross-sectional study evaluated both the current attitude of Dutch pharmacists towards PGx and their own perceived abilities to interpret PGx data, as well as actual adoption of PGx testing by pharmacists. The data indicate that approximately 80% of the pharmacists are moderately hopeful that PGx could prevent patients receiving wrong drug or doses; could detect which drug or dose is the most effective for a patient and that PGx could minimize the risk of adverse events. The survey of de Denu et al. shows similar ratings of 80.0%, 82.6% and 79.1% of Canadian pharmacists who are at least moderately hopeful on these subjects respectfully. Dutch pharmacists also have similar worries

(moderate to high) compared with their Canadian colleagues (7).

The results from this survey further show that adoption of PGx is particularly low in the community pharmacy setting, although previous research has shown that implementation of PGx in primary care is feasible (19). In the Netherlands, pharmacists working in primary care are considered the gatekeepers of patients' medication records. Prescriptions from multiple prescribers for the same patient converge in community or outpatient pharmacies. Therefore, community and outpatient pharmacists are the designated healthcare professionals to perform medication surveillance based on therapeutic recommendations from the G-standard, the nationwide electronic drug database. In our opinion PGx is an integral part of medication surveillance and therefore these pharmacists should understand a PGx test result at a phenotype level and to be able to optimize therapy based on CDS alerts. In addition, as experts in the area of pharmacology pharmacists could take a leading role in genotyped guided therapy by recommending or ordering test when this is indicated.

The low adoption might be explained by the lack of consensus on which specific patients should be tested. For several drug treatments typically applied in the hospital setting i.e. fluoropyrimidine derivatives or purine-antimetabolites, there is high quality evidence from prospective studies showing that genotyping for mutations in *DPYD* and *TPMT* improves outcome of drug treatment (20, 21). However, for many drugs frequently used in primary care such evidence is still lacking. In addition, currently available pharmacogenetic guidelines by the DPWG and the CPIC only provide recommendations for patients with a known genotype and do not indicate which patients should be genotyped. Future versions of the DPWG guidelines containing information on when genotyping is indicated in combination with clinical rules or pre-test alerts could further help with the implementation of PGx testing.

For this cross-sectional survey a voluntary basis was used introducing the potential risk of bias as strongly opinionated (both in a positive and in negative manner) or experienced individuals are more likely to respond introducing selection bias. From "The Dutch Foundation for Pharmaceutical Statistics (SFK)", an organization which gathers data from a panel which includes 95% of the community and outpatient pharmacies in the Netherlands, demographic data on pharmacists working in the community and outpatient setting was obtained. The distribution of responders to this survey working in a community or outpatient setting between the different age groups (≤ 29 , 30–39, 40–49, 50–59, and ≥ 60 years) differed significantly from the distribution of all Dutch pharmacists among these age groups ($p < 0.001$). In the surveyed population a higher relative count of pharmacist was found in the combined group of 20–39 (44.0% vs. 37.8%) compared to the combined group of 40+ (56.0% vs. 62.2%) in a χ^2 analysis ($p = 0.009$). The male / female ratio and

the ratio between specialty trained and in training did not differ significantly (22). The age distribution of responders working in a hospital setting among the five age groups also differed significantly from the age distribution of the whole population of hospital pharmacists over the five age groups ($p < 0.001$). Responders were also significantly younger compared to all pharmacists working in a hospital setting when the age groups were combined in the groups 20–39 (56.2% vs. 45.1%) and 40+ (43.5% vs. 54.9%) and analysed with a chi² analysis ($p < 0.012$). Demographic data on the population of hospital pharmacists was obtained from the professional society of hospital pharmacists, (www.nvza.nl) (23). Likewise, no differences in gender and the ratio between specialty trained and in training were observed. Therefore, our data may not be fully applicable to the older pharmacists. The percentage of responders among the hospital pharmacists was significantly higher compared to the other two groups. In the Netherlands, several hospitals have prospective genotyping programmes for *TPMT* and *DPYD* and hospital pharmacists may therefore have more experience with PGx testing (24). As a result, our estimate of adoption of PGx among the whole population of Dutch pharmacists may be too optimistic. However, compared to the two previous surveys our study shows a higher response rate (18.8% vs. 2.59% and 6.76%) that reduces the risk of selection bias (7, 8).

Conclusion

This survey shows that adoption of PGx among Dutch pharmacists is still low and despite the nationwide availability of interruptive CDS containing the DPWG guidelines Dutch pharmacists still perceive a lack of knowledge on the subject that remains to be an important barrier for PGx test adoption.

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Supplementary Document S8.1: Questionnaire + results per question

Baseline			
		N	%
Q1: What is your gender?	☐ Male	305	45.7
	☐ Female	362	54.3
		N	%
Q2: What is your age?	☐ 20–29	105	15.7
	☐ 30–39	209	31.3
	☐ 40–49	144	21.6
	☐ 50–59	158	23.7
	☐ ≥ 60	51	7.6
		N	%
Q3: Are you in possession of a specialty or are you in training for a specialty?	☐ Yes, I am in possession of a specialty	549	82.3
	☐ Yes, I am in training for specialty	85	12.7
	☐ No, I do not possess a specialty and are not in training for a specialty	33	4.9
		N	%
Q4: What is your practice setting?	☐ Community pharmacy	464	69.6
	☐ Hospital pharmacy	164	24.6
	☐ Outpatient pharmacy	32	4.8
	☐ Other ...	7	1.0
		N	%
Q5: Which department carries out the majority of the pharmacogenetic test for diagnostic purposes?	☐ Hospital pharmacy laboratory	19	11.6
	☐ Clinical chemistry laboratory	30	18.3
	☐ Human genetics laboratory	11	6.7
	☐ Samples are determined externally	87	53.0
	☐ Samples are not determined in the hospital and not determined externally	8	4.9
	☐ Other ...	4	2.4
	☐ Hospital pharmacy laboratory	5	3.0
	☐ Unknown	19	11.6
Belief and expectations towards PGx			
		N	%
Q6: Do you believe that a patient's genetic profile may influence his/her response to drug therapy?	☐ Yes	665	99.7
	☐ No	2	0.3
		N	%
Q7: Do you expect that pharmacogenetic testing will prevent your patient from taking the wrong medicine (or the wrong dose)? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	11	1.6
	☐ 1	117	17.5
	☐ 2	310	46.5
	☐ 3	229	34.3

Supplementary Document S8.1: Continued

		N	%
Q8: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will be more efficacious in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	5	0.7
	☐ 1	96	14.4
	☐ 2	309	46.3
	☐ 3	257	38.5
		N	%
Q9: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will cause less side effects in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	11	1.6
	☐ 1	124	18.6
	☐ 2	313	46.9
	☐ 3	219	32.8
Worries toward PGx testing			
		N	%
Q10: Are you worried that a PGx test might show there is no suitable drug for your patient? (0 = not worried / 3 = very worried)?	☐ 0	268	40.2
	☐ 1	210	31.5
	☐ 2	150	22.5
	☐ 3	39	5.8
		N	%
Q11: Are you worried that a PGx test could reveal that your patient also has risk factors for another disease that he/she does not know about? (0 = not worried / 3 = very worried)?	☐ 0	92	13.8
	☐ 1	162	24.3
	☐ 2	236	35.4
	☐ 3	177	26.5
		N	%
Q12: Are you worried that a health insurance could obtain information about an individual's genotype based on the drug/dose prescribed? (0 = not worried / 3 = very worried)	☐ 0	25	3.7
	☐ 1	32	4.8
	☐ 2	116	17.4
	☐ 3	494	74.1
		N	%
Q13: Are you worried that one of your patient's PGx test results could be passed to an unauthorized person? (0 = not worried / 3 = very worried)	☐ 0	53	7.9
	☐ 1	147	22.0
	☐ 2	187	28.0
	☐ 3	280	42.0
		N	%
Q14: Do you think that your patient's unfavorable test results could have adverse psychological consequences on him and his family?	☐ Yes	425	63.7
	☐ No	105	15.7
	☐ No opinion	137	20.5
		N	%
Q15: Are you more concerned about the loss of privacy of a patient's genetic information from the results of pharmacogenetic tests than from the results of other laboratory or diagnostic tests?	☐ Yes	154	23.1
	☐ No	513	76.9

Supplementary Document S8.1: Continued

		Yes		No		
		N	%	N	%	
Q16: Among the following health professionals, which ones should have access to patients' pharmacogenetic information (select all that apply)	☐ Physician	662	99.3	5	0.7	
	☐ Pharmacist	649	97.3	18	2.7	
	☐ Genetic counsellor	517	77.5	150	22.5	
	☐ Clinical chemist	319	47.8	348	52.2	
	☐ Nurse practitioner	113	16.9	554	83.1	
	☐ Psychologist	41	6.1	626	93.9	
	☐ General nurse	13	1.9	654	98.1	
	☐ Social worker	1	0.1	666	99.9	
☐ Dietician	27	4.0	640	96.0		
				N	%	
Q17: Do you believe that health insurers should provide full coverage for pharmacogenetic tests?	☐ Always			204	30.6	
	☐ Sometimes			461	69.1	
	☐ Never			2	0.3	
PGx test adoption						
				N	%	
Q18: At any time in the past 6 months, have you ordered or recommended a pharmacogenetic test?	☐ Yes			98	14.7	
	☐ No			569	85.3	
				N	%	
Q19: Within the past 6 months, with what average frequency have you ordered or recommended a pharmacogenetic test?	☐ 1 time /mo.			77	78.6	
	☐ 2–5 times /mo.			22	22.4	
	☐ >5 times /mo.			7	7.1	
	☐ N/A					
				N	%	
Q20: At any time in the past 6 months, have you ordered or recommended a pharmacogenetic test for ... (select all that apply)		Yes		No		
		N	%	N	%	
		☐ A patient	91	92.9	7	7.1
		☐ Yourself	2	2.0	96	98.0
		☐ A colleague or friend	5	5.1	93	94.9
		☐ A family member?	3	3.1	95	96.9
☐ Other	3	3.1	95	96.9		
☐ N/A						

Supplementary Document S8.1: *Continued*

		Yes		No		
		N	%	N	%	
Q21: Pharmacogenetic tests have benefited your patients by ... (select all that apply)	☐ Improving drug effectiveness	51	52.0	47	48.0	
	☐ Reducing drug toxicity	73	74.5	25	25.5	
	☐ Increasing patients' understanding of their disease/therapy	18	18.4	80	81.6	
	☐ Improving patients' adherence to therapy	6	6.1	92	93.9	
	☐ No tests ordered	6	6.1	92	93.9	
	☐ Patients have not benefited	11	11.2	87	88.8	
	☐ Other	51	52.0	47	48.0	
Q22: Do you anticipate ordering or recommending more, less or no pharmacogenetic tests for patients within the next 6 months				N	%	
				☐ More PGx tests	78	79.6
				☐ Less PGx test	12	12.2
				☐ No tests	8	8.2
Q23: Do you anticipate ordering or recommending a pharmacogenetic test for a patient within the next 6 months?				N	%	
				☐ Yes	181	27.1
				☐ No	486	72.9
		Yes		No		
		N	%	N	%	
Q24: If you have not ordered or recommended a pharmacogenetic test in the past 6 months, or do not anticipate ordering one in the next 6 months, please indicate the main reason why (select one):	☐ Concern over privacy	10	1.7	567	98.3	
	☐ Little-to-no or uncertain value in testing	8	1.4	569	98.6	
	☐ Lack of insurance coverage for testing	81	14.0	496	86.0	
	☐ Not enough knowledge about testing/genomic markers	348	60.3	229	39.7	
	☐ Patients' resistance to genetic testing	11	1.9	566	98.1	
	☐ I do not dispense drugs with PGx tests available or recommended	148	25.6	429	74.4	
	☐ Other	184	31.9	393	68.1	
	☐ N/A					
Q25: Currently, various (pharmaco)genetic tests are available directly to consumers. At any time in the past 6 months, has a patient brought into your office the results of a genome-wide scan obtained on his or her own?				N	%	
				☐ Yes	10	1.5
				☐ No	657	98.5

Supplementary Document S8.1: Continued

Education & training			
		Yes	No
		N	N
Q26: What kind of genetics test(s) had these patients performed? A "whole genome scan" is a scan of the patient's DNA that reveals markers associated with diseases and/or altered response to drug therapy	☐ A test of a single (pharmaco) gene	2	8
	☐ A test of multiple (pharmaco)genes	4	6
	☐ A "whole genome scan"	4	6
		N	%
Q27: At which University did you obtain your PharmD?	☐ University of Groningen	221	33.1
	☐ University of Leiden	38	5.7
	☐ University of Utrecht	357	53.5
	☐ University of Amsterdam	32	4.8
	☐ Other	19	2.8
		N	%
Q28: Was PGx instruction included in your graduate pharmacy education curriculum?	☐ Yes	265	39.7
	☐ No	402	60.3
		N	%
Q29: Was PGx instruction included in your postgraduate specialty training ?	☐ Yes	163	24.4
	☐ No	504	75.6
		N	%
Q30: Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy?	☐ Yes	94	14.1
	☐ No	573	85.9
		N	%
Q31: Would you like to participate in extra training on pharmacogenetics	☐ Yes	592	88.8
	☐ No	75	11.2
Attitude towards own ability to interpret PGx test results			
		N	%
Q32: Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice?	☐ Yes	180	27.0
	☐ Yes, but after having had training on the subject	442	66.3
	☐ No, this is not my responsibility	45	6.7

Supplementary Document S8.1: Continued

		N	%		
Q33: Would you feel comfortable to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case?	☐ Yes	323	48.4		
	☐ No	164	24.6		
	☐ Undecided	180	27.0		
		N	%		
Q34: If a pharmacogenetic test revealed that the only available drug to treat your patient’s disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?	☐ Yes	23	3.4		
	☐ No	317	45.7		
	☐ Yes, only if he/she had a life-threatening disease	327	49.0		
		N	%		
Q35: Would you feel comfortable to recommend genetic testing to your patients if those tests could reveal which diseases are liable to affect them in the future	☐ Yes	52	7.8		
	☐ Yes, but only if that disease could be treated	84	12.6		
	☐ No	339	50.8		
	☐ Undecided	192	28.8		
Access to and use of PGx information					
		N	%		
Q36: Do you obtain extra information on genetic testing and its application in the context of drug therapy? (if “No” proceed to Q38)	☐ Yes	258	38.7		
	☐ No	409	61.3		
		Yes		No	
		N	%	N	%
Q37: Where do you obtain information on genetic testing and its application in the context of drug therapy? (select all that apply)	☐ Drug labeling (package insert)	203	30.4	464	69.6
	☐ Internet	163	24.4	504	75.6
	☐ Genetic testing laboratory	62	9.3	605	90.7
	☐ Colleague	100	15.0	567	85.0
	☐ Post-academic education and pharmacotherapeutic meetings	79	11.8	588	88.2
	☐ Other	66	9.9	601	90.1

Supplementary Document S8.1: Continued

Q38: What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test		Authority approval of recommendation		☐ Very unimportant	N	%
				☐ Unimportant	7	1.0
				☐ Un-decided	10	1.5
				☐ Important	114	17.1
				☐ Very important	310	46.5
		Speciality guidelines		☐ Very unimportant	226	33.9
				☐ Unimportant	4	0.6
				☐ Un-decided	3	0.4
				☐ Important	40	6.0
				☐ Very important	328	46.9
		Scientific journal		☐ Very unimportant	292	43.8
				☐ Unimportant	4	0.6
				☐ Un-decided	5	0.7
				☐ Important	116	17.4
				☐ Very important	374	56.1
		Recommendation or experience of thought leaders or respected colleagues		☐ Very unimportant	168	25.2
				☐ Unimportant	3	0.4
				☐ Un-decided	30	4.5
				☐ Important	256	38.4
				☐ Very important	328	49.2
		☐ Very important	50	7.5		
Q39: Where do you obtain information to make a choice about the drug and dose in case of a known genotype?		Yes		No		
		N	%	N	%	
		☐ Drug labeling (package insert)	407	61.0	260	39.0
		☐ Registration authority	173	25.9	494	74.1
		☐ Scientific literature	389	58.3	278	41.7
		☐ Colleague	153	22.9	514	77.1
		☐ Pharmaceutical Compass	228	34.2	439	65.8
		☐ Informatorium Medicamentorum	587	88.0	80	12.0
		☐ Other ...	37	5.5	630	94.5
Q40: Were you aware that in the Netherlands dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?				N		%
				☐ Yes	474	74.1
				☐ No	173	25.9
Q41: Were you aware that in the Netherlands medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?				N		%
				☐ Yes	436	65.4
				☐ No	231	34.6

Supplementary Document S8.2: Comparison between specialties

	Community + other		Outpatient		Hospital	
	N	%	N	%	N	%
Response	p < 0.001					
Yes	471	16.9	32	18.4	164	27.5
No	2316	83.1	142	81.6	432	72.5
	2787	100.0	174	100.0	596	100.0
Age	p = 0.003					
20–29	71	15.1	8	25.0	26	15.9
30–39	130	27.6	12	37.5	67	40.9
40–49	99	21.0	7	21.9	38	23.2
50–59	128	27.2	5	15.6	25	15.2
≥ 60	43	9.1	0	0.0	8	4.9
	471	100.0	32	100.0	164	100.0
Do you expect that... PGx will allow detect of which drug or dose will be more efficacious	p = 0.017					
0	2	0.4	0	0.0	3	1.8
1	57	12.1	6	18.8	33	20.1
2	225	47.8	9	28.1	75	45.7
3	187	39.7	17	53.1	53	32.3
	471	100.0	32	100.0	164	100.0
At any time in the past 6 months, have you ordered or recommended a pharmacogenetic test	p < 0.001					
No	454	96.4	30	93.8	85	51.8
Yes	17	3.6	2	6.2	79	48.2
	471	100.0	32	100.0	164	100.0
Do you anticipate ordering or recommending a pharmacogenetic test for a patient within the next 6 months	p < 0.001					
no past testing, no future testing	407	86.4	23	71.9	48	29.3
no past testing, future testing	47	10.0	7	21.9	37	22.6
past testing	17	3.6	2	6.2	79	48.2
	471	100.0	32	100.0	164	100.0

Supplementary Document S8.2: Continued

	Community + other		Outpatient		Hospital	
	N	%	N	%	N	%
At which University did you obtain your PharmD	p = 0.015					
Groningen	153	32.5	8	25.0	60	36.6
Utrecht	241	51.2	22	68.8	94	57.3
Leiden	29	6.2	1	3.1	8	4.9
Amsterdam	30	6.4	0	0.0	2	1.2
Other	18	3.8	1	3.1	0	0.0
	471	100.0	32	100.0	164	100.0
Was education on PGx included in your postgraduate specialty training	p < 0.001					
No	411	87.3	28	87.5	65	39.6
Yes	60	12.7	4	12.5	99	60.4
	471	100.0	32	100.0	164	100.0
Would you like to participate in extra training on pharmacogenetics	p < 0.001					
No	33	7.0	2	6.2	40	24.4
Yes	438	93.0	30	93.8	124	75.6
	471	100.0	32	100.0	164	100.0
Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice	p < 0.001					
No	34	7.2	2	6.2	9	5.5
Yes	77	16.3	6	18.8	97	59.1
Yes, after training	360	76.4	24	75.0	58	35.4
	471	100.0	32	100.0	164	100.0
Would you feel comfortable to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case	p < 0.001					
No	134	28.5	11	34.4	19	11.6
Yes	189	40.1	12	37.5	122	74.4
Undecided	148	31.4	9	28.1	23	14.0
	471	100.0	32	100.0	164	100.0

Supplementary Document S8.2: Continued

	Community + other		Outpatient		Hospital	
	N	%	N	%	N	%
Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy	p < 0.001					
No	444	94.3	31	96.9	98	59.8
Yes	27	5.7	1	3.1	66	40.2
	471	100.0	32	100.0	164	100.0
Where do you obtain information on genetic testing and its application in the context of drug therapy?						
Drug labelling (package insert)	p < 0.001					
No	359	76.3	23	71.9	82	50.0
Yes	112	23.7	9	28.1	82	50.0
	471	100.0	32	100.0	164	100.0
Colleague	p < 0.001					
No	429	91.1	25	78.1	113	68.9
Yes	42	8.9	7	21.9	51	31.1
	471	100.0	32	100.0	164	100.0
Internet	p < 0.001					
No	379	80.5	26	81.2	99	60.4
Yes	92	19.5	6	18.8	65	39.6
	471	100.0	32	100.0	164	100.0
Genetic testing laboratory	p < 0.001					
No	451	95.8	31	96.9	123	75.0
Yes	20	4.2	1	3.1	41	25.0
	471	100.0	32	100.0	164	100.0
Other	p < 0.001					
No	446	94.7	29	90.6	126	76.8
Yes	25	5.3	3	9.4	38	23.2
	471	100.0	32	100.0	164	100.0
What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test?						
Speciality guidelines	p = 0.034					
Important/ very important	5	1.1	0	0.0	2	1.2
Undecided	37	7.9	1	3.1	2	1.2
Unimportant/ very unimportant	429	91.1	31	96.6	160	93.0
	471	100.0	32	100.0	164	100.0

Supplementary Document S8.2: Continued

	Community + other		Outpatient		Hospital	
	N	%	N	%	N	%
Where do you obtain information to make a choice about the drug and dose in case of a known genotype						
Drug labelling (package insert)	p = 0.027					
No	199	42.3	10	31.2	51	39.0
Yes	272	57.7	22	68.8	113	61.0
	471	100.0	32	100.0	164	100.0
Scientific literature	p < 0.001					
No	223	47.3	16	50.0	39	23.8
Yes	248	52.7	16	50.0	125	76.2
	471	100.0	32	100.0	164	100.0
Colleague	p < 0.001					
No	396	84.1	21	65.6	97	59.1
Yes	75	15.9	11	34.4	67	40.9
	471	100.0	32	100.0	164	100.0
Pharmaceutical Compass	p < 0.001					
No	264	56.1	26	81.2	149	90.9
Yes	207	43.9	6	18.8	15	9.1
	471	100.0	32	100.0	164	100.0
Were you aware that in the Netherlands ...						
dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?	p < 0.001					
No	154	32.7	5	15.6	14	8.5
Yes	317	67.3	27	84.4	150	91.5
	471	100.0	32	100.0	164	100.0
medication surveillance based on the genotype of a patient in incorporated in the automated drug dispensing systems?	p < 0.001					
No	187	39.7	11	34.4	33	20.1
Yes	284	60.3	21	65.6	131	79.9
	471	100.0	32	100.0	164	100.0

Supplementary Document S8.2: Continued

	Community + other		Outpatient		Hospital	
	N	%	N	%	N	%
Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?	p = 0.002					
Yes	58	12.3	7	21.9	40	24.4
No	316	67.1	21	65.6	88	53.7
Undecided	97	20.6	4	12.5	36	22.0
	471	100.0	32	100.0	164	100.0
A PGx test might show there is no suitable drug for your patient	p < 0.001					
0	161	34.2	15	46.9	92	56.1
1	157	33.3	9	28.1	44	26.8
2	118	25.1	7	21.9	25	15.2
3	35	7.4	1	3.1	3	1.8
	471	100.0	32	100.0	164	100.0
Which of the following health professionals should have access to the patient's PGx test results						
Nurse-practitioner	p = 0.012					
No	382	81.1	24	75.0	148	90.2
Yes	89	18.9	8	25.0	16	9.8
Are you worried that ...						
A health insurance could obtain information about an individual's genotype based on the drug/dose prescribed	p = 0.003					
0	16	3.4	2	6.2	7	4.3
1	21	4.5	5	15.6	6	3.7
2	68	14.4	7	21.9	41	25.0
3	366	77.7	18	56.2	110	67.1
	471	100.0	32	100.0	164	100.0

Supplementary Document S8.3: Results of the multivariate analysis

	Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
	n = 667		n = 569		n = 667		n = 667	
What is your main practice environment								
Community pharmacy + other	1.00		1.00		1.00		1.00	
Outpatient pharmacy	1.58 (0.31–8.16)	0.586	3.01 (1.05–8.57)	0.039	0.48 (0.05–4.21)	0.505	1.02 (0.21–4.86)	0.985
Hospital pharmacy	9.44 (4.13–21.57)	< 0.001	5.85 (2.67–12.82)	< 0.001	3.67 (1.57–8.55)	0.003	0.53 (0.23–1.23)	0.139
Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy								
No	1.00		1.00		Not applicable		1.00	
Yes	1.99 (0.99–3.99)	0.052	1.07 (0.47–2.47)	0.866			2.40 (1.18–4.90)	0.016
Was education on PGx included in your postgraduate specialty training								
No	1.00		1.00		1.00		1.00	
Yes	0.95 (0.48–1.89)	0.879	1.01 (0.50–2.01)	0.983	1.65 (0.86–3.17)	0.130	1.17 (0.59–2.33)	0.651
Would you like to participate in extra training on pharmacogenetics								
No	1.00		Variable not significant in the univariate analysis		1.00		Not applicable	
Yes	0.98 (0.43–2.25)	0.967			2.96 (1.38–6.33)	0.005		

Supplementary Document S8.3: Continued

	Variables associated with past adoption (ordered or recommended a PGx test in the last six months)	Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)	Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy	Variables associated with the need of extra training on pharmacogenetics
	n = 667	n = 569	n = 667	n = 667
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	P value	P value	P value	P value
	1.00	1.00	1.00	1.00
No	7.17 (0.76–67.85)	1.34 (0.30–5.94)	1.29 (0.32–5.17)	0.66 (0.25–1.78)
Yes	2.89 (0.31–26.83)	1.41 (0.35–5.68)	0.34 (0.09–1.31)	3.71 (1.41–9.76)
Yes, after training				0.008
Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice				
No	1.00	1.00	1.00	1.00
Yes	7.17 (0.76–67.85)	1.34 (0.30–5.94)	1.29 (0.32–5.17)	0.66 (0.25–1.78)
Yes, after training	2.89 (0.31–26.83)	1.41 (0.35–5.68)	0.34 (0.09–1.31)	3.71 (1.41–9.76)
Would you feel comfortable to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case				
No	1.00	1.00	1.00	Variable not significant in the univariate analysis
Yes	1.29 (0.50–3.30)	2.18 (1.03–4.64)	2.37 (0.88–6.42)	
Undecided	0.76 (0.25–2.30)	1.03 (0.45–2.39)	1.16 (0.38–3.51)	
If a pharmacogenetic test revealed that the only available drug to treat your patient's disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?				
No	Variable not significant in the univariate analysis	Variable not significant in the univariate analysis	1.00	1.00
Yes			8.54 (2.25–32.42)	0.51 (0.16–1.63)
Yes, only if he/she had a life-threatening disease			0.46 (0.24–0.86)	1.37 (0.76–2.44)

Supplementary Document S8.3: *Continued*

		Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
		n = 667		n = 569		n = 667		n = 667	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?									
No		1.00		Variable not significant in the univariate analysis		1.00		Variable not significant in the univariate analysis	
Yes		0.51 (0.25–1.07)	0.076			0.69 (0.32–1.49)	0.349		
Undecided		0.59 (0.25–1.43)	0.246			1.32 (0.54–3.18)	0.541		
Do you expect that PGx can prevent you patient from taking the wrong drug or dose									
Low expectations (0 / 1)	Variable not significant in the univariate analysis	1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis	
High expectations (2 / 3)		2.20 (0.98–4.92)		0.056					
Do you expect that PGx will allow detect of which drug or dose will be more efficacious									
Low expectations (0 / 1)	Variable not significant in the univariate analysis	1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		1.00	
High expectations (2 / 3)		1.51 (0.62–3.70)		0.365				0.99 (0.76–1.30)	
Do you expect that PGx will allow detection of which drug or dose will cause less side effects									
Low expectations (0 / 1)	Variable not significant in the univariate analysis	1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		1.00	
High expectations (2 / 3)		1.29 (0.55–3.02)		0.557				1.20 (0.94–1.53)	
0.137									

Supplementary Document S8.3: *Continued*

		Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
		n = 667		n = 569		n = 667		n = 667	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Are you worried that a PGx test might show there is no suitable drug for your patient									
Low worries (0 / 1)		1.00		1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis	
High worries (2 / 3)		0.99 (0.46–2.13)	0.973	1.31 (1.06–1.61)	0.011				
What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test? – Speciality guidelines									
Unimportant/ very unimportant		1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis	
Undecided		0.89 (0.17–4.61)	0.886						
Important/ very important		1.84 (0.38–9.02)	0.451						
What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test? – Authority approval or recommendation									
(very) unimportant		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		1.00		1.00	
Undecided						42.28 (3.59–498.48)		3.12 (0.80–12.15)	
(very) important						13.38 (1.33–155.55)		3.28 (1.00–10.78)	
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Drug labelling (package insert)									
No		1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes		1.34 (0.61–2.96)	0.469	1.61 (0.72–3.59)	0.246	1.44 (0.66–3.15)		0.362	

Supplementary Document S8.3: Continued

	Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
	n = 667		n = 569		n = 667		n = 667	
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Colleague								
No	1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes	1.73 (0.76–3.97)	0.195	1.21 (0.54–2.72)	0.650	1.08 (0.49–2.35)	0.854	Variable not significant in the univariate analysis	
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Post-academic education and pharmacotherapeutic meetings								
No	1.00		1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis	
Yes	1.23 (0.53–2.87)	0.631	2.91 (1.36–6.23)	0.006				
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Internet								
No	1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes	1.44 (0.71–2.89)	0.311	1.64 (0.80–3.38)	0.176	1.05 (0.51–2.15)	0.892	Variable not significant in the univariate analysis	
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Genetic Laboratories								
No	1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes	2.16 (0.99–4.71)	0.054	1.09 (0.41–2.85)	0.867	0.57 (0.24–1.33)	0.191		
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Other								
No	1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes	0.88 (0.40–1.94)	0.747	1.19 (0.52–2.76)	0.679	0.73 (0.33–1.63)	0.445	Variable not significant in the univariate analysis	

Supplementary Document S8.3: *Continued*

	Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
	n = 667		n = 569		n = 667		n = 667	
Where do you obtain information to make a choice about the drug and dose in case of a known genotype – Drug labelling (package insert)								
No	Variable not significant in the univariate analysis		1.00		Variable not significant in the univariate analysis		1.00	
Yes			0.86 (0.46–1.61)	0.647			1.85 (1.05–3.26)	0.032
Where do you obtain information to make a choice about the drug and dose in case of a known genotype – Scientific literature								
No	1.00		1.00		1.00		Variable not significant in the univariate analysis	
Yes	1.50 (0.75–3.02)	0.255	1.16 (0.96–1.41)	0.132	1.41 (0.71–2.78)	0.322		
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Colleague								
No	1.00		1.00		Variable not significant in the univariate analysis		Variable not significant in the univariate analysis	
Yes	0.89 (0.42–1.87)	0.759	0.99 (0.83–1.18)	0.886				
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Pharmaceutical Compass								
No	1.00		1.00		1.00		1.00	
Yes	0.88 (0.38–2.07)	0.777	0.97 (0.85–1.10)	0.586	1.68 (0.77–3.65)	0.192	1.05 (0.54–2.06)	0.889
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Informatorium Medicamentorum								
No	Variable not significant in the univariate analysis		Variable not significant in the univariate analysis		1.00		Variable not significant in the univariate analysis	
Yes					1.88 (0.58–6.14)	0.294		

Supplementary Document S8.3: Continued

		Variables associated with past adoption (ordered or recommended a PGx test in the last six months)		Variables associated with future adoption (planned to order or recommend a PGx test in the coming six months)		Variables associated with feeling adequately informed about the availability of genetic testing and its application in the context of drug therapy		Variables associated with the need of extra training on pharmacogenetics	
		n = 667		n = 569		n = 667		n = 667	
		OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
		1.00		Variable not significant in the univariate analysis		1.00			
		2.93 (0.98–8.71)	0.054			2.32 (0.77–7.05)	0.136		
Where do you obtain information on genetic testing and its application in the context of drug therapy? – Other									
No		1.00		Variable not significant in the univariate analysis		1.00		Variable not significant in the univariate analysis	
Yes									
Were you aware that in the Netherlands dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?									
No		1.00		1.00		1.00		1.00	
Yes		1.00 (0.37–2.67)	0.999	1.82 (0.79–4.22)	0.162	1.36 (0.51–3.65)	0.537	0.89 (0.41–1.95)	0.770
Were you aware that in the Netherlands medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?									
No		1.00		1.00		1.00		1.00	
Yes		1.23 (0.57–2.69)	0.596	1.81 (0.93–3.53)	0.082	1.84 (0.82–4.13)	0.139	1.03 (0.51–2.08)	0.927
At any time in the past 6 months, have you ordered or recommended a pharmacogenetic test?									
No		Not applicable		Not applicable		1.00		1.00	
Yes						1.27 (0.55–2.93)	0.574	1.22 (0.56–2.67)	0.623
Do you anticipate ordering or recommending a pharmacogenetic test for a patient within the next 6 months?									
No		Not applicable		Not applicable		1.00		Variable not significant in the univariate analysis	
Yes						1.65 (0.75–3.65)	0.213		

Supplementary Document S8.4: Comparison between responders with past adoption, future adopters and non-adopters (1)

	Non-adopters		Future adopters		Past adoption	
	N	%	N	%	N	%
Specialty	p < 0.001					
Community pharmacy	407	85.1	47	51.6	17	17.3
Outpatient pharmacy	56	4.8	7	7.7	2	2.0
Hospital pharmacy	48	10.0	37	40.7	79	80.6
	478	100.0	91	100.0	98	100.0
Do you expect that...						
PGx can prevent you patient from taking the wrong drug or dose	p = 0.002					
0	8	1.7	3	3.3	0	0.0
1	97	20.3	8	8.8	12	12.2
2	229	47.9	37	40.7	44	44.9
3	144	30.1	43	47.3	42	42.9
	478	100.0	91	100.0	98	100.0
PGx will allow detection of which drug or dose will cause less side effects	p = 0.002					
0	9	1.9	2	2.2	0	0.0
1	97	20.3	11	12.1	16	16.3
2	235	49.2	31	34.1	47	48.0
3	137	28.7	47	51.6	35	35.7
	478	100.0	91	100.0	98	100.0
Was education on PGx included in your postgraduate specialty training	p < 0.001					
No	404	84.5	59	64.8	41	41.8
Yes	74	15.5	32	35.2	57	58.2
	478	100.0	91	100.0	98	100.0
Would you like to participate in extra training on pharmacogenetics	p < 0.001					
No	47	9.8	4	4.4	24	24.5
Yes	431	90.2	87	95.6	74	75.5
	478	100.0	91	100.0	98	100.0
	p < 0.001					
No	41	8.6	3	3.3	1	1.0
Yes	85	17.8	28	30.8	67	68.4
Yes, after training	352	73.6	60	65.9	30	30.6
	478	100.0	91	100.0	98	100.0

Supplementary Document S8.4: Continued

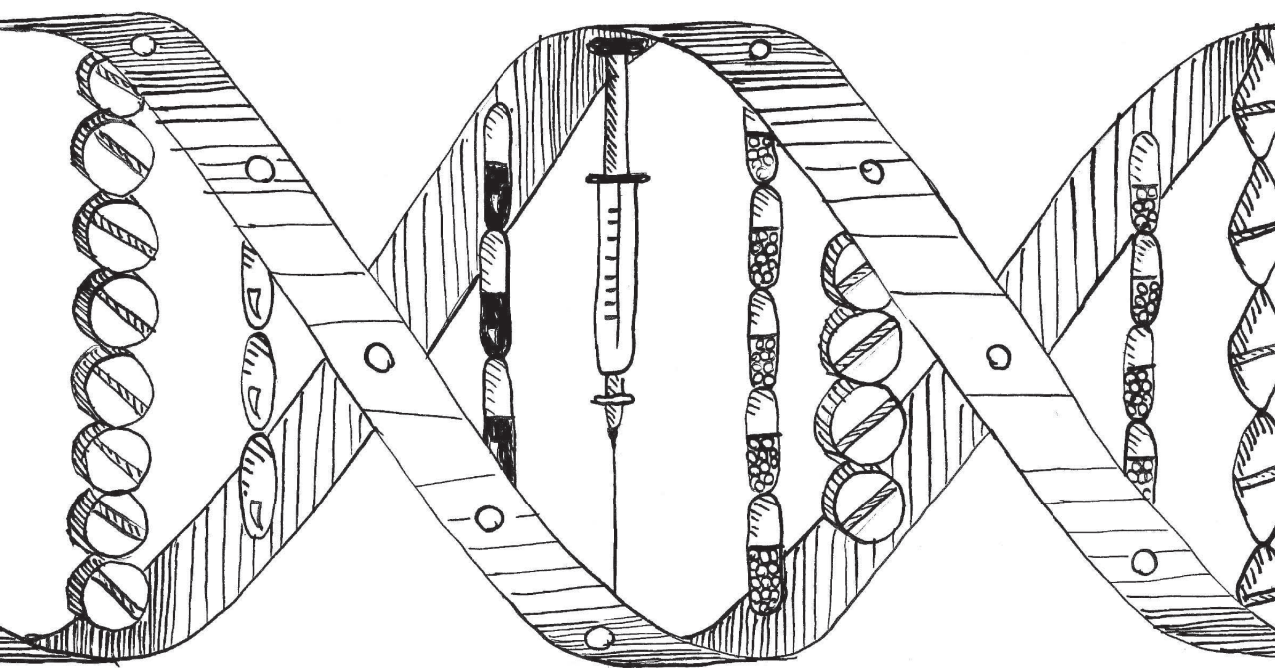
	Non-adopters		Future adopters		Past adoption	
	N	%	N	%	N	%
Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice	p < 0.001					
No	41	8.6	3	3.3	1	1.0
Yes	85	17.8	28	30.8	67	68.4
Yes, after training	352	73.6	60	65.9	30	30.6
	478	100.0	91	100.0	98	100.0
Would you feel comfortable to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case	p < 0.001					
No	143	29.9	13	14.3	8	8.2
Yes	183	38.3	60	65.9	80	81.6
Undecided	152	31.8	18	19.8	10	10.2
	478	100.0	91	100.0	98	100.0
Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy	p < 0.001					
No	446	93.3	74	81.3	53	54.1
Yes	21	6.7	17	18.7	45	45.9
	478	100.0	91	100.0	98	100.0
Do you obtain extra information on genetic testing and its application in the context of drug therapy?	p < 0.001					
No	357	74.7	27	29.7	25	25.5
Yes	121	25.3	64	70.3	73	74.5
	478	100.0	91	100.0	98	100.0
Where do you obtain information on genetic testing and its application in the context of drug therapy?						
Drug labelling (package insert)	p < 0.001					
No	385	80.5	38	41.8	41	41.8
Yes	93	19.5	53	58.2	57	58.2
	478	100.0	91	100.0	98	100.0

Supplementary Document S8.4: Continued

	Non-adopters		Future adopters		Past adoption	
	N	%	N	%	N	%
Colleague	p < 0.001					
No	439	91.8	64	70.3	64	65.3
Yes	39	8.2	27	29.7	34	34.7
	478	100.0	91	100.0	98	100.0
Post-academic education and pharmacotherapeutic meetings	p < 0.001					
No	443	92.7	66	72.5	79	80.6
Yes	35	7.3	25	27.5	19	19.4
	478	100.0	91	100.0	98	100.0
Internet	p < 0.001					
No	404	84.5	49	53.8	51	52.0
Yes	74	15.5	42	46.2	47	48.0
	478	100.0	91	100.0	98	100.0
Genetic testing laboratory	p < 0.001					
No	463	96.9	77	84.6	65	66.3
Yes	15	3.1	14	15.4	33	33.7
	478	100.0	91	100.0	98	100.0
Other	p < 0.001					
No	451	94.4	75	82.4	75	76.5
Yes	27	5.6	16	17.6	23	23.5
	478	100.0	91	100.0	98	100.0
What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test?						
Speciality guidelines	p = 0.041					
Important/ very important	26	5.4	4	4.4	3	3.1
Undecided	197	41.2	33	36.3	26	26.5
Unimportant/ very unimportant	255	53.3	54	59.3	69	70.4
	478	100.0	91	100.0	98	100.0
Scientific literature	p < 0.001					
No	232	48.5	25	27.5	21	21.4
Yes	246	51.5	66	72.5	77	78.6
	478	100.0	91	100.0	98	100.0
Colleague	p = 0.001					
No	386	80.8	63	69.2	65	66.3
Yes	92	19.2	28	30.8	33	33.7
	478	100.0	91	100.0	98	100.0

Supplementary Document S8.4: Continued

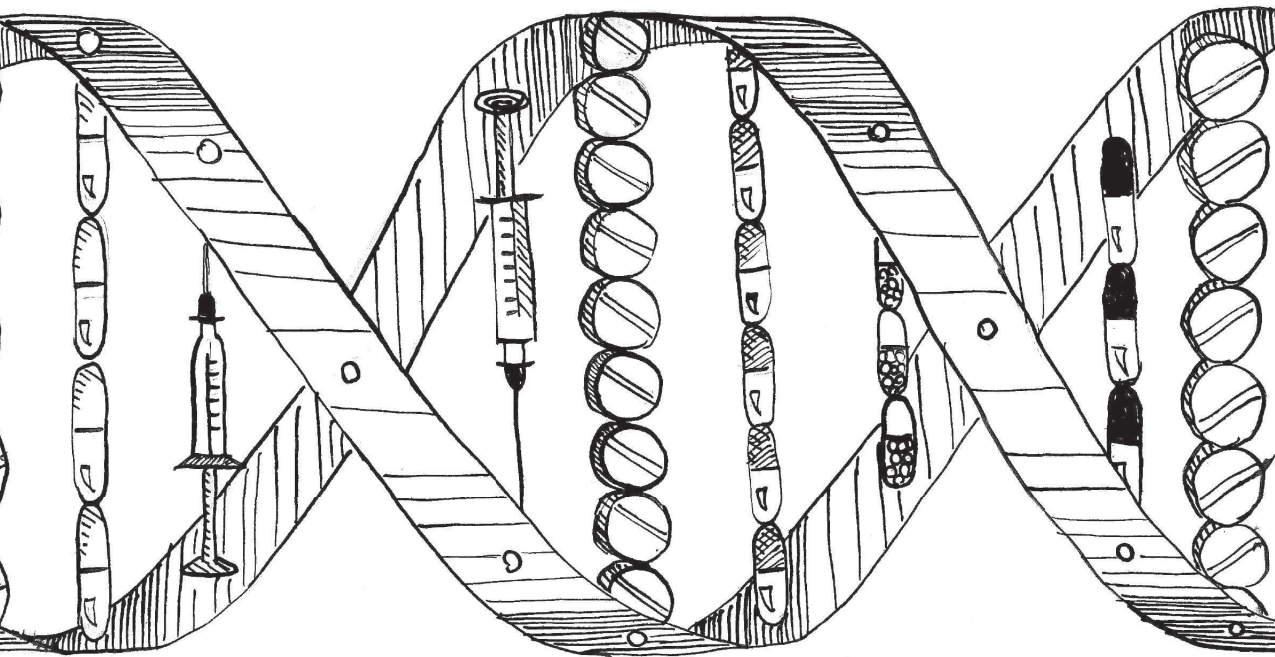
	Non-adopters		Future adopters		Past adoption	
	N	%	N	%	N	%
Pharmaceutical Compass	p < 0.001					
No	290	60.7	66	72.5	83	84.7
Yes	188	39.3	25	27.5	15	34.2
	478	100.0	91	100.0	98	100.0
Other	p = 0.029					
No	456	95.4	87	95.6	87	88.8
Yes	22	4.6	4	4.4	11	11.2
	478	100.0	91	100.0	98	100.0
Were you aware that in the Netherlands ...						
dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?	p < 0.001					
No	157	32.8	9	9.9	7	7.1
Yes	321	67.2	82	90.1	91	92.9
	478	100.0	91	100.0	98	100.0
medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?	p < 0.001					
No	198	41.4	18	19.8	15	15.3
Yes	280	58.6	73	80.2	83	84.7
	478	100.0	91	100.0	98	100.0
Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?	p = 0.002					
Yes	64	13.4	14	15.4	27	27.6
No	312	65.3	64	70.3	49	50.0
Undecided	102	21.3	13	14.3	22	22.4
	478	100.0	91	100.0	98	100.0
Are you worried that ...						
A PGx test might show there is no suitable drug for your patient	p = 0.012					
0	179	37.4	34	37.4	55	56.1
1	157	32.8	28	30.8	25	25.5
2	108	22.6	25	27.5	17	17.3
3	34	7.1	4	4.4	1	1.0
	478	100.0	91	100.0	98	100.0



Chapter 9

A nationwide cross-sectional survey of
pharmacy students on knowledge, experience,
and attitudes towards pharmacogenetic
testing in the Netherlands

Bank PCD, Swen JJ, Guchelaar HJ



Abstract

To benchmark knowledge and attitude of pharmacy students towards pharmacogenetics (PGx) and PGx-testing and compare the results to practicing colleagues. All pharmacy students in The Netherlands were invited to participate in a web-based survey consisting of 28 questions. Of the 824 invited students, 148 individuals (18.0%) completed the questionnaire. All responders believed in the concept of PGx and had high expectations towards PGx. The majority (96.6%) had received some form of education on PGx, but only 12.8% felt adequately informed. When compared to practicing pharmacists' differences were observed in the use of information and feeling qualified to recommend PGx-testing. More education on PGx is required in the curriculum to fill the perceived knowledge gap among future pharmacists.

Introduction

In recent years the field of pharmacogenetics (PGx) has developed rapidly and this has translated to an increasing number of drug labels containing information on genetic biomarkers (1, 2). In addition, the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have created widely recognized guidelines with therapeutic recommendations for patients with a known genotype (3-5). Consequently, healthcare professionals need to develop their knowledge of pharmacogenetics to be able to optimize patient care based on pharmacogenetic markers. Previous studies have shown that physicians and pharmacists in the United States, Canada and the Netherlands have high expectations of PGx to improve the efficacy and safety of drugs. However, despite the enthusiasm of physicians and pharmacists towards PGx, a knowledge gap on this subject appears to be present (6-8). This knowledge gap potentially hinders the adoption of PGx into clinical care and may be the consequence of a lack of education on PGx in their curriculum (8). To solve the lack of knowledge among healthcare professionals additional PGx related education could be essential. Pharmacy students represent the next generation of pharmacists and are bound to come into contact with the field of PGx in their later career path. Limited knowledge among these students may impede PGx application in clinical care. In a statement issued in 2015 the American Society of Health-System Pharmacists has encouraged the embedding of education on PGx in college of pharmacy curricula and Specialties certification programs (9). In the Netherlands The Royal Dutch Pharmacist's Association (KNMP) has incorporated PGx in their view of the future for care in 2020, but no clear recommendation to incorporate PGx in the pharmacy curricula exist (10).

Currently, it is unknown whether pharmacy students receive education on PGx and what their expectations and attitudes of pharmacy students towards PGx and PGx-testing are. In this study we set out to investigate whether pharmacy students believe in the concept of PGx, what expectations they have towards PGx, to research whether a knowledge gap on PGx is present among these students and to analyse whether there are differences between pharmacy students and practising pharmacists.

Methods

Study design

Similar to a previous survey of practicing pharmacists, a web-based survey was performed with NetQ [101]. In brief, a list with the email addresses of all students of pharmacy in The

Netherlands was obtained from the KNMP and an email with a link to the survey was sent to 824 students. After two weeks a reminder was sent. The students could complete the survey between December 15th 2014 and February 1st 2015. Participation was completely voluntary and no reimbursement was offered. All responses were analysed anonymously. For the comparison with Dutch practicing pharmacists the results of a cohort of 667 pharmacists that completed an identical set of the questions (see below) were used (8).

Questionnaire

A questionnaire previously described in detail was used (6-8). Questions not applicable for students were removed (e.g. questions relating to PGx tests ordered or recommended in a clinical setting). In the first part of the survey a brief overview of the topics covered and an explanation for pharmacogenetics was provided. In total the questionnaire consisted of 28 questions divided among five sections. In the first section five questions were asked to gather baseline information on the participants. The second part of the questionnaire (Q6–9) surveyed the responders' belief in the concept of PGx and their expectations towards PGx. In the third section (Q10–13) participants were asked questions relating to attitudes of toward their own abilities. Q14–20 (section 4) surveyed sources of information of PGx used by candidates. In the final section (Q1–28) of the survey the participants were asked questions relating to ethics and test coverage (see Supplementary Document S9.1).

Survey analysis

Survey responses were automatically tabulated and stored by Netq. For the analysis of the responses only complete questionnaires were included. In order to compare the results of the pharmacy students with the previously surveyed pharmacists age was recoded in a six-level categorical variable (≤ 29 , 30–39, 40–49, 50–59, ≥ 60 years) and the answers of Q17 (see Supplementary Document S9.1) were condensed to a three level variable ((very) unimportant, undecided, (very) important) (8). The χ^2 test was used to test for univariate associations. Binary logistic regression, multinomial logistic regression and ordinal logistic regression were used for the multivariate analyses using gender and age-groups as covariates. For the analysis of question 12 (see Supplementary Document S9.1) age was condensed from a six-level to a five-level categorical variable (≤ 29 , 30–39, 40–49, ≥ 50 years). Statistical analyses were performed with SPSS version 20 (SPSS, Inc., Illinois, USA) with $p < 0.05$ considered significant.

Results

Characterization of responders

Out of the 824 pharmacy students who received an invitation to participate in the survey 148 students (18.0%) completed the questionnaire. Of the responders 70.3% was female and the median age was 24. The survey included students from the second through the sixth year of the study with a large majority of the responders being master students (93.9%). Of the students 96.6% had received some education in PGx as part of the curriculum (see Table 9.1).

Belief in the concept of PGx & expectations towards PGx(-testing)

All students included in the analysis indicated to believe in the concept of (partially) hereditary drug response. To benchmark the expectation of the students towards PGx and PGx-testing they were asked to rate three statements on a scale from 0 (no expectation) to 3 (high expectation). To the question whether they expected a PGx test could prevent

Table 9.1: Characteristics of responders

	N	%
Gender		
Male	44	29.7
Female	104	70.3
Age		
20	4	2.7
21	11	7.4
22	15	10.1
23	30	20.3
24	35	23.6
25	31	20.9
26	15	10.1
27	1	0.7
28	4	2.7
29	2	1.4
In which year of the program do you currently follow courses?		
Second year	1	0.7
Third year	8	5.4
Forth year	27	18.2
Fifth year	42	28.4
Sixth year	70	47.3
Has received education on PGx as part of their curriculum?		
Yes	143	96.6
No	5	3.4

a patient from receiving the wrong choice of drug or dose of a given treatment 86.5% of the students scored at least 2. For the statements “I expect that a PGx test will detect the most efficacious drug or dose” and “I expect that a PGx test will allow for detection the drug or dose that will cause less side effects” 87.2% and 73.7% of the student rated with a score ≥ 2 (see Figure 9.1).

Attitude towards own expected ability to interpret PGx test results

Of the surveyed students 27.7% feels qualified to receive the PGx result of a patient, interpret genotype(s) and advise a treating healthcare professionals or patient on the choice of the drug regimen based on the results. The large majority (70.9%) see themselves qualified to receive and interpret a genotype and advise a patient or colleague based on the results, but only after receiving additional training on the subject, while 1.4% does not think this is part of their (future) job description. 75.0% sees him/herself qualified to recommend PGx testing to patients if the PGx test can reveal whether a drug is effective, whereas 8.1% does not feel qualified and 16.9% does not know. If the PGx test could also reveal a disease the patient is susceptible to in the future 20.9% would feel qualified to recommend the test and 23.0% would feel qualified only if the disease could be treated. In contrast, 31.1% would not feel qualified to recommend a PGx test if that could reveal a disease and 25.0% does

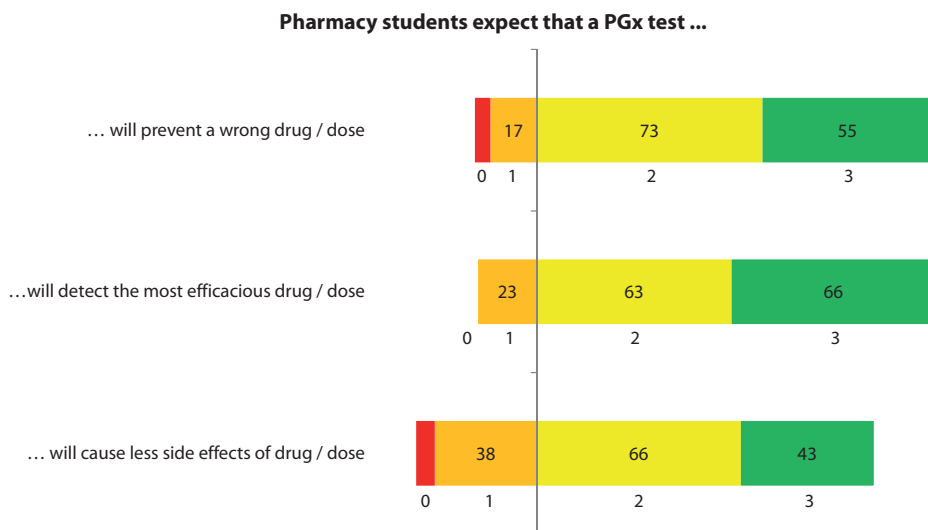


Figure 9.1: Expectations of pharmacy students towards PGx testing.

Red = I have a very low expectation that PGx ..., orange = I have a low expectation that PGx ..., yellow = I have a high expectation that PGx ..., green = have a very high expectation that PGx ... (the size of the bar is proportional to the number of respondents).

not know if they would feel qualified in that case. When a PGx test would reveal that the only available drug therapy for a patient will not work or would lead to severe side effects 31.1% of the surveyed student would not treat the patient with that drug and 64.2% would only give the treatment if the patient was suffering from a life-threatening condition. Only 4.7% of the responders would continue with the drug even though the results of the PGx would indicate no efficacy.

Access to and use of PGx information

Although 96.6% of the students indicated that they had received education on PGx only 12.8% of all students currently feels adequately informed about the availability of PGx-tests and how to apply PGx in treatment of patients. Among students in the final year of their curriculum (n = 70) 17.1% of the responders felt adequately informed about PGx testing. 90.5% of the responders indicated they would use additional sources of information on how to apply PGx testing in pharmacotherapy of patients. The different sources of information used by students to obtain information about the use of PGx in relation to treatment or to support a choice in drug and dose in case of patient with an actionable phenotype predicted from a PGx test can be found in Supplementary Document S9.2.

Worries related toward PGx testing, privacy & coverage of PGx tests

In the last section of the questionnaire the students were benchmarked on potential worries towards the results of PGx testing, privacy and insurance of the PGx tests. Similar to the assessment of the expectations the students were asked to rate four questions on a four point scale from very low worries (0) to very high worries (3). To the question whether they were worried that a PGx might show that there is no suitable treatment for their patient 44.0% scored at least 2. Slightly more students (57.5%) were at least moderately worried (score ≥ 2) that a PGx test could show that a patient carries additional risk factors for another disease. 71.7% scored a 2 or 3 on the question whether they were worried that PGx test results could fall in the hands of unauthorized individuals. Almost all of the surveyed students (91.2%) were at least moderately worried that insurance companies could infer a patients genotype based on the drug or dose a patient is prescribed (see Figure 9.2). Students also showed worries concerning the potential impact of unfortunate PGx test results, as 87.2% believed this could have negative psychological effects on the patients and their family. And 23.0% of the responders were more worried for loss of privacy of the results of a PGx test compared to other diagnostic or laboratory tests. In their opinion the treating physician (98.0%) and pharmacist (99.3%) should have access to PGx data, whereas only a small portion of the surveyed students thought psychologists (8.8%), dieticians (4.7%), nurses

Pharmacy students are worried that ...

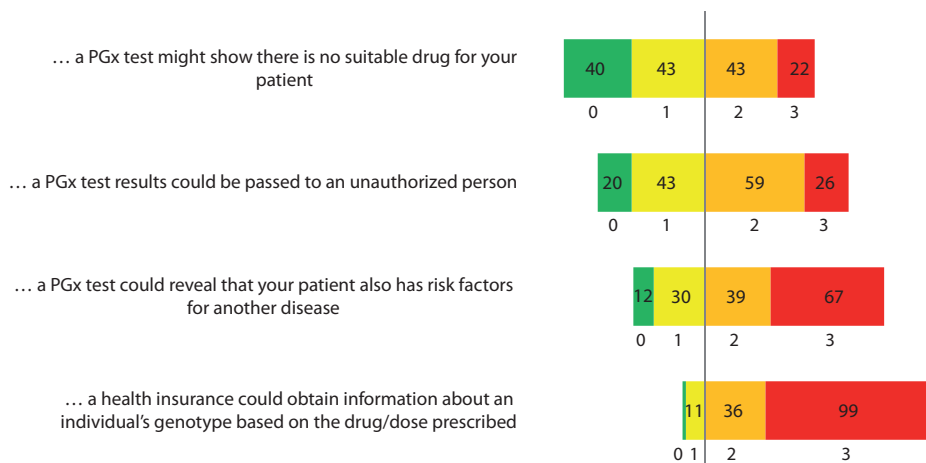


Figure 9.2: Worries of pharmacy students towards PGx testing.

Green = I have very low worries that ... (0), yellow = I have a low worries that ... (1), orange = I have a high worries that PGx ... (2), red = have a very high worries that PGx ... (3) (the size of the bar is proportional to the number of respondents).

(3.4%) and social workers (1.4%) were allowed to access to results of PGx tests. Among the students there was no consensus on whether clinical geneticists (78.4%), clinical chemist (43.2%) or nurse-practitioners (16.9%) should be allowed to see a patients' PGx-data. Finally, the students were asked if insurance companies should reimburse PGx-tests. All students were of the opinion that this indeed should be the case, but thought differently about the frequency in which PGx tests should be reimbursed. According to 78.4% of the students this should only be in certain occasions, whereas 21.6% thinks PGx tests should always be covered.

Differences between pharmacy students and practicing pharmacists

In a secondary analysis the responses of the pharmacy students were compared to the results of a previous survey among practicing pharmacists. In the univariate analyses between the two groups differences could be observed in multiple questions. In comparison, practicing pharmacists more often felt that interpreting PGx test results and advise patients and other healthcare professionals based on genotypes was not part of their job description (6.7% vs. 1.4%, $p = 0.038$). Additionally, practicing pharmacists less often felt qualified to recommend PGx testing to predict the efficacy of drug treatments (48.4% vs. 75.0%, $p < 0.001$) and less

often felt qualified recommending a genetic test if that test could reveal information about a disease a patient was susceptible to (7.8% vs. 20.9%, $p < 0.001$). Practicing pharmacists were more likely to stop a treatment if a PGx test would indicate if the only available drug was not effective or would lead to severe side-effects (49.0% vs. 31.1%, $p < 0.001$). Differences were also seen in the use of information sources on how to apply PGx testing in pharmacotherapy of patients. In general pharmacy students more often indicated to use additional sources of information to determine the application of PGx in relation to pharmacotherapy (90.5% vs. 38.7%, $p < 0.001$). Pharmacy students more often believed that an unfavourable result from a PGx test could have negative psychological consequences on a patient and his/her family (87.2% vs. 63.7%, $p = 0.034$) and were more often at least moderately worried that PGx could show that there is no suitable treatment for a patient (44.0% vs. 28.3%, $p < 0.001$). Finally, a difference was observed in whether social workers should have access to PGx data, as pharmacy students more often agreed with this statement compared to practicing pharmacists (1.4% vs. 0.1%, $p = 0.029$). In other questions no significant differences were visible in the univariate analysis (Supplementary Document S9.3). Using gender and age groups as co-variants the multivariate analysis revealed that pharmacy students more often would feel qualified to recommend PGx testing to predict drug efficacy (odd's ratio (OR) = 5.25 (confidence interval (CFI) = 2.47–11.16, $p < 0.001$), more often obtain extra information on genetic testing and its application in the context of drug therapy (OR = 12.61 (CFI = 6.42–24.77), $p < 0.001$) and more often think that an unfavourable test results could have adverse psychological consequences on him and his family (OR = 2.92 (1.08–7.89), $p = 0.034$). In contrast, pharmacy students are less often aware of the incorporation of medication surveillance based on genotype in electronic drug dispensing systems (OR = 0.12(0.07–0.22), $p < 0.001$) (Supplementary Document S9.4).

Discussion

This study shows that pharmacy students believe in the concept of (partially) heritable drug response. The surveyed students had high expectations of PGx in making pharmacotherapy safer and more effective even though some concerns were also present among the responders of this survey. Despite almost all responders received some sort of education on PGx as part of their curriculum, the majority of students did not feel adequately informed about PGx. This effect remained visible in the responders who were in the last year of their education. Also worries that unauthorized individuals could obtain a patients' genotype or that insurance companies can infer a genotype from a prescribed dose or alternate choice of drug scored relatively high.

When the results of the pharmacy students are compared with the results of practicing pharmacists it can be seen that the results are quite similar although there are some differences. The differences between the students and their practicing colleagues are mainly present in feeling qualified to recommend PGx testing to predict efficacy of a specific drug, whether individuals would use additional information to support the use of PGx test in therapy, the sort of sources of information used to support PGx testing within therapy and the information sources to support changes in drug and dose in case of a known actionable genotype. Differences in feeling qualified to recommend PGx to predict efficacy of a treatment may be explained by clinical experience gained in the field or a degree of selection bias in the previous survey where pharmacist who had adopted a PGx test (and as a result had more confidence in their abilities to recommend testing) were more likely to respond to the survey as they were familiar with the topic. The differences in use sources of information may be the result of an ideal situation in case of the student group vs. the actual situation in practice in the group of the pharmacists. Finally, differences in knowledge of the incorporation of medication surveillance in electronic medication surveillance systems may be explained by the fact that pharmacy students do have gained experiences using this form of clinical decision support in clinical practice.

In this cross-sectional study of pharmacy students were benchmarked to a number of PGx-related topics including expectations and worries towards PGx-testing. The expectations of the students seem to be generally high with over 80% of the students scoring at least ≥ 2 prevent receiving a wrong regimen and predict which regimen is the most effective. Furthermore, 72.7% of the student scored at least ≥ 2 on the same scale to rate their expectation that PGx will provide the ability to predict which regimen will give the lowest chance of side effects. In addition to similarities to Dutch practicing pharmacists the expectations benchmarked in this study are also comparable with a survey of Canadian pharmacists where 80.0, 82.6 and 79.1% scored moderately hopeful on the three statements respectively and the results of a survey of Jordanian pharmacists who also have similar high expectations of PGx in relation to pharmacotherapy (7, 8, 11).

From Table 9.1 it can be observed that 70.3% of the responders is female compared to 29.7% of male responders. In a previous study among Dutch pharmacists a (M:F) ratio of 45.5%:54.3% was observed. Although this difference in male-female ratio can be interpreted as selection bias, the increase of females is in line with other research and likely a trend toward a more female profession (12). Additionally, as with any other questionnaire with no incentive for participating in the survey, there is risk for systematic bias as individuals with a strong opinion in both a positive or negative way are more likely to respond. In this survey the response rate among the pharmacy students was 18.0% which relatively high

compared to previous surveys (6, 7). As a result of a relatively high response rate the risk of systematic bias in this study will be likely be low.

A striking finding in this survey is that only 12.8% of the students feel adequately informed about how to apply PGx in pharmacotherapy despite 96.6% of responders stating that PGx was part of their education which may result in a knowledge gap among future healthcare professionals. The percentage of students that felt adequately informed about PGx was similar to their older colleagues (14.1%) of whom only 39.7% had received education as part of their curriculum (8). One explanation may be found in the manner in how information on PGx is integrated in the curriculum. At this moment information on PGx and its applicability in pharmacotherapy is still taught in a traditional form using lectures. If the current practicing pharmacists had received any education as part of their curriculum, this was likely taught in a similar manner. With the decrease of the costs of sequencing it is anticipated that in the next years more and more patients will have a copy of their own genome. Pharmacogenetics is currently one of area's within genetics that is relatively easy to implement in the clinic as guidelines exist providing PGx test interpretation and recommendations for actionable genotypes. The healthcare professionals of tomorrow are bound to come in contact with PGx test results and should be able to interpret these results and use them to improve pharmacotherapy.

Although this survey identified a potential future knowledge gap among pharmacy students, the survey did not contain questions relating to the current implementation PGx in the curriculum (which year, which courses and credit hours etc.), the students' perception on the clinical utility of PGx, their views on how PGx should be implemented within the PharmD curriculum and potential outcomes of a structured PGx program. An assessment among 715 healthcare US students, including 328 pharmacy students, showed that 75.3% (strongly) agreed PGx should be an important part of the curriculum, whereas only 13.1% (strongly) agreed that PGx had indeed been an important part of the curriculum. Furthermore, Adams et al. developed the "Test2Learn" program in which a cohort of pharmacy students underwent personal genomics testing and as a result gained confidence in understanding PGx test and increased their self-perceived ability to empathize with potential patients (13). Similarly, initiatives such as reported by Weitzel et al, in which students genotype themselves and use this hands on experience in an educational setting increases understanding of PGx testing and comfort levels of student regarding acting on PGx data (14). Additional research should investigate whether Dutch students also would like hands-on experience with PGx during the Dutch pharmacy program.

A similar elective course is present as a part of master Bio-Pharmaceutical Sciences at the Leiden University. In this course on clinical pharmacology students genotyped themselves,

interpret their own genotypes and learn how to adjust medication based on their genetic predicted phenotype. A similar program as part of a course on medication surveillance could help pharmacy students with understanding the current state of field, the clinical utility of PGx and their ability to interpret and act on genetic data. Further studies should investigate whether this form of education and/or in combination with other methods such as specialized residencies can reduce the PGx knowledge gap in the current pharmacy curriculum.

Conclusion

This study shows that pharmacy students believe in the concept of hereditary drug response and have high expectations towards PGx. In a comparison with practicing pharmacists' differences in elements of feeling qualified to recommend PGx testing, the use of information on the applicability of PGx in pharmacotherapy and opinions about the possible negative impact of PGx tests were observed. Similar to their future colleagues the surveyed students perceive a knowledge gap despite having received education on the subject.

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Supplementary Document S9.1: Questionnaire

Questions	Answer options
Section 1: Baseline information	
Q1: What is your gender?	☐ Male ☐ Female
Q2: What is your age?	...
Q3: At which University do you currently follow your curriculum?	☐ University of Groningen ☐ University of Leiden ☐ University of Utrecht ☐ Other
Q4: In which year of the program do you currently follow courses?	☐ First year ☐ Second year ☐ Third year ☐ Fourth year ☐ Fifth year ☐ Sixth year
Q5: Has PGx been part of any course that you have followed as part of your curriculum	☐ Yes ☐ No
Section 2: Belief and expectations towards PGx	
Q6: Do you believe that a patient's genetic profile may influence his/her response to drug therapy?	☐ Yes ☐ No
Q7: Do you expect that pharmacogenetic testing will prevent your patient from taking the wrong medicine (or the wrong dose)? (0 = no expectations... / 3 = very high expectations ...)	☐ 0 ☐ 1 ☐ 2 ☐ 3
Q8: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will be more efficacious in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0 ☐ 1 ☐ 2 ☐ 3
Q9: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will cause less side effects in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0 ☐ 1 ☐ 2 ☐ 3

Supplementary Document S9.1: Continued

Questions	Answer options
Section 3: Attitude towards own ability to interpret PGx test results	
Q10: Do you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice?	☐ Yes ☐ Yes, but after having had training on the subject ☐ No, this is not my responsibility
Q11: Would you feel qualified to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case?	☐ Yes ☐ No ☐ I don't know
Q12: If a pharmacogenetic test revealed that the only available drug to treat your patient's disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?	☐ Yes ☐ No ☐ Yes, only if he/she had a life-threatening disease
Q13: Would you feel qualified to recommend genetic testing to your patients if those tests could reveal which diseases are liable to affect them in the future	☐ Yes ☐ Yes, but only if that disease could be treated ☐ No ☐ I don't know
Section 4: Access to and use of PGx information	
Q14: Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy?	☐ Yes ☐ No
Q15: Would you obtain extra information on genetic testing and its application in the context of drug therapy?	☐ Yes ☐ No
Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy? (select all that apply)	☐ Drug labelling (package insert) ☐ Colleague ☐ Post-academic education and pharmacotherapeutic meetings ☐ Internet ☐ Genetic testing laboratory ☐ Other ...

Supplementary Document S9.1: Continued

Questions				Answer options	
Q17: What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test					
	Very unimportant	Unimportant	Un-decided	Important	Very important
Authority approval or recommendation					
Speciality guideline					
Scientific journal					
Recommendation or experience of thought leaders or respected colleagues					
Q18: Where do you obtain information to make a choice about the drug and dose in case of a known genotype?					
				☐ Drug labelling (package insert)	
				☐ Registration authority	
				☐ Scientific literature	
				☐ Colleague	
				☐ Farmaceutisch Kompas	
				☐ Kennisbank / Informatorium medicamentorum	
				☐ Other ...	
Q19: Were you aware that in the Netherlands dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?					
				☐ Yes	
				☐ No	
Q20: Were you aware that in the Netherlands medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?					
				☐ Yes	
				☐ No	
Section 5: Worries toward PGx testing & coverage of PGx testing					
Q21: Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?				☐ Yes	
				☐ No	
				☐ No opinion	
Q22: Are you worried that a PGx test might show there is no suitable drug for your patient? (0 = not worried / 3 = very worried)?					
				☐ 0	
				☐ 1	
				☐ 2	
				☐ 3	

Supplementary Document S9.1: Continued

Questions	Answer options
Q23: Are you worried that a PGx test could reveal that your patient also has risk factors for another disease that he/she does not know about? (0 = not worried / 3 = very worried)?	☐ 0 ☐ 1 ☐ 2 ☐ 3
Q24: Are you worried that one of your patient's PGx test results could be passed to an unauthorized person? (0 = not worried / 3 = very worried)	☐ 0 ☐ 1 ☐ 2 ☐ 3
Q25: Are you more concerned about the loss of privacy of a patient's genetic information from the results of pharmacogenetic tests than from the results of other laboratory or diagnostic tests?	☐ Yes ☐ No
Q26: Among the following health professionals, which ones should have access to patients' pharmacogenetic information (select all that apply)	☐ Physician ☐ Pharmacist ☐ Genetic counsellor ☐ Clinical chemist ☐ Nurse practitioner ☐ Psychologist ☐ General nurse ☐ Social worker ☐ Dietician
Q27: Are you worried that a health insurance could obtain information about an individual's genotype based on the drug/dose prescribed? (0 = not worried / 3 = very worried)	☐ 0 ☐ 1 ☐ 2 ☐ 3
Q28: Do you believe that health insurers should provide full coverage for pharmacogenetic tests?	☐ Always ☐ Sometimes ☐ Never

Supplementary Document S9.2: Results per question

Question	Answer	N	%
Section 1: Baseline information			
Q1: What is your gender?	☐ Male	44	29.7
	☐ Female	104	70.3
Q2: What is your age?	☐ 20	4	2.7
	☐ 21	11	7.4
	☐ 22	15	10.1
	☐ 23	30	20.3
	☐ 24	35	23.6
	☐ 25	31	20.9
	☐ 26	15	10.1
	☐ 27	1	0.7
	☐ 28	4	2.7
	☐ 29	2	1.4
Q3: At which University do you currently follow your curriculum?	☐ University of Groningen	47	31.8
	☐ University of Utrecht	101	68.2
	☐ Other	0	0.0
Q4: In which year of the program do you currently follow courses?	☐ Second year	1	0.7
	☐ Third year	8	5.4
	☐ Fourth year	18.2	18.2
	☐ Fifth year	28.4	28.4
	☐ Sixth year	47.3	47.3
Q5: Has PGx been part of any course that you have followed as part of your curriculum	☐ Yes	143	96.6
	☐ No	5	3.4
Section 2: Belief and expectations towards PGx			
Q6: Do you believe that a patient's genetic profile may influence his/her response to drug therapy?	☐ Yes	148	100.0
	☐ No	0	0.0
Q7: Do you expect that pharmacogenetic testing will prevent your patient from taking the wrong medicine (or the wrong dose)? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	5	3.4
	☐ 1	15	10.1
	☐ 2	73	49.3
	☐ 3	55	37.2
Q8: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will be more efficacious in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	0	0.0
	☐ 1	19	12.8
	☐ 2	63	42.6
	☐ 3	66	44.6

Supplementary Document S9.2: Continued

Question	Answer	N	%
Q9: Do you expect that pharmacogenetic testing will allow detecting which drug (or which dose) will cause less side effects in your patient? (0 = no expectations... / 3 = very high expectations ...)	☐ 0	6	4.1
	☐ 1	33	22.3
	☐ 2	66	44.6
	☐ 3	43	29.1
Section 3: Attitude towards own ability to interpret PGx test results			
Q10: Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice?	☐ Yes	41	27.7
	☐ Yes, but after having had training on the subject	105	70.9
	☐ No, this is not my responsibility	2	1.4
Q11: Would you feel qualified to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case?	☐ Yes	111	75.0
	☐ No	12	8.1
	☐ Un-decided	25	16.9
Q12: If a pharmacogenetic test revealed that the only available drug to treat your patient's disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?	☐ Yes	7	4.7
	☐ Yes, only if he/she had a life-threatening disease	95	64.2
	☐ No	46	31.1
Q13: Would you feel qualified to recommend genetic testing to your patients if those tests could reveal which diseases are liable to affect them in the future	☐ Yes	31	20.9
	☐ Yes, but only if that disease could be treated	34	23.0
	☐ No	46	31.1
	☐ Un-decided	37	25.0
Section 4: Access to and use of PGx information			
Q14: Do you feel that you are adequately informed about the availability of genetic testing and its application in the context of drug therapy?	☐ Yes	19	87.2
	☐ No	129	87.2
Q15: Would you obtain extra information on genetic testing and its application in the context of drug therapy? (if "No" proceed to Q17)	☐ Yes	134	90.5
	☐ No	14	9.5

Supplementary Document S9.2: Continued

Question		Answer	N	%
Q16: Where would you obtain information on genetic testing and its application in the context of drug therapy? (select all that apply)		☐ Drug labeling (package insert)	102	68.9
		☐ Colleague	75	50.7
		☐ Post-academic education and pharmacotherapeutic meetings	79	53.4
		☐ Internet	97	65.5
		☐ Genetic testing laboratory	68	45.9
		☐ Other	23	15.5
Q17: What level of evidence is of importance to you in consideration of ordering a pharmacogenetic test	authority approval of recommendation	☐ Very unimportant	0	0.0
		☐ Unimportant	1	0.7
		☐ Un-decided	23	15.5
		☐ Important	75	50.7
		☐ Very important	49	33.1
	Speciality guidelines	☐ Very unimportant	0	0.0
		☐ Unimportant	0	0.0
		☐ Un-decided	13	8.8
		☐ Important	88	59.5
		☐ Very important	47	31.8
	Scientific journal	☐ Very unimportant	0	0.0
		☐ Unimportant	1	0.7
		☐ Un-decided	25	16.9
		☐ Important	75	50.7
		☐ Very important	47	31.8
	Recommendation or experience of thought leaders or respected colleagues	☐ Very unimportant	0	0.0
		☐ Unimportant	12	8.1
		☐ Un-decided	67	45.3
		☐ Important	61	41.2
		☐ Very important	8	5.4
Q18: Where would you obtain information to make a choice about the drug and dose in case of a known genotype?		☐ Drug labeling (package insert)	81	54.7
		☐ Registration authority	49	33.1
		☐ Scientific literature	115	77.7
		☐ Colleague	29	19.6
		☐ Pharmaceutical Compass	51	34.5
		☐ Informatorium Medicamentorum	135	91.2
		☐ Other ...	1	0.7

Supplementary Document S9.2: Continued

Question	Answer	N	%
Q19: Were you aware that in the Netherlands dosing guidelines are available with information on the choice and dose of drugs based on the genotype of a patient?	☐ Yes	115	77.7
	☐ No	33	22.3
Q20: Were you aware that in the Netherlands medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?	☐ Yes	35	23.6
	☐ No	113	76.4
Section 5: Worries toward PGx testing			
Q21: Do you think that your patient's unfavorable test results could have adverse psychological consequences on him and his family?	☐ Yes	129	87.2
	☐ No	7	4.7
	☐ No opinion	12	8.1
Q22: Are you worried that a PGx test might show there is no suitable drug for your patient? (0 = not worried / 3 = very worried)?	☐ 0	40	27.0
	☐ 1	43	29.1
	☐ 2	43	29.1
	☐ 3	22	14.9
Q23: Are you worried that a PGx test could reveal that your patient also has risk factors for another disease that he/she does not know about? (0 = not worried / 3 = very worried)?	☐ 0	20	13.5
	☐ 1	43	29.1
	☐ 2	59	39.9
	☐ 3	26	17.6
Q24: Are you worried that one of your patient's PGx test results could be passed to an unauthorized person? (0 = not worried / 3 = very worried)	☐ 0	12	8.1
	☐ 1	30	20.3
	☐ 2	39	26.4
	☐ 3	67	45.3
Q25: Are you more concerned about the loss of privacy of a patient's genetic information from the results of pharmacogenetic tests than from the results of other laboratory or diagnostic tests?	☐ Yes	34	23.0
	☐ No	114	77.0
Q26: Among the following health professionals, which ones should have access to patients' pharmacogenetic information (select all that apply)	☐ Physician	145	98.0
	☐ Pharmacist	147	99.3
	☐ Nurse practitioner	25	16.9
	☐ General nurse	5	3.4
	☐ Genetic counsellor	116	78.4
	☐ Clinical chemist	64	43.2
	☐ Social worker	2	1.4
	☐ Psychologist	13	8.8
	☐ Dietician	7	4.7

Supplementary Document S9.2: *Continued*

Question	Answer	N	%
Q27: Are you worried that a health insurance could obtain information about an individual's genotype based on the drug/dose prescribed? (0 = not worried / 3 = very worried)	☐ 0	2	1.4
	☐ 1	11	7.4
	☐ 2	36	24.3
	☐ 3	99	66.9
Q28: Do you believe that health insurers should provide full coverage for pharmacogenetic tests?	☐ Always	32	21.6
	☐ Sometimes	116	78.4
	☐ Never	0	0.0

Supplementary Document S9.3: Comparison between pharmacy students and pharmacists

	Pharmacy students		Practicing pharmacists		
	N	%	N	%	P value
Response					
Yes	148	18.0	667	18.8	p = 0.620
No	676	82.0	2883	81.2	
Total	824	100.0	3550	100.0	
Q1: What is your gender?					
Male	44	29.7	305	45.7	p < 0.001
Female	104	70.3	362	54.3	
Total	148	100	667	100.0	
Q2: What is your age?					
20–29	148	100.0	105	15.7	p < 0.001
30–39	0	0.0	209	31.3	
40–49	0	0.0	144	21.6	
50–59	0	0.0	158	23.7	
≥ 60	0	0.0	51	7.6	
Total	148	100.0	667	100.0	
Q3: At which University do you currently follow your curriculum / did you follow your curriculum?					
University of Groningen	47	31.8	221	33.1	p < 0.001
University of Leiden	0	0.0	38	5.7	
University of Utrecht	101	68.2	537	53.5	
University of Amsterdam	0	0.0	32	4.8	
Other	0	0.0	19	2.8	
Total	148	100.0	667	100.0	
Q5: Did you receive education on PGx during your curriculum					
Yes	143	96.6	265	60.3	p < 0.001
No	5	3.4	402	39.7	
Total	148	100.0	667	100.0	
Q10: Would you feel qualified to receive your patient’s pharmacogenetic testing results, interpret them and advise your patient on a treatment choice					
No	2	1.4	45	6.7	p = 0.038
Yes	41	27.7	180	27.0	
Yes, after training	105	70.9	442	66.3	
Total	148	100.0	667	100.0	

Supplementary Document S9.3: Continued

	Pharmacy students		Practicing pharmacists		
	N	%	N	%	P value
Q11: Would you feel qualified to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case					
No	12	8.1	164	24.6	p < 0.001
Yes	111	75.0	323	48.4	
Undecided	25	16.9	180	27.0	
Total	148	100.0	667	100.0	
Q12: If a pharmacogenetic test revealed that the only available drug to treat your patient's disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?					
No	46	31.1	327	49.0	p < 0.001
Yes	7	4.7	23	3.4	
Yes, only if he/she had a life-threatening disease	95	64.2	317	47.5	
Total	148	100.0	667	100.0	
Q13: Would you feel qualified to recommend genetic testing to your patients if those tests could reveal which diseases are liable to affect them in the future					
No	46	31.1	339	50.8	p < 0.001
Yes	31	20.9	52	7.8	
Yes, but only if that disease could be treated	34	23.0	84	12.6	
Undecided	37	25.0	192	28.8	
Total	148	100.0	667	100.0	
Q15: Would you obtain extra information on genetic testing and its application in the context of drug therapy?					
No	14	9.5%	409	61.3%	p < 0.001
Yes	134	90.5%	258	38.7%	
Total	148	100.0	667	100.0	
Q16: Where would you obtain information on genetic testing and its application in the context of drug therapy?					
Drug labelling (package insert)					
No	46	31.1	464	69.6	p < 0.001
Yes	102	68.9	203	30.4	
Total	148	100.0	667	100.0	
Colleague					
No	73	49.3	567	85.0	p < 0.001
Yes	75	50.7	100	15.0	
Total	148	100.0	667	100.0	

Supplementary Document S9.3: Continued

	Pharmacy students		Practicing pharmacists		
	N	%	N	%	P value
Post-academic education and pharmacotherapeutic meetings					
No	69	46.6	588	88.2	p < 0.001
Yes	79	53.4	79	11.8	
Total	148	100.0	667	100.0	
Internet					
No	51	34.5	504	75.6	p < 0.001
Yes	97	65.5	163	24.4	
Total	148	100.0	667	100.0	
Genetic testing laboratory					
No	80	54.1	605	90.7	p < 0.001
Yes	68	45.9	62	9.3	
Total	148	100.0	667	100.0	
Other					
No	125	84.5	601	90.1	p = 0.046
Yes	23	15.5	66	9.9	
Total	148	100.0	667	100.0	
Q18: Where do you obtain information to make a choice about the drug and dose in case of a known genotype					
Scientific literature					
No	33	22.3	278	41.7	p < 0.001
Yes	115	77.7	389	58.3	
Total	148	100.0	667	100.0	
Other					
No	147	99.3	630	94.5	p = 0.011
Yes	1	0.7	37	5.5	
Total	148	100.0	667	100.0	
Q20: Were you aware that in the Netherlands ...					
medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?					
No	113	76.4	231	34.6	p < 0.001
Yes	35	23.6	436	65.4	
Total	148	100.0	667	100.0	

Supplementary Document S9.3: Continued

	Pharmacy students		Practicing pharmacists		
	N	%	N	%	P value
Q21: Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?					
No	7	4.7	105	15.7	p < 0.001
Yes	129	87.2	425	63.7	
No opinion	12	8.1	137	2.5	
Total	148	100.0	667	100.0	
Q22: Are you worried that ...					
A PGx test might show there is no suitable drug for your patient					
0	40	27.0	268	40.2	p < 0.001
1	43	29.1	210	31.5	
2	43	29.1	150	22.5	
3	22	14.9	39	5.8	
Total	148	100.0	667	100.0	
Q27: Which of the following health professionals should have access to the patient's PGx test results					
Social worker					
No	146	98.6	666	99.9	p = 0.029
Yes	2	1.4	1	0.1	
Total	148	100.0	667	100.0	

Supplementary Document S9.4: Result of the multivariate analysis of differences between pharmacy students and pharmacists

To determine whether other covariates as age and gender could explain possible differences in answers found between the two groups the significant results of the univariate analysis were analysed using a multivariate model including age and gender. Questions with a dichotomous (YES/NO) answer model were analysed using a logistic regression model (Q15, 16, 18, 20), whereas for questions with 3 or more answer options (Q10, 11, 13, 21) a multinomial regression model was used.

Result of logistic regression analysis

Q15: Would you obtain extra information on genetic testing and its application in the context of drug therapy?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	12.61 (6.42–24.77)	< 0.001
Gender (female vs. male)	0.60 (0.43–0.83)	0.002
Age		
30–39 (vs. 20–29)	0.85 (0.53–1.38)	0.510
40–49 (vs. 20–29)	0.64 (0.38–1.08)	0.098
50–59 (vs. 20–29)	0.66 (0.39–1.12)	0.123
60–69 (vs. 20–29)	0.50 (0.24–1.04)	0.064

Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy – Drug labelling / package insert		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	3.41 (2.02–5.77)	< 0.001
Gender (female vs. male)	0.77 (0.56–1.06)	0.108
Age		
30–39 (vs. 20–29)	0.64 (0.39–1.05)	0.080
40–49 (vs. 20–29)	0.51 (0.30–0.89)	0.017
50–59 (vs. 20–29)	0.59 (0.35–1.02)	0.057
60–69 (vs. 20–29)	0.56 (0.27–1.18)	0.130

Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy – Colleague		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	3.28 (1.88–5.70)	< 0.001
Gender (female vs. male)	0.94 (0.64–1.38)	0.768
Age		
30–39 (vs. 20–29)	0.53 (0.29–0.96)	0.037
40–49 (vs. 20–29)	0.40 (0.20–0.79)	0.009
50–59 (vs. 20–29)	0.56 (0.29–1.07)	0.077
60–69 (vs. 20–29)	0.34 (0.12–0.96)	0.042

Supplementary Document S9.4: Continued

Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy – Post-academic education and pharmacotherapeutic meetings		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	13.70 (6.21–30.21)	< 0.001
Gender (female vs. male)	0.69 (0.46–1.04)	0.076
Age		
30–39 (vs. 20–29)	1.20 (0.51–2.84)	0.678
40–49 (vs. 20–29)	1.12 (0.45–2.82)	0.808
50–59 (vs. 20–29)	2.56 (1.11–5.91)	0.028
60–69 (vs. 20–29)	1.58 (0.53–4.75)	0.411

Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy – Internet		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	4.47 (2.59–7.69)	< 0.001
Gender (female vs. male)	0.55 (0.39–0.77)	0.001
Age		
30–39 (vs. 20–29)	0.72 (0.43–1.23)	0.234
40–49 (vs. 20–29)	0.56 (0.31–1.00)	0.051
50–59 (vs. 20–29)	0.61 (0.34–1.09)	0.093
60–69 (vs. 20–29)	0.42 (0.18–0.97)	0.041

Q16: Where do you obtain information on genetic testing and its application in the context of drug therapy – Other		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	2.46 (1.01–5.99)	0.048
Gender (female vs. male)	0.46 (0.29–0.73)	0.001
Age		
30–39 (vs. 20–29)	2.29 (0.97–5.42)	0.059
40–49 (vs. 20–29)	1.09 (0.41–2.90)	0.861
50–59 (vs. 20–29)	0.61 (0.22–1.74)	0.358
60–69 (vs. 20–29)	1.01 (0.30–3.44)	0.988

Q18: Where do you obtain information to make a choice about the drug and dose in case of a known genotype – Scientific literature		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	1.88 (1.08–3.28)	0.027
Gender (female vs. male)	0.87 (0.64–1.18)	0.369
Age		
30–39 (vs. 20–29)	0.91 (0.56–1.49)	0.709
40–49 (vs. 20–29)	0.64 (0.38–1.09)	0.098
50–59 (vs. 20–29)	0.53 (0.31–0.89)	0.016
60–69 (vs. 20–29)	0.72 (0.36–1.46)	0.364

Supplementary Document S9.4: Continued

Q18: Where do you obtain information to make a choice about the drug and dose in case of a known genotype – Other		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	0.11 (0.01–0.94)	0.044
Gender (female vs. male)	0.92 (0.46–1.84)	0.823
Age		
30–39 (vs. 20–29)	1.08 (0.40–2.95)	0.882
40–49 (vs. 20–29)	0.58 (0.17–1.98)	0.390
50–59 (vs. 20–29)	1.20 (0.41–3.47)	0.741
60–69 (vs. 20–29)	0.65 (0.12–3.46)	0.609

Q20: Were you aware that in the Netherlands medication surveillance based on the genotype of a patient is incorporated in the automated drug dispensing systems?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	0.12 (0.07–0.22)	< 0.001
Gender (female vs. male)	0.94 (0.68–1.29)	0.680
Age		
30–39 (vs. 20–29)	1.29 (0.76–2.20)	0.348
40–49 (vs. 20–29)	0.66 (0.38–1.14)	0.133
50–59 (vs. 20–29)	0.49 (0.28–0.84)	0.010
60–69 (vs. 20–29)	0.32 (0.15–0.65)	0.002

Results of multinomial regression

Q10: Would you feel qualified to receive your patient's pharmacogenetic testing results, interpret them and advise your patient on a treatment choice? (multinomial regression)		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	2.59 (0.47–14.26)	0.274
Gender (female vs. male)	0.27 (0.13–0.55)	< 0.001
Age		
30–39 (vs. 20–29)	0.80 (0.26–2.49)	0.697
40–49 (vs. 20–29)	0.71 (0.19–2.72)	0.619
50–59 (vs. 20–29)	0.10 (0.03–0.34)	< 0.001
60–69 (vs. 20–29)	0.10 (0.02–0.43)	0.002
Answer 2: Yes, after training (vs. reference no)		
Cohort (students vs. pharmacist)	3.97 (0.75–21.07)	0.106
Gender (female vs. male)	0.56 (0.29–1.10)	0.090
Age		
30–39 (vs. 20–29)	0.79 (0.26–2.38)	0.673
40–49 (vs. 20–29)	1.51 (0.42–5.43)	0.531
50–59 (vs. 20–29)	0.40 (0.14–1.16)	0.092
60–69 (vs. 20–29)	0.34 (0.09–1.26)	0.106

Supplementary Document S9.4: Continued

Q11: Would you feel qualified to recommend pharmacogenetic testing to your patients if those tests could predict that a specific drug could be efficacious in their case?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	5.25 (2.47–11.16)	< 0.001
Gender (female vs. male)	0.57 (0.39–0.85)	0.006
Age		
30–39 (vs. 20–29)	1.48 (0.82–2.66)	0.188
40–49 (vs. 20–29)	1.35 (0.72–2.53)	0.351
50–59 (vs. 20–29)	0.59 (0.32–1.09)	0.093
60–69 (vs. 20–29)	0.53 (0.23–1.20)	0.127
Answer 2: Undecided (vs. reference no)		
Cohort (students vs. pharmacist)	2.33 (0.98–5.56)	0.056
Gender (female vs. male)	1.10 (0.71–1.71)	0.669
Age		
30–39 (vs. 20–29)	1.46 (0.75–2.86)	0.267
40–49 (vs. 20–29)	1.40 (0.68–2.87)	0.363
50–59 (vs. 20–29)	1.26 (0.63–2.49)	0.512
60–69 (vs. 20–29)	0.90 (0.36–2.27)	0.821

Q12: If a pharmacogenetic test revealed that the only available drug to treat your patient's disease is ineffective or leads to severe side effects, would you still advise your patient to take that medicine?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	0.66 (0.20–2.19)	0.499
Gender (female vs. male)	0.30 (0.13–0.68)	0.004
Age		
30–39 (vs. 20–29)	0.31 (0.09–1.01)	0.051
40–49 (vs. 20–29)	0.37 (0.12–1.19)	0.096
50–69 (vs. 20–29)	0.05 (0.01–0.25)	< 0.001
Answer 2: Yes, only if he/she had a life-threatening disease (vs. reference no)		
Cohort (students vs. pharmacist)	0.88 (0.50–1.54)	0.646
Gender (female vs. male)	1.42 (1.04–1.93)	0.025
Age		
30–39 (vs. 20–29)	0.58 (0.35–0.98)	0.041
40–49 (vs. 20–29)	0.28 (0.16–0.49)	< 0.001
50–69 (vs. 20–29)	0.30 (0.17–0.51)	< 0.001

Supplementary Document S9.4: Continued

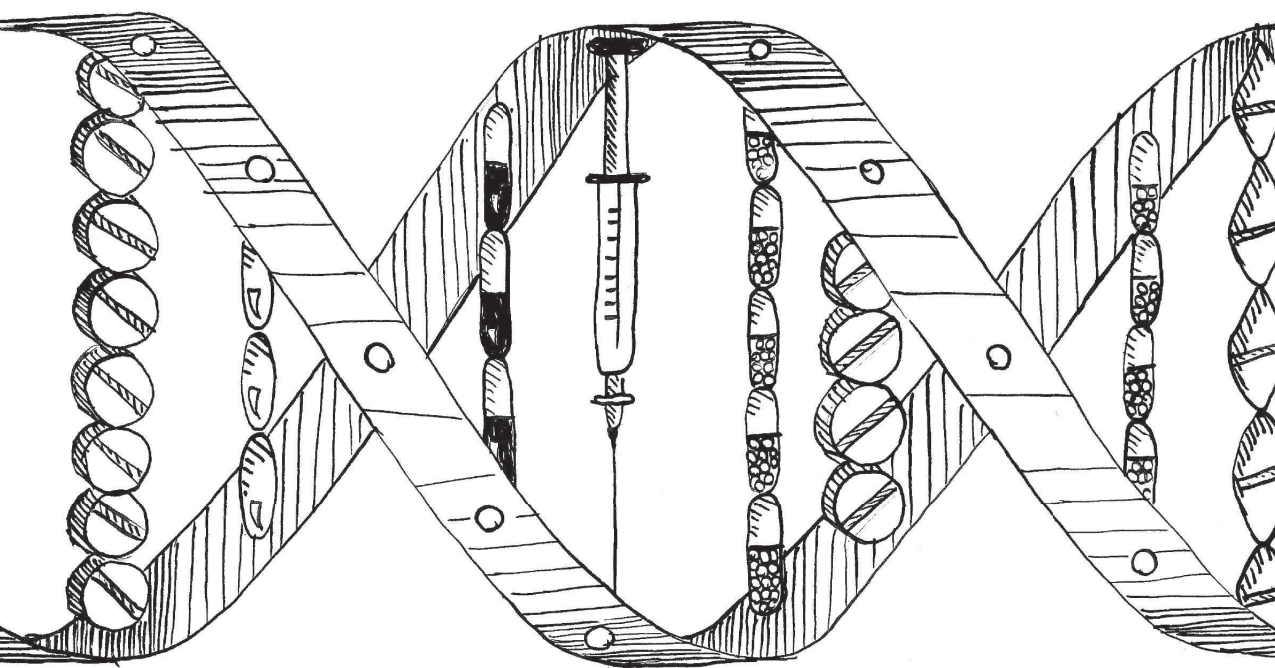
Q13: Would you feel qualified to recommend genetic testing to your patients if those tests could reveal which diseases are liable to affect them in the future?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	3.64 (1.63–8.12)	0.002
Gender (female vs. male)	0.30 (0.18–0.51)	< 0.001
Age		
30–39 (vs. 20–29)	0.76 (0.33–1.78)	0.532
40–49 (vs. 20–29)	0.44 (0.16–1.19)	0.107
50–59 (vs. 20–29)	0.48 (0.19–1.22)	0.124
60–69 (vs. 20–29)	0.62 (0.19–2.05)	0.434
Answer 2: Yes, but only if that disease could be treated (vs. reference no)		
Cohort (students vs. pharmacist)	7.41 (2.86–19.20)	< 0.001
Gender (female vs. male)	0.49 (0.31–0.77)	0.002
Age		
30–39 (vs. 20–29)	2.28 (0.88–5.92)	0.090
40–49 (vs. 20–29)	2.04 (0.76–5.50)	0.158
50–59 (vs. 20–29)	2.54 (0.97–6.71)	0.059
60–69 (vs. 20–29)	2.62 (0.82–8.38)	0.105
Answer 3: Undecided (vs. reference no)		
Cohort (students vs. pharmacist)	1.81 (0.97–3.39)	0.063
Gender (female vs. male)	0.90 (0.63–1.28)	0.541
Age		
30–39 (vs. 20–29)	1.56 (0.90–2.71)	0.112
40–49 (vs. 20–29)	1.14 (0.63–2.06)	0.675
50–59 (vs. 20–29)	1.19 (0.65–2.18)	0.567
60–69 (vs. 20–29)	1.15 (0.50–2.66)	0.737

Supplementary Document S9.4: Continued

Q21: Do you think that your patient's unfavourable test results could have adverse psychological consequences on him and his family?		
	Odd's ratio (confidence interval)	P value
Answer 1: Yes (vs. reference no)		
Cohort (students vs. pharmacist)	2.92 (1.08–7.89)	0.034
Gender (female vs. male)	1.41 (0.91–2.17)	0.121
Age		
30–39 (vs. 20–29)	0.49 (0.24–1.02)	0.058
40–49 (vs. 20–29)	0.69 (0.31–1.51)	0.353
50–59 (vs. 20–29)	0.81 (0.36–1.81)	0.606
60–69 (vs. 20–29)	0.85 (0.29–2.46)	0.764
Answer 2: No opinion (vs. reference no)		
Cohort (students vs. pharmacist)	0.86 (0.26–2.79)	0.797
Gender (female vs. male)	1.96 (1.16–3.31)	0.012
Age		
30–39 (vs. 20–29)	0.52 (0.22–1.21)	0.131
40–49 (vs. 20–29)	0.58 (0.23–1.47)	0.255
50–59 (vs. 20–29)	1.02 (0.41–2.57)	0.962
60–69 (vs. 20–29)	1.17 (0.35–3.95)	0.795

Part V

General discussion
& Summary

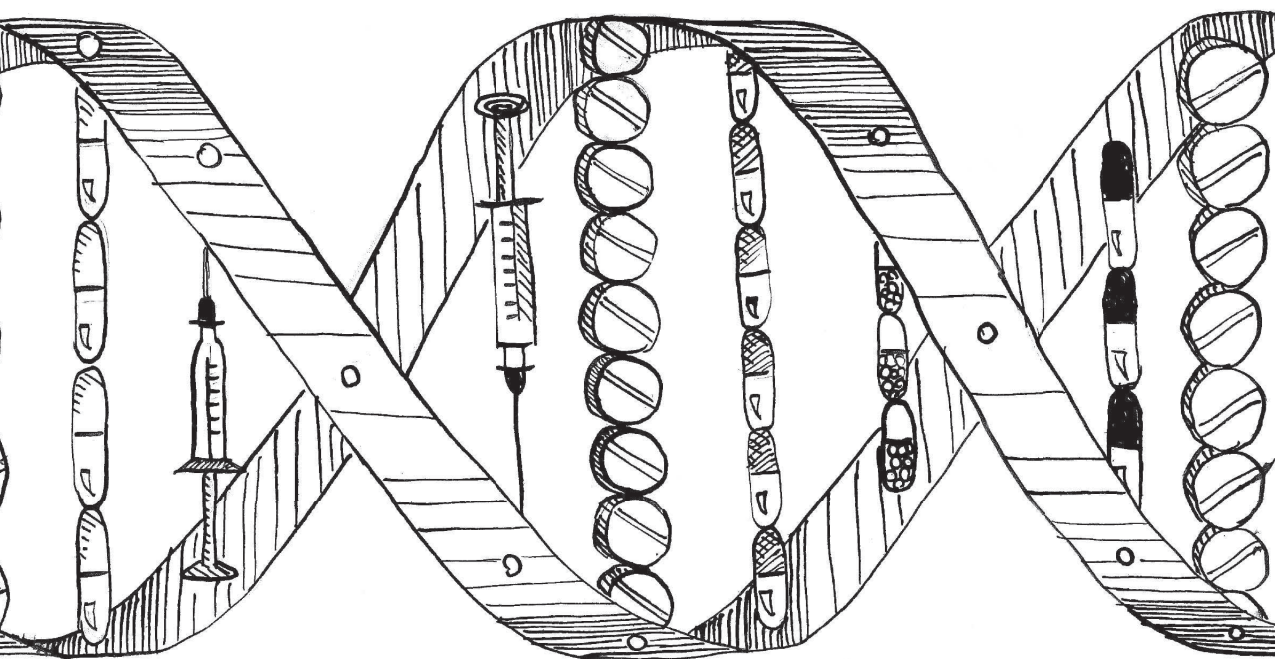


Chapter 10

General discussion

Adapted from: Implementation of
pharmacogenetics in everyday clinical settings

Bank PCD, Swen JJ, Guchelaar HJ



Abstract

Currently, germline pharmacogenetics (PGx) is successfully implemented within certain specialties in clinical care. With the integration of PGx in pharmacotherapy multiple stakeholders are involved, which are identified in this chapter. Clinically relevant pharmacogenes with their related PGx test are discussed, along with diagnostic test criteria to guide clinicians and policy makers in PGx-test selection. The chapter further reviews the similarities and the differences between the guidelines of the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) which both support healthcare professionals in understanding PGx test results and help guiding pharmacotherapy by providing evidence-based dosing recommendations. Finally, clinical studies which provide scientific evidence and information on cost-effectiveness supporting clinical implementation of PGx in clinical care are discussed along with the remaining barriers for adoption of PGx-testing by healthcare professionals.

Introduction

In the last decade pharmacogenetics (PGx) has made the transition from a field within clinical pharmacology research to an integral part of pharmacotherapy. Barriers obstructing the integration of PGx in clinical care included a lack of clinical grade tests, a lack of evidence based guidelines providing interpretation and therapeutic recommendations and a lack of integration into the workflow of healthcare professionals (1). Currently, many of these barriers have been successfully addressed. Multiple genotyping tests suitable for clinical use have been developed. Consortia such as the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) both have provided widely acclaimed guidelines (2-4). Clinical decision support (CDS) integrating electronic medication surveillance based on genotype into the workflow has become available on institutional or national level (3-7). For several PGx genes large studies have provided evidence that information on germline variants in single genes can lead to more effective or safer therapy. The first part of this review identifies the parties that play a role in the wide-spread implementation of PGx in clinical care. The second part covers various topics regarding current implementation of PGx in clinical practice, including: clinically relevant tests; diagnostic criteria; guidelines containing evidence based therapeutic recommendations; multiple implementation programs; and barriers still hindering the full and widespread integration of PGx in clinical care.

Players in the field

On the road to wide-spread integration of PGx into clinical care multiple parties play a role in PGx becoming a part of routine practice. These roles include making decisions to adopt PGx testing, enforcing research on genetic biomarkers, providing the materials for clinical laboratories to test patients, or reviewing available evidence to provide healthcare professionals the tools to optimize pharmacotherapy. Different stakeholders can be involved in the implementation process including the biotechnology industry, governments, clinicians, healthcare insurers and patients.

Research institutions and the analytical industry are developing new methods for detection of genetic variants. Although this technology is mostly developed to detect mutations responsible for genetic diseases, clinical laboratories can optimize the developed assays to detect relevant (germline) PGx variants. In licensed medicine, information on PGx markers in drug labels increasingly will lead to ordering a PGx test before the use of the drug. However, the pharmaceutical industry still is somewhat divided regarding the concept of personalized

medicine and as long as development of “one-size-fits-all” drugs is an aim, pharmaceutical companies will probably consider the need for PGx markers as a disadvantage. Currently, PGx is mainly used by the pharmaceutical industry as a manner to salvage drugs that do not appear effective or safe in clinical trials. Erlotinib is an example of a drug that in the initial phase appeared not to show the expected results in terms of overall survival in phase II and III studies whereas a small subgroup performed exceptionally well. Further research revealed specific mutations in the EGFR receptor that could explain the gain in survival (8). Although mandatory genetic testing before prescribing is mainly seen in combination with somatic mutations, genetic testing for germline variants is required before starting certain treatments, such as eliglustat which requires *CYP2D6* testing with an FDA-cleared test. For the actionable phenotype ‘poor metabolizer’ the dosage has to be halved, while for the phenotype ‘ultra-rapid metabolizer’ this therapy is not recommended as patients with the UM phenotype might not achieve adequate levels of concentrations of eliglustat (9). However, patents have expired for many drugs with gene interactions and producers of generic drugs are not interested in advancing the research in (germline) biomarkers for the drugs in their portfolio. For multiple gene-drug pairs the regulators had to step in and issue boxed warnings to enforce healthcare professionals to take gene-pairs interactions into account when prescribing (e.g. *CYP2C19* and clopidogrel and *CYP2C9/VKORC1* and warfarin). Additionally, because of the efforts of EMA and FDA policy innovative pharmaceutical companies are enforced to investigate the influence of PGx variants. As an outcome, the FDA reported an increase in the number of registered drugs with information on genetic biomarkers in corporate in drug labels (10). Similarly, in 2015 Ehmann et al. showed an increased uptake of information on PGx biomarkers in drug labels of drugs registered in the European Union in the last decades (11).

Based on information from drug labels and guidelines, healthcare professionals are expected to adjust pharmacotherapy if the genotype of the patient is known and is actionable. However, currently the clinically relevant genotypes are not known for the majority of the patients. Since no solid evidence is available which supports the use of upfront panel-based (or pre-emptive) genotyping, the use of PGx is restricted mainly to single-gene testing mostly performed retrospectively in order to explain a lack of efficacy or adverse reactions. Many physicians and pharmacists are not yet fully aware of the potential of PGx notwithstanding the fact that in the last decades clinical research has generated data on the existence and frequencies of genetic variants in a variety of pharmacogenes. In addition, several multidisciplinary initiatives of healthcare professionals and researchers have translated the effects of aberrant phenotypes into widely acclaimed clinical guidelines providing therapeutic recommendations (2-4).

Obviously, patients play an important role in the uptake of PGx in clinical care. Relative to secondary care, in primary care patients have a large impact on the decisions that are made concerning pharmacotherapy. Using a survey Haga et al. showed that the majority of patients are positive towards PGx as this diagnostic in the eyes of the surveyed patients can lead towards selecting the right drug by healthcare professionals and ultimately to safer and more effective pharmacotherapy. However, concerns whether third-parties can access the results of a PGx test result or the fear that PGx results can be used for nefarious reasons by employers or healthcare insurance are reasons to reject a PGx test (12).

Finally, one of the players with a large influence in primary care regarding implementation of PGx-testing are health insurers. This stakeholder determines whether costs of PGx and other laboratory test used in primary care are reimbursed, whereas in secondary care laboratory test are usually part of integral treatment plans that are reimbursed. Lack of coverage of PGx testing by insurance companies is an important demotivator in patients' uptake of PGx testing. Haga et al. have shown that 81% of patient in their study are likely to decline if the test was not reimbursed (12). Additionally, the current (lack of) policy regarding coverage of PGx testing is also a major reason why the adoption of PGx testing among healthcare professionals is low. In a recent survey 14.0 % of the pharmacists indicate that uncertainty regarding the reimbursement is a reason for not having ordered or recommended a PGx test in the previous six months (**Chapter 8**).

Challenges for implementation of PGx

In the process of widespread implementation of PGx in clinical care several challenges still play a role. These hurdles include the answers to the question which pharmacogenes should be incorporated in routine care and which known variants in the gene should be tested for. The decision on whether genetic testing for a pharmacogene is implemented is dependent on several aspects including the diagnostic criteria of the assays, available therapeutic recommendations for patients with an aberrant phenotype and evidence whether PGx testing improves patient care. Additionally, in certain healthcare settings data on cost-effectiveness and cost-consequences also can play a role. These topics will be covered in the next sections.

Selecting clinically relevant pharmacogenes

Regarding the above-mentioned challenges, the first question to be asked is which pharmacogenes are clinically relevant and should be tested. The answer can partially be found in the guidelines of the CPIC and the DPWG. Although the guidelines created by both

consortia are written on the premise that the genotype of the patient is already known, and the guidelines do not provide a recommendation who to test, the consortia have already selected gene-drug pairs with a clinically relevant impact on outcome of pharmacotherapy. Testing for genetic variants in genes other than described in these guidelines can be considered to be of very limited clinical use. The selection of pharmacogenes discussed below is based on the pharmacogenes most frequently determined in the Netherlands.

One of the examples of a clinically relevant pharmacogene where genetic variation results in a difference in specific treatment outcomes is *CYP2D6*, a gene that metabolizes 20–25% of the drugs that have gained market approval (13). It was described in one of the first reports of hereditary influence on drug metabolism (14, 15). Currently, more than 100 variants (including single nucleotide polymorphisms, insertions and deletions) resulting in 113 possible combinations of genetic variations (haplotypes) have been described in literature. Furthermore, reports have shown that the entire gene can be deleted or duplicated [<http://www.cypalleles.ki.se/>] (16). In the last decade multiple commercial platforms have been developed to genotype *CYP2D6*.

In addition to *CYP2D6* the CPIC and DPWG guidelines have described many more clinically relevant pharmacogenes including *CYP2C9*, *CYP2C19*, *CYP3A5*, *DPYD*, *TPMT*, and *VKORC1* (for the associated drugs with these genes see Table 10.1). For these pharmacogenes research has shown that the number of common genetic variants that have a well-known clinical relevant impact on the function of the protein are much lower and range from one in case of *VKORC1* to four for *DPYD*. As a result, for the limited number of variants per gene it is relatively simple to design and validate assays based on methodologies such as Taqman, pyrosequencing or high-resolution melting. An important aspect with home-brew assays is quality assurance of the assay by means of the use of multiple techniques, controls using plasmids or reference samples from the Coriell Institute for Medical Research and participation in proficiency testing (17, 18).

CYP2D6

One commercial test to genotype *CYP2D6* was the Roche Amplichip (Hoffmann-La Roche, Basel, Swiss) which was one of the first genetic tests to receive FDA approval. The assay was based on the Affymetrix Genechip platform (Affymetrix, Santa Clara, CA)(19) and allowed for testing of 2 variant alleles of *CYP2C19* and 32 variant alleles of *CYP2D6* including deletion (*5) and duplication of the gene. At the time it was on the market it was considered the gold standard in *CYP2C19* and *CYP2D6* genotyping in clinical practice. A disadvantage of the platform, partly a result of the FDA-approval, was that sometimes

Table 10.1: Overview of the guidelines of the CPIC and the DPWG

Gene	Drugs
<i>COMT</i>	methylphenidate [†]
<i>CYP1A2</i>	clozapine [‡] , olanzapine [‡]
<i>CYP2B6</i>	efavirenz [‡]
<i>CYP2C9</i>	acenocoumarol [‡] , phenprocoumon [‡] , phenytoin, glibenclamide [‡] , gliclazide [‡] , glimepiride [‡] , tolbutamide [‡] , warfarin
<i>CYP2C19</i>	amitriptyline, citalopram, clomipramine, clopidogrel, desipramine [‡] , doxepin, escitalopram, esomeprazole [‡] , fluvoxamine [‡] , imipramine, lansoprazole [‡] , mirtazapine [‡] , moclobemide [‡] , omeprazole [‡] , pantoprazole [‡] , prasugrel [‡] , rabeprazole [‡] , sertraline, ticagrelor [‡] , trimipramine [‡] , voriconazole
<i>CYP2D6</i>	amiodaron [‡] , amitriptyline, aripiprazole [‡] , atenolol [‡] , atomoxetine [‡] , bisoprolol [‡] , carvedilol [‡] , citalopram [‡] , clomipramine, clonidine [‡] , clozapine [‡] , codeine, desipramine [‡] , disopyramide [‡] , doxepin, duloxetine [‡] , eliglustat [‡] , escitalopram [‡] , flecainide [‡] , fluphenazine [‡] , fluoxetine [‡] , flupentixol [‡] , fluvoxamine, gefitinib [‡] , haloperidol [‡] , imipramine, quinidine [‡] , methylphenidate [‡] , metoprolol [‡] , mirtazapine [‡] , nortriptyline, olanzapine [‡] , ondansetron [‡] , oxycodone [‡] , paroxetine, pimozide [‡] , propafenone [‡] , quetiapine [‡] , risperidone [‡] , sertraline [‡] , sotalol [‡] , tamoxifen, tramadol [‡] , trimipramine [‡] , tropisetron [‡] , venlafaxine [‡] , zuclopenthixol [‡]
<i>CYP3A4</i>	quetiapine [‡]
<i>CYP3A5</i>	tacrolimus
<i>CFTR</i>	ivacaftor [‡]
<i>CYP4F2</i>	warfarin [‡]
<i>DPYD</i>	capecitabine, 5-fluorouracil, tegafur
<i>G6PD</i>	rasburicase [‡]
<i>Factor V Leiden</i>	oral contraceptives containing estrogen derivatives [‡]
<i>HLA-A and HLA-B</i>	abacavir, allopurinol, carbamazepine, flucloxacillin [‡] , phenytoin [‡] , ribavirin [‡]
<i>IFNL3</i>	peginterferon alfa-2a [‡] , peginterferon alfa-2b [‡] , ribavirin [‡]
<i>MTHFR</i>	methotrexate [‡]
<i>SLCO1B1</i>	atorvastatin [‡] , simvastatin
<i>TPMT</i>	azathioprine, 6-mercaptopurine, thioguanine
<i>UGT1A1</i>	atazanavir [‡] , irinotecan [‡]
<i>VKORC1</i>	warfarin, phenprocoumon [‡] , acenocoumarol [‡]

[‡]CPIC only; [†]DPWG only.

analyses resulted in a no-call (20-22). Additionally, problems with the calling of the *CYP2D6**41 by the Amplichip have been reported in literature (18, 23) (**Chapter 3**). The miscalling of the *41 by the Amplichip can be explained by the manner that is used to call this allele by this platform. It uses a combination of the detected presence of 1661G>C, 2850C>T and 4180G>C SNPs and the absence of the -1584C>G SNP. Platforms that have been developed at a later stage using oligonucleotide markers for SNP detection call the

*41 allele solely based on the 2988G>A SNP (19) (**Chapter 3**). After the discovery of the 2988G>A SNP in 2004 (after the development of the Roche Amplichip) the definition of the *41 changed as The Human Cytochrome P450 Allele Nomenclature Committee decided the 2988G>A SNP was indicative of the *41 allele (16, 24, 25). As a result of the FDA approval, this new SNP could not be (directly) incorporated into the platform resulting in erroneous calling of the *41. Despite this known problem the assay was still recommended for genotyping *CYP2D6* until Roche discontinued the platform in 2016, partly because the assay was not used frequently in clinical practice because of its high costs. At the moment xTag 2D6 assay by Luminex (Luminex Molecular Diagnostics, Austin, TX) which uses the Luminex® 100/200™ platform is one of the *CYP2D6* assays most frequently used in the clinic. The third version of the assay currently on the market detects 16 variant alleles of the *CYP2D6* gene. In this assay two separate multiplex long-range PCR reactions are carried out followed by a process of multiplex allele-specific primer extension.

The downside to the assays described above is that the required platforms are relatively expensive. The major disadvantage of using commercial assays is the limited number of variant alleles which can be detected. Based on the total number of variants in *CYP2D6* one can deduce that using assays that can detect from 11 up to 32 variant alleles do not cover all known genetic variants in the *CYP2D6* gene and patients might be designated an incorrect genetic predicted phenotype based on a *1 auto call by the platform (16). A solution for this problem is sequencing of *CYP2D6* to read all nucleotides and compare it to a reference gene. In 2013 Dodgen et al. described a platform that combines *CYP2D6* long-range PCR with multiple rounds of Sanger sequencing per sample. The long-range PCR were used for an amplicon used in Sanger sequencing as well as products to identify deletion and duplication of the gene (23, 26). Attempts have been made by analysing the *CYP2D6* gene directly in a single sequencing reaction using second-generation sequencing, however due to existence of the pseudogenes *CYP2D7* and *CYP2D8* and the homology with other genes encoding for CYP450 enzymes this has proven difficult. Buermans et al. have circumvented this problem by using a long-range PCR in combination with the Pacific Biosciences RSII which allows for reliable full-length sequencing of *CYP2D6*. As result of the full-length read complete phasing of the sequencing data is possible resulting in calling of complete haplotype across the entire *CYP2D6* gene which includes up- and downstream regions, introns and exons (27). Although sequencing is considered an improvement upon genotyping assays, as all the genetic variants in the gene are uncovered, in a clinical setting this could present a problem. With sequencing new genetic variants within the *CYP2D6* gene will be found of unknown functionality and unknown influence on the function of the metabolizing enzyme. Attempts have been made to quantify the effects of new genetic variants on the metabolizing capacity of enzymes using in silico prediction (28). However, with different settings of the analyses concordance for

predicted effects of newly found variants have been low (29). In the case of implementing next generation sequencing in a clinical setting the discovery of novel uncharacterized variants in the *CYP2D6* gene could present a challenge. A solution for this problem would be to limit the results of the sequencing analysis to known mutations that are fully characterized.

In conclusion, despite the complexity of *CYP2D6* multiple commercial assays are available for a broad coverage of genetic variants in this gene. Furthermore, research will likely lead to novel techniques which in the future can be used in the clinic for a more detailed analysis of this pharmacogene.

CYP2C9 + VKORC1

The *CYP2C9* gene will mostly be tested in combination with *VKORC1* to guide dosing of coumarins or explain adverse reactions to vitamin K antagonists. Testing for *CYP2C9**2 and *3 along with the -1639G>A mutation in the promotor of *VKORC1* appears to be sufficient in patients of non-African ancestry to adjust the maintenance dose of coumarins or to determine the starting dose in case the INR is measured routinely at follow up. In case of patients of African descent, the additional variants of *5, *6, *8, and *11 appear relevant as a reduction of 15–30% of the maintenance dose is required if patients are carriers of these alleles (4, 30).

CYP2C19

Currently, genetic testing for *CYP2C19* is usually performed to predict response to clopidogrel therapy and multiple studies are ongoing to investigate whether genotyping of *CYP2C19* can lead to an improvement in antiplatelet therapy with clopidogrel (31). The most frequently tested variants in the *CYP2C19* gene are *2, *3 (which were also found on the Amplichip platform) and *17. Commercially available assays for *CYP2C19* such as the INFINITI platform by AutoGenomics (AutoGenomics, Inc., Vista, CA), the xTag 2C19 Mutation Detection Kit by Luminex (Luminex Molecular Diagnostics, Austin, TX) and the Genmark (GenMark Diagnostics, Carlsbad, CA) have a broad coverage of genetic variants, whereas inhouse assays developed by clinical laboratories usually include *2, *3 and *17.

CYP3A5

The most frequent variants found among the (Caucasian) population are the *3 and *6 alleles, encoding for a non-functional protein whereas the functional variant *1 is relatively uncommon in this population with a MAF of 0.078. In African Americans the *1 is more common than the *3 and *6 alleles (4, 32). A systematic review of the literature has shown

that patients that are hetero- or homozygous carriers of the *1 variant of the *CYP3A5* gene require a significantly higher dose (175–250%) than patient carrying two non-functional copies (4, 32). However, clinical studies investigating the use of the *CYP3A5* genotype to personalize tacrolimus dosing have shown mixed results whether genotyping can improve pharmacotherapy with tacrolimus compared to standard care (often comprising of therapeutic drug monitoring) (33, 34). Currently, AutoGenomics (AutoGenomics, Inc., Vista, CA) and Genmark provide commercial platforms for *CYP3A5* in combination with the *CYP3A4* gene with both platforms providing a coverage of 8 alleles for *CYP3A5*, whereas the assays developed in laboratories usually include the *3 and *6 alleles.

DPYD

Although genetic testing for *DPYD* is widespread in routine care in some countries for multiple years (e.g. The Netherlands), it has only recently been incorporated into the Clinical Practice Guidelines of the European Society for Medical Oncology or the American Society for Clinical Oncology. Screening for relevant genetic variants in *DPYD* before starting capecitabine or 5-fluorouracil is increasingly becoming part of the standard-of-care when treating patients with fluoropyrimidine derivatives (35, 36). In recent years, literature has been accumulated on genetic variants in *DPYD* and their effects on the functionality of the DPD enzyme. For instance, the evidence for a 50% dose reduction among heterozygous carriers of the IVS14+1G>A (*2A) has been established in many studies (**Chapter 6**). Efforts by Meulendijks et al., Offer et al. and initiatives as the DPWG and CPIC have resulted in a consensus on the variants on the *2A, 1679T>G (*13) and the 2846A>T variants having no (*2A and *13) or reduced activity (2846A>T) respectively (4, 37–39). Until recently there was a discordance between the CPIC and the DPWG concerning the classification of the 1236G>A/HapB3 variant. In the guidelines of the DPWG is mentioned that this variant leads to decreased fluoropyrimidine clearance and to increased toxicity, while in their 2013 guideline the CPIC had not yet assigned a status to this variant (4, 39) (**Chapter 7**). However, this difference has currently been resolved by an update of the *DPYD* guideline published in February 2018 in which the gene-activity score for *DPYD* has also been adopted (40). In the Netherlands assays developed by clinical laboratories usually included the four SNPs described by Meulendijks et al. (35, 37).

TPMT

The first guidelines published by the CPIC covered the interpretation of genetic variations found in the *TPMT* gene to genetic predicted phenotypes and therapeutic recommendations for dosing of azathioprine, 6-mercaptopurine and thioguanine in case of actionable *TPMT*

genotypes (41). In an update of the guideline published in 2013 a total of 33 variants in the *TPMT* gene are described, however the alleles *2, *3A, *3B and *3C appear to be the most relevant as this set of alleles explains 95% of the variance found in *TPMT* in terms of frequency (42). Most clinical laboratories that have developed their own assay will commonly use the three SNPs found in *2, *3B and *3C. As the *TPMT**3A haplotype is comprised of both rs1800460 (*3B) and rs1142345 (*3C) this combination of SNPs can thus also be used to detect the *3A haplotype (42).

Panel based approach

With the advancement of the field of biotechnology with a concurrent decrease of costs of techniques the screening of multiple (pharmaco)genes simultaneously (panel-based) approach has become available for use in a clinical setting. As multiple large implementation studies in secondary care have shown that patients are often (> 90–95%) carriers of at least one actionable phenotype, a panel based screening of multiple pharmacogenes appears to be the next step in PGx testing. The genes *CYP2C19*, *CYP2C6*, *SLCO1B1* and *TPMT* were included in panels by all large studies, whereas *CYP2C9/VKORC1* and *CYP3A5* are included two of the three implementation projects (6, 7, 43, 44). Van Driest et al. have shown in the Vanderbilt Pharmacogenomic Resource for Enhanced Decisions in Care and Treatment (PREDICT) program that using a panel reduced the number of tests by 60% compared to a reactive single gene assay (7). Currently, multiple platforms can be used for a pre-emptive panel approach. One of the first high-throughput genotyping methods available for analysis of multiple pharmacogenes was the Drug Metabolizing Enzymes and Transporters (DMET) Plus array by Affymetrix using Genechip platform. (Affymetrix, Santa Clara, CA). Fernandez et al. showed that high concordance with other genotyping methods (45). The disadvantages of the DMET plus array include the length of a single run (~48 hours spread out over three days), the inability to detect certain copy-number variants (e.g. heterozygous carriers of the *CYP2D6**5 and duplications of the *CYP2D6*) and recently discovered but clinically relevant alleles such as the 2846A>T and 1236G>A/HapB3 in the *DPYD* gene are missing. In case the DMET plus platform is used to guide pharmacotherapy in a clinical setting this platform has to be supplemented with additional test depending on the genes incorporated in the desired panel (44) (**Chapter 4**).

In the PREDICT program the VeraCode ADME core panel (Illumina, San Diego, CA, USA) was used (7). This platform detects 184 genetic markers in 34 ADME (46). Similar to the DMET platform the VeraCode platform is not able to detect duplications of this gene (although it can detect the *5 allele). Furthermore, important SNPs in the *DPYD* gene (2846A>T and 1236G>A/HapB3 and *13) are absent in the panel of 184 genetic variants

and would have to be supplemented if the panel would be used for the characterization of *CYP2D6* and *DPYD* (**Chapter 4**).

In addition to fixed selections of genetic variants found on the DMET plus and the VeraCode ADME core panel laboratories may choose to select the pharmacogenes and an associated panel of genetic variants based on their own criteria. Although Thermo Fisher (Thermo Fisher, Waltham, Massachusetts, USA) did offer a prefabricated array that tests for 158 variants in 29 Phase I/II and transporter genes, a custom platform based on Taqman assays can also be designed. A downside to the array similar to the DMET platform is that the Taqman cannot detect copy number variants and an additional qPCR has to be performed if genes with known duplication and/or deletions as *CYP2D6* and *UGT2B7* are included in the desired panel.

Genome wide approaches

In the last decade whole exome screening (WES) and whole genome screening (WGS) have become available for use in the clinical setting as a result of the advances in the field of biotechnology. Although WES and WGS are still relatively costly and therefore mainly used by clinical geneticists as an end-of-line method to diagnose hereditary diseases, information on genetic variants found in pharmacogenes can be considered a useful by-product of the use of these methods. Along with Genome-Wide Association (GWA) methods, WES and WGS are currently mostly used in a research setting to detect novel genetic variants in pharmacogenes.

Providing data on diagnostic criteria of PGx testing

Currently, outcomes of (genome-wide association) studies related to PGx are often reported as odds/hazard ratios or associations, where an individual carrying a certain genetic variant in a gene has a higher risk on a negative outcome of therapy or a lack of response. However, odds/hazards ratios and associations do not provide information about the analytical validity, clinical validity and clinical utility of genetic tests or panels which are important objective factors in determining whether the test can lead to a clinically relevant improvement in healthcare (47). The analytical validity is the ability to identify the genetic variants of interest and is expressed as the analytical sensitivity and specificity of the test. The analytical sensitivity and specificity are generally required to be higher than 98% and 99.5%, respectively (48, 49). The clinical validity concerns the correlation of the genetic marker with a clinical phenotype or outcome. Parameters for clinical validity are clinical sensitivity, clinical specificity and the amount of change in a person's risk for a certain

event between the wildtype genotype and a variant genotype. The final parameter for the reliability of a genetic marker is the clinical utility. Clinical utility relates to whether the genetic information would lead to change in treatment that improves drug efficacy or safety (i.e. the impact on healthcare) (50). Currently, relevant data concerning clinical validity and clinically utility is scarcely reported despite the need for this information to answer the question whether implementation of a specific genetic test or a panel in clinical practice can lead to a significant benefit of healthcare (47). A manner to obtain information regarding the clinical validity (such as negative and positive predicting values) is to reassess existing literature and extract this relevant data. With this information clinicians and other decision makers in healthcare can more objectively guide the decision-making process regarding the question whether genetic tests are useful in clinical practice (47, 51). Jansen et al. have shown for example that for the gene-drug pair *SLCO1B1* and statins, a systematic review of the literature could provide the desired information on diagnostic criteria.

Guidelines directing the clinical use of PGx test results

In 2010 the most important barrier for the implementation of PGx into clinical practice identified by clinicians was the translation of PGx-test results into clinical action (1, 2, 52). Two consortia, the Clinical Pharmacogenetics Implementation Consortium (CPIC®) and the Dutch Pharmacogenetics Working Group (DPWG), both anticipated a near future where patients would confront their treating physician and pharmacists with their own genotype and started with the translation of information found in the scientific literature into tools usable by clinicians. The guidelines of both groups provide recommendations for treatment of patients where an actionable genetic predicted phenotype is known at start of pharmacotherapy, rather than indicating which gene should be tested (see Table 10.1) (2, 3). Up to date, CPIC has published guidelines which cover 40 gene-drug pairs and the DPWG has reviewed 86 candidate gene-drug pairs in their guidelines and provide therapeutic recommendations for 47 gene-drug pairs (**Chapter 7**).

When guidelines of the CPIC and the DPWG on the same gene-drug pairs are compared there is substantial agreement between the guidelines in terms of therapeutic recommendations for certain phenotypes. The most common explanation for observed discordances in therapeutic recommendations between the CPIC and the DPWG is the use of different methodologies to support dosing advices. Whereas the CPIC used a consensus based approach from a panel from experts on the subject, the DPWG calculates dosing advices with aid of pharmacokinetic data. Other explanations for differences can be found based on the date of literature review (**Chapter 7**).

Another important difference is the discordance in the translation of genotypes to genetic predicted phenotypes. Similar to the therapeutic recommendations these discordances in translation can be explained by a difference of interpretation of *CYP2D6* genetic predicted phenotypes in the literature combined with the use of different model substrates by the CPIC and the DPWG. This in turn led to a difference in the interpretation of the *CYP2D6* intermediate metabolizer phenotype. Currently an international working group of experts on the subject including members from the CPIC and the DPWG has recently begun to standardize the translation from genotype to phenotype (**Chapter 7**).

To aid in the personalization of pharmacotherapy the guidelines should not only be available in the form of published articles or chapters in books, but be an integral part of the workflow of healthcare professionals. In the Netherlands the first therapeutic dose recommendations of the DWPG were integrated in the national drug database (G-standard) in 2006, making medication surveillance based on actionable genetic predicted phenotypes available on a national level for healthcare professionals in both primary and secondary care through clinical decision support in drug prescribing and dispensing systems (3). In other nations where there is no national drug database available, multiple reports describe the integration of PGx into clinical decision support at an institutional level in both primary and secondary care (53-58). Alternatively, in situations where electronic medication surveillance is not (yet) possible a mobile enable clinical decision support could aid healthcare professionals in adjusting pharmacotherapy based on a patients PGx profile (59, 60). Evidence suggest that medication surveillance based on a patients PGx profile indeed leads to a change in behavior among physicians reducing the number of high PGx risk (61).

Providing scientific evidence for improvement in patient care by PGx testing

The body of evidence that is required to justify the implementation of routine PGx testing in clinical practice has been subject of debate (62-64). The evidence-based medicine paradigm would require evidence to prove that a new technique or treatment would indeed lead to increased safety or efficacy or a same performance for lower costs before it can be implemented into clinical practice with a randomized controlled trial (RCT) considered the gold standard. The evidence-based model would require RCTs for genotype guided therapy for each individual gene-drug pair or a panel approach. However, there are multiple arguments against the demand for RCTs to deliver evidence for genotype-guided therapy for each gene-drug pair (**Chapter 2**).

From one point of view there is no pharmacological argument to see adjustments for a gene-drug pair any different than adjustments for many other relevant clinical factors such as impaired renal function. Dosing adjustments for clinical factors are usually based on data from observational studies and case-reports/series, however regulatory authorities do not enforce a requirement of RCTs to deliver evidence that adjustments based on clinical parameters lead to an improvement of clinical outcomes (64). Additionally, if there is sufficient observational evidence available that pharmacogenetic screening can reduce significant morbidity or prevent mortality RCT's can be considered unethical as patients with non-wildtype genotypes in the standard arm are knowingly exposed to suboptimal treatment which can lead to adverse events (65). Another argument against a RCT for each gene-drug pair is the cost aspect. As RCTs are highly expensive this would require an enormous investment to finance the required trials for all relevant gene-drug pairs. As stated earlier, in case of novel drugs RCTs are financed as part of the research and development by the innovative pharmaceutical companies that hold the patent for the marketed drug. However, many of the drugs with a known interaction with a polymorphic pharmacogene are drugs with a well-known pharmacology which are already produced by generic pharmaceutical companies which do not have any gain by financing additional research concerning their pharmaceutical in their portfolio (**Chapter 2**).

However, despite this rationale clinicians seemed hesitant to implement this new concept of pharmacology in clinical practice as evidence for an improvement of pharmacotherapy by PGx in the previous decade was lacking (66). Fortunately, in the last years several clinical studies for single gene-drug pairs have already been performed providing evidence for implementation of genotype-guided therapy in case of these gene-drug pairs. A landmark RCT performed by Mallal et al. in 2008 showed that genotyping for the *5701 allele in the *HLA-B* gene could lower the incidence of abacavir-induced hypersensitivity reactions from 7.8% in the control group to 3.4% in the control group ($p < 0.001$) (67). Additionally, Coenen et al. studied in a multicenter study whether genotyping for the *TPMT* would lead to a reduction of hematologic adverse drug reactions (ADRs) in treatment of inflammatory bowel disease using thiopurines. Although the study could not detect a reduction in the proportion in hematologic ADRs between the screening arm and the control arm (7.4% vs. 7.9%, 95% confidence interval: 0.57–1.52) a tenfold reduction in the hematological ADRs among carriers of *TPMT* variants was observed in the intervention group compared to the same population in the control group (2.6% vs. 22.9%, 95% confidence interval 0.01–0.85) (68). A third example of a multicenter study to investigate the clinical utility of a PGx test was performed by Deenen et al. Unlike the two previous studies this group did not use a RCT design, but compared a prospectively genotype-guided cohort to a historical cohort to show that screening for the *2A variant in the *DPYD* gene can reduce the risk of toxicity

of grade 3 or higher from 73% to 28% ($p < 0.001$). Furthermore, the proportion of patients that died because of the fluoropyrimidine was reduced from 10% to 0% (69). In case of the gene-drug pairs *HLA-B*57:01* and abacavir, *DPYD* and fluoropyrimidines and *TPMT* and thiopurines the results of the performed studies helped to provide evidence to justify implementation of routine screening in clinical practice.

In addition to studies investigating whether routine genetic screening of a single pharmacogene can lead to an improvement of healthcare, a number of studies tests for a panel of multiple pharmacogenes simultaneously (a panel approach). Using a case-control study consisting of 205 genotyped patients receiving genotype-guided dosing through clinical decision support and 820 matched controls Brixner et al. showed that a preemptive screening using a panel approach resulted in a significant reduction in hospitalizations (9.8% vs. 16.1%, $p = 0.027$) and patient visits to the emergency department (4.4% vs. 15.4%, $p = 0.0002$) (70). Similar to the results of Brixner et al. a prospective pilot RCT performed by Elliot et al. found a reduction of in the number of rehospitalizations at 60 days in the intervention group compared to the group receiving regular care. In the intervention arm of the study patients were pre-emptively genotyped for *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4* and *CYP3A5* followed by optimization of pharmacotherapy using clinical decision support whereas the control population received regular care (53). In addition, the Ubiquitous PGx consortium is currently enrolling patients in the PREemptive PGx testing for prevention of Adverse drug Reactions (PREPARE) study in sites across seven European countries as part of a Horizon2020 funded program with the aim to provide evidence to support routine implementation of pre-emptive panel-based PGx screening (71). Overall, the body of evidence supporting the implementation of PGx in clinical practice is growing, mostly by the efforts of academic hospitals or specialized research institutions.

Providing information on cost-effectiveness and cost-consequences of PGx testing

In addition to information about the analytical validity, clinical validity and clinical utility an important aspect for the implementation in clinical care is data about cost-effectiveness and cost-consequences of PGx tests. The information on the cost-effectiveness is especially relevant in the case of implementation of PGx testing in primary as health insurers will require this information in their decision to reimburse specific (pharmaco)genomic testing. In secondary and tertiary care cost-effectiveness is slightly less relevant as in a hospital setting a laboratory test is usually part of integral treatment plans and are not reimbursed directly by health insurers.

Ironically, most of the cost-effectiveness analysis (CEAs) relating to PGx tests have been performed for gene-drug interactions concerning drugs that are mostly initiated in a hospital setting. For fluoropyrimidines the studies performed by Deenen et al. and Cortejoso et al. show that genotyping for variants in *DPYD* can reduce the number of patients admitted to the intensive care unit with severe fluoropyrimidine induced toxicity (69, 72). Other gene-drug pairs where the body of evidence concerning the cost-effectiveness is growing are *HLA-B*5701* and abacavir and *CYP2C19* and clopidogrel by reducing the numbers of patients suffering from hypersensitivity reaction or stent-thrombosis respectively (73-76).

Up to date the evidence supporting cost-effectiveness of routine screening for genetic variants in pharmacogenes associated with prescription drugs which are frequently prescribed in primary care such as antidepressants (*CYP2D6* and *CYP2C19*), statins (*SLCO1B1*), and coumarins (*CYP2C9* and *VKORC1*) has only been shown in a number of studies despite the amount of literature published on these gene-drug pairs (77, 78). As a result, health insurance companies are not likely to reimburse routine PGx testing for these single gene-drug pairs in primary care. However, evidence for cost-effectiveness for each individual gene-drug may not be necessary when using a panel approach. Brixner et al. have shown in their study that simultaneous screening for *CYP2C19*, *CYP2C9*, *CYP2D6*, *CYP3A4* and *CYP3A5* can lead to savings in healthcare as a result of a reduction in hospitalizations and patient visits to the emergency department (70). Similarly, the U-PGx project will also perform an analysis to determine whether pre-emptive screening of 50 variants in 13 pharmacogenes will lead to a decrease in costs in the healthcare system (71). Finally, with the rapid developments in biotechnology and the concurrent rapid decrease in costs of genotyping techniques such as genome-wide screening as WES or WGS might become cost-efficient in the next decade.

Improving acceptance of PGx testing

In addition to the scientific evidence, the integration of any laboratory test or clinical procedure is dependent on the adoption by healthcare professionals and patients. With the rapid progression of the field of PGx in the last decades healthcare professionals are advent of an era where there is a significant chance they will be confronted with (pharmaco)genomic information from patients and are expected to optimize the patients' treatment based on this information. A survey performed among US physicians has shown that although doctors believe in the concept of heritable drug response only 10.3% feels adequately informed about the availability of PGx testing and the place of PGx testing within pharmacotherapy (66). Similarly, a questionnaire among Canadian pharmacists revealed a high expectation

of PGx to improve safety and effectiveness of drug therapy only 7.7% of the responders felt comfortable interpreting and advising upon PGx test results. Furthermore, virtually all (96.6%) of the surveyed pharmacist would like additional education on the subject (79). Both studies indicate a knowledge gap relating to PGx is present among healthcare professionals.

With the integration of therapeutic recommendations for patients with known actionable genotypes into clinical decision support in electronic drug prescribing and dispensing systems it was anticipated that a knowledge gap relating to PGx among healthcare professionals would be circumvented. In contrast, a questionnaire performed among Dutch pharmacists showed that this was not the case. Knowledge of the guidelines of the DPWG and their integration in the clinical decision support was not associated with feeling informed about PGx testing or PGx test adoption (**Chapter 8**). Additionally, the percentages of responders that felt informed about PGx testing (14.7%), that would like to follow additional training on the subject (88.8%) and that had adopted a PGx test (14.1%) were similar to previous studies (66, 79) (**Chapter 8**). Further research performed among pharmacy students revealed a similar percentage of 12.8% of participants that felt adequately informed despite almost all of the students (96.6%) having followed some form of education on the subject (**Chapter 9**). The results from the latter study indicate that the current traditional form education on PGx as part of the curriculum is not sufficient and may result in a future knowledge gap.

The results from these studies indicate that a lack of knowledge on PGx testing is one of the remaining barriers hindering implementation. To remove this barrier more education on the subject for (future) healthcare professionals is required and should be presented in an alternate manner. In one initiative reported by Weitzel et al. students obtain their own pharmacogenetic profile and use this information in an education setting. This form of education using hands-on experience based learning appears to increase understanding of PGx markers and allows students to get used to the idea to act on (pharmaco) genomic markers (80). Training on the subject for practicing healthcare professionals could be provided in the form of post-academic education where in addition to basic information about (pharmaco)genomics, the existence of guidelines containing therapeutic recommendations and evidence for implementation of genetic testing is discussed. One survey found a link between this form of education and the future use of PGx testing (**Chapter 8**).

A current challenge is that certain practicing healthcare professionals do not often receive the opportunity to gain experience in adjusting medication based on PGx markers. As the body of evidence for genetic screening for gene-drug pairs occurring in primary care is

not as large compared to fields in secondary care (e.g. oncology) general practitioners and community pharmacists do not routinely use PGx markers to adjust therapy. As a result, they probably do not feel qualified to adjust pharmacotherapy based on PGx markers (**Chapter 8**). In the Implementation of Pharmacogenetics in to Primary care Project (IP3) community pharmacists were offered free ordering of a PGx panel consisting of 8 genes (*CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* and *VKORC1*) and gain experience with genotype-guided dosing of ten drugs frequently prescribed in primary care (**Chapter 4**). In line with previous implementation projects the IP3 showed that the majority of patients (90%) carried at least one actionable genotype in the panel and combinations of actionable genotypes with relevant drugs were common (31.0%). And although not significant ($p = 0.112$) there was an increase in the percentage of surveyed community pharmacists and general practitioners that felt qualified to interpret and act on PGx data (15.2% before vs. 28.3% after) (**Chapter 4**).

The combination of the frequencies of actionable genotypes from this panel of 8 genes along with prescription data from 95% of the community pharmacies in the Netherlands projects that 23.6% of all patients receiving incident prescriptions from a selection of 45 drugs associated with the genes in the panel experience a gene-drug. In a possible future scenario where patients are routinely genotyped for this panel of clinically relevant pharmacogenes community pharmacists are likely required to perform an intervention in 5.4% of therapy and strictly monitor an additional 18.2%. To execute this role as key user of PGx data pharmacists will need to be comfortable with adjusting therapy based on genetic markers (**Chapter 5**).

Conclusion

In the last decades the field of PGx has provided a wealth of information from research on SNPs in candidate genes up to studies using hypothesis free testing in the form of GWA studies and next generation sequencing powered by the advances in molecular technology. In the last years assays have been developed that are usable in the clinical settings and by efforts of the CPIC and the DPWG the vast amount of scientific literature concerning many gene-drug pairs has been reviewed and condensed into actionable therapeutic recommendations usable in the clinic. Multiple studies have shown that implementation of PGx-testing (using a panel approach) in clinical practice is feasible and several RCTs have delivered evidence for the routine implementation of PGx-testing for a number of gene-drug pairs such as *DPYD* and *TPMT*. Currently, studies providing the clinical utility and cost-effectiveness of panel-based screening are ongoing which might deliver the final evidence required for widespread implementation of PGx in clinical care. One barrier which

still appears to be remaining are healthcare providers themselves which indicate that they hindered by a knowledge gap on the subject. A change in the current curriculum might prevent lack of knowledge among future healthcare professionals whereas post-academic education will resolve the knowledge gap among current practicing colleagues.

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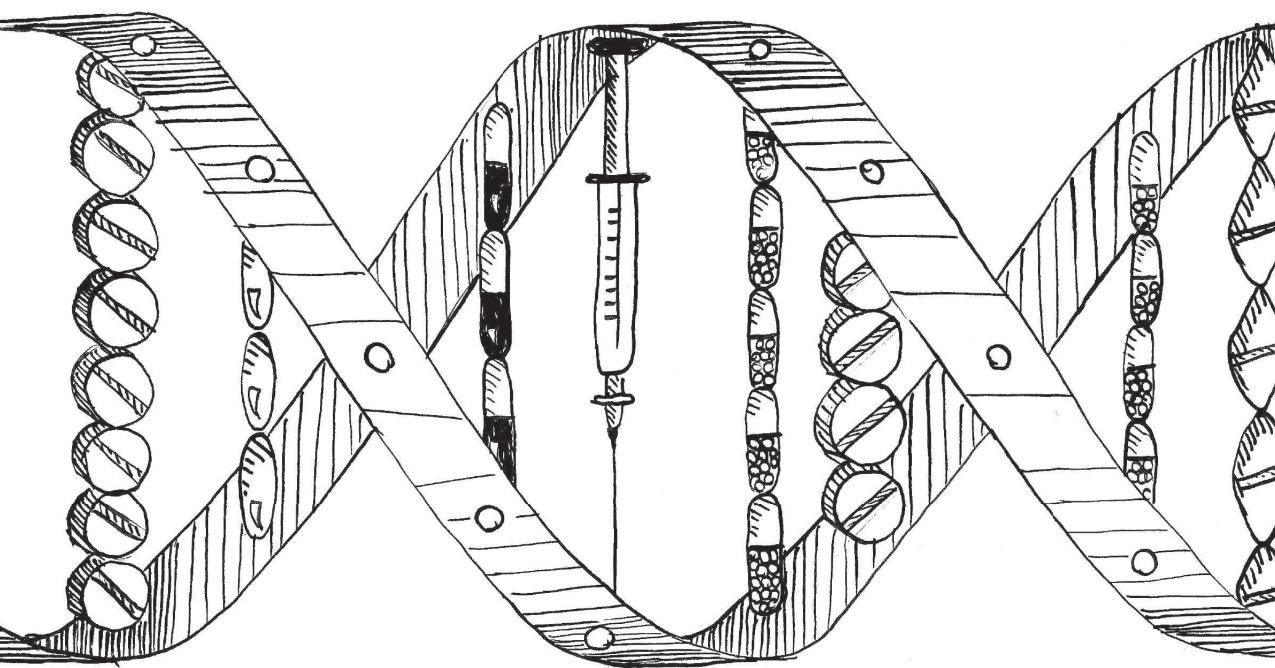
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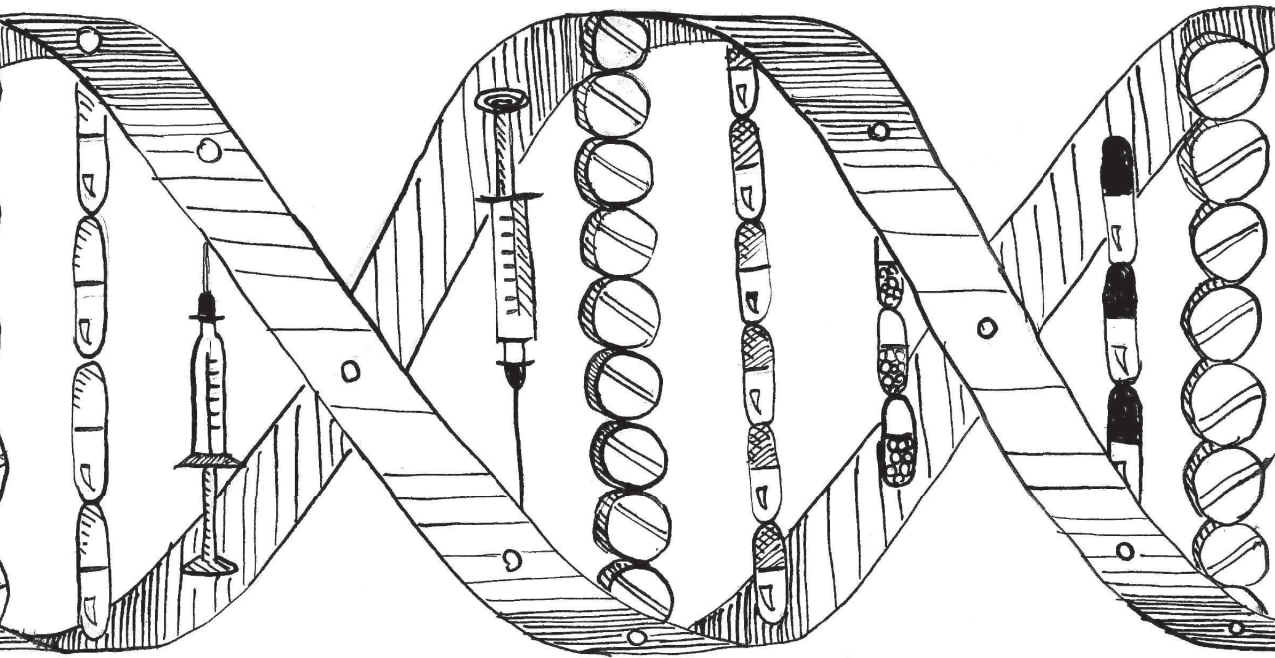
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Chapter 11

English summary



Introduction

This thesis covers scientific research with the overall aim to improve clinical acceptance of pharmacogenetics in clinical care. **Chapter 1** describes how in the last decades clinical studies have generated evidence that a one-size-fits-all approach to drug therapy is often not applicable and large inter-patient variability exists in both risk of side effects and efficacy of pharmaceuticals. By aid of the advancements made in biotechnology, proof was delivered that absorption, distribution, metabolism and excretion of pharmaceuticals (and endogenous substrates) is in part under control by the genome. Recent clinical studies have indeed shown that the use of genetic information can improve drug efficacy and toxicity. Additionally, the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) have provided clinicians with evidence-based therapeutic recommendations and in The Netherlands guidelines of the DPWG are incorporated in the electronic drug-prescribing and -dispensing systems. Currently, pharmacogenetics (PGx) is routinely used to adjust pharmacotherapy with high risk medication in some secondary and tertiary centers. However, in primary care PGx is still scarcely used, while a large majority of the drugs that are reviewed by CPIC and the DPWG are (primarily) prescribed and dispensed in primary healthcare centers. Moreover, small pilot studies and large PGx implementation projects have shown that a large majority of individuals carry at least one actionable phenotype. This portrays the need to implement a pre-emptive panel based pharmacogenetic screening in primary healthcare centers.

The first part of this thesis provides answers to frequently asked questions by clinicians regarding PGx. The second part describes the clinical implementation of PGx in clinical practice and its impact on primary care on a national level. Harmonization of PGx guidelines and therapeutic recommendations are described in the third part of the thesis. In the fourth part knowledge, experience and attitudes towards PGx of pharmacists and pharmacy students are described. The final part contains the general discussion.

Part I: Answers to frequently asked question by clinicians regarding PGx

In **Chapter 2**, six frequently asked questions by clinicians are answered which can be applied to the implementation of PGx in clinical practice.

Q1: Do we need pharmacogenetic markers to improve pharmacotherapy? Yes!

As randomized clinical trials that are required for registration purposes generally include highly selected individuals, the efficacy and safety of prescription drugs is often lower than found in the clinical studies. Safety of pharmacotherapy can be increased by slowly titrating patients to a tolerable dose with sufficient efficacy. Using predictive pharmacogenetic biomarkers at the start of therapy, healthcare professionals can potentially use drugs in a more efficacious manner without requiring the so called ‘trial-and-error prescribing’.

Q2: What are the sources of genetic variation that influence drug response?

Pharmacokinetics: Genetic variations come in form of single nucleotide polymorphisms, insertions and deletions that occur in the encoding genes, which can have consequences for the activity of the protein. Increased enzyme activity, by duplication of the encoding gene, can also occur.

Pharmacodynamics: When genetic variation occur in the genes that encode for a receptor or enzymes involved in the signal-transduction this can lead to altered downstream signaling and associated processes and ultimately can lead to altered pharmacodynamics of a drug.

Idiosyncratic drug reactions: In some cases, genetic variation can lead to altered drug response that does not appear to be related to exposure to the drug. These mutations are often found in the genetic regions that code for the Human Leukocyte Antigen (HLA) and seem to modulate immune-responses to drugs.

Q3: To what extent is variability in drug response explained by pharmacogenetics? Variable!

Using the R^2 parameter, the contribution of pharmacogenetic biomarkers to an outcome can be compared to conventional clinical factors used to adjust therapy. Dependent on the studied phenotype PGx biomarkers should be combined with clinical factors, rather than used standalone.

Q4: Are pharmacogenetic test results actionable? Yes!

However, the majority of drug labels do not yet contain information on how to adjust therapy based on genetic biomarkers, although this is increasing. If genetic biomarkers are to be used by clinicians as part of routine care the results from clinical studies have to be translated to therapeutic recommendations, scoring systems or decision trees.

Q5: What level of evidence is required for implementation? Still debated!

From the evidence-based paradigm the best supporting evidence for implementation of pharmacogenetics in clinical practice would be obtained from randomized clinical trials (RCT's). However, when gene-drug interactions are considered in the same way as drug-drug interactions or dose-adjustment for clinical factors as impaired renal or liver function, observational evidence should be sufficient to support implementation. In case proof of principle is established with a number of RCT's investigating PGx markers providing positive results, additional evidence in the form of well replicated observational studies should be sufficient to justify implementation of PGx markers into clinical care.

Q6: Where can I find sources for up-to-date information regarding interpretation of PGx test results? PharmGKB!

The DPWG and CPIC both have provided clinicians with dosing recommendations based on systematic reviews of the literature since 2008 and 2011 respectively. With this information these two consortia have provided tools which facilitate physicians and pharmacists with the interpretation of PGx tests and adjustment of pharmacotherapy based on genotypes. The guidelines of both consortia can be found on pharmgkb.org.

Part II: Implementation of PGx in clinical practice and its impact on a national level

To ensure clinical acceptance of PGx-testing healthcare professionals should be able to order clinical grade tests for clinically relevant genes. One pharmacogene particularly relevant in pharmacotherapy is *CYP2D6*. At the start of this thesis the gold standard in *CYP2D6* testing in clinical practice was the Roche AmpliChip. In **Chapter 3** the concordance between the novel GenoChip *CYP2D6* macroarray and the AmpliChip was investigated. The study was performed by genotyping 200 samples with germline DNA samples from the CYPTAM study, a prospective multicenter study performed in the Netherlands and Belgium which includes 671 female patients with breast cancer which were screened with the Roche AmpliChip. Of the tested samples 99% (n = 198) of the results of the GenoChip were concordance with the results of the AmpliChip. In two samples the genotyping results from the GenoChip *CYP2D6* macroarray were discordant from the results from the AmpliChip as the AmpliChip identified a *41 allele, while the GenoChip did not. Sanger sequencing of the two discordant samples revealed that the 2988G>A mutation, considered by The Human Cytochrome P450 Allele Nomenclature Committee as the key mutation for *41, was not present. Based on the results of this comparison it was concluded that

the CYP2D6 GenoChip Macroarray is a valid method for detecting 13 genetic variants in the *CYP2D6* gene.

In addition to the development of clinical grade assays adoption of PGx test by healthcare professionals is an important factor in its implementation in clinical practice. At the start of this thesis PGx was increasingly used in hospitals, while the adoption of PGx tests by general practitioners and community pharmacists remained low. To further improve adoption of PGx-testing in primary care the **Implementation of Pharmacogenetics in Primary care Project (IP3)** was initiated. **Chapter 4** describes the IP3-study in which general practitioners and community pharmacists could implement PGx testing in their own practice. Patients with an incident prescription for a drug with known gene-drug interactions (amitriptyline, atomoxetine, atorvastatin, (es)citalopram, clomipramine, doxepin, nortriptyline, simvastatin or venlafaxine) were eligible for inclusion. The patients were screened for 40 genetic variants in the genes *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* and *VKORC1* using the DMET platform in combination with a qPCR Taqman to detect copy-number variation in the *CYP2D6* gene. The genotypes were translated to predicted phenotypes and combined with a therapeutic recommendation from the DPWG guidelines in a written report. This report was then transferred to the treating general practitioner and pharmacist with the request to archive the results in their electronic drug prescribing and -dispensing systems in order to perform medication surveillance using clinical decision support (CDS). A total of 200 patients were included by clinicians in the period from November 2014 to July 2016, accounting to an adoption of PGx-testing in 18.0% of the eligible patients. The majority of the patients was included on an incident prescription of a statin, being atorvastatin (57.5%) or simvastatin (14.5%). The remainder of the patients (28.0%) was included on an antidepressant (amitriptyline, (es)citalopram, nortriptyline, or venlafaxine). In 90% of the patients at least one actionable genotype in the selected panel was found. More importantly, in 31.0% of the incident prescriptions a combination of one of the selected drugs of inclusion with the associated actionable genotype was present and a therapeutic recommendation was provided based on the DPWG guidelines to the clinicians to change the regimen and/or monitor the patient more closely for ineffective or unsafe therapy. The study showed that genotype guided dosing in primary care is feasible and allowed general practitioners and community pharmacists to gain experience with PGx in the context of their own practice. Additionally, it demonstrated that actionable genotypes in the selected panel are common among the population. In **Chapter 5** an estimate was made of the national clinical impact of implementing a preemptive panel based PGx consisting of *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* and *VKORC1* in primary care throughout The Netherlands. The frequencies of the actionable genotypes for the selection of 8 genes

from the IP3-study were used a representative sample for the Dutch population and were combined with national prescription data in primary care obtained from the Foundation of Pharmaceutical Statistics (SFK). Our estimate shows that for 45 drugs associated with the 8 genes a gene-drug interaction as described in the guidelines of the DPWG is present in 23.6% of all incident prescriptions ($n = 856,002$ prescriptions/year). Additionally, in one in every 19 ($= 5.4\%$) incident prescriptions this would result in an adjustment of the dose or switch to an alternate drug at the start of therapy. Based on this estimation we predict that in a near future where clinicians will be confronted with patients with a known PGx profile, general practitioners and community pharmacists will routinely have to adjust pharmacotherapy based on actionable genotypes.

Part III: Harmonization of PGx-test interpretation and therapeutic recommendations

An important goal is to achieve a worldwide uniform interpretation of PGx-test results and clinical actions based on actionable pharmacogenes. However, due to differences in size of studies, study population, chosen methodology and chance investigators might find differences in effect size of studied genotype-phenotype relationships. **Chapter 6** provides a response to Magnani et al. who proposed a further reduction of the starting dose of fluoropyrimidine regimens in patients who are heterozygous carriers of the *DPYD* IVS14+1G>A variant. This proposal was based on a single prospective study with 180 patients that were candidate for therapy with a fluoropyrimidine-based regimen. Patients were screened for the *DPYD* IVS14+1G>A splice site variant as part of the study and were routinely evaluated for fluoropyrimidine-related toxicities. In our response, based on literature reviews performed by the CPIC and the DPWG and pharmacokinetic study performed by Deenen et al, we argue that a 50% dose reduction followed by dose titration based upon toxicity is the best approach to fluoropyrimidine dosing in patients who are heterozygous carriers of the IVS14+1G>A variant.

Despite the global availability of scientific literature different research groups interpret the available evidence slightly differently resulting in differences in interpretation of PGx test results and therapeutic recommendations. **Chapter 7** describes the initiative to harmonize the guidelines released by the CPIC and DPWG by an inventory of existing differences. This was executed by a review of the methodologies incorporated by these two consortia, a systematic comparison of the genotype to phenotype translations and therapeutic recommendations for gene-drug interactions shared between the guidelines of both groups. The comparison shows that there is a substantial agreement between

the therapeutic recommendations provided in the guidelines of the two consortia. The discordances in dosing advices or recommendations to switch to alternate drugs can be explained by differences in applied methodologies, the differences at which literature search for the recommendations for the drug-gene pairs were carried out or differences in clinical practices between countries. An important difference between the guidelines of the two consortia is the translation of genotype to phenotype in case of the gene *CYP2D6*. CPIC classifies the combination of a normal *CYP2D6* allele with a null allele as a normal metabolizer, while according to the DWPG this is an intermediate metabolizer. Currently, an international group of experts in the field, including members of CPIC and DPWG is trying to find consensus on how *CYP2D6* genotypes should be translated.

Part IV: Knowledge, experience and attitudes towards PGx of pharmacists and pharmacy students

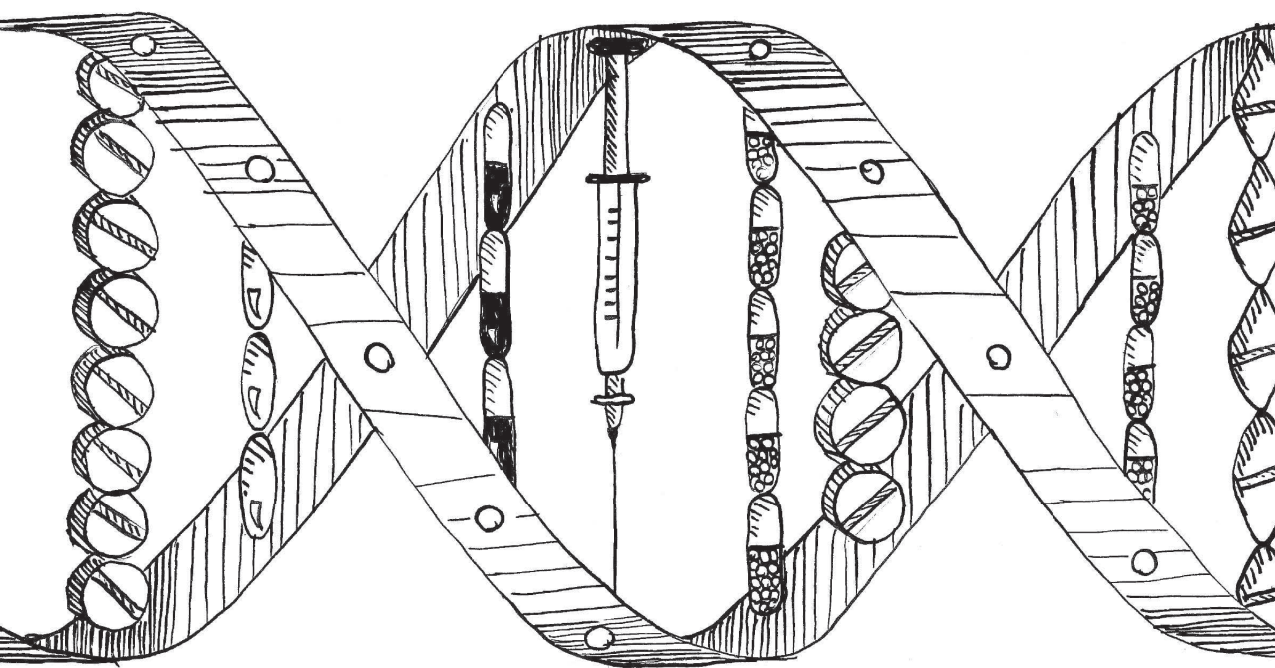
The fourth part of this thesis is dedicated to the mindset of (future) healthcare professionals toward PGx.

In addition to evidence, the opinion of clinicians of a new laboratory test is also of major importance for its implementation in routine care. Available research done among USA physicians and Canadian pharmacists has shown that clinicians have found themselves unprepared by the swift pace of evolution of (pharmaco)genetic research driven by the advances in biotechnology. The adoption of PGx by clinicians can be hampered if they do not believe in the concept of (partially) heritable drug response, do not know where to apply for PGx testing or are not comfortable in their abilities to interpret PGx test results and act on (pharmaco)genetic data. In **Chapter 8** the results of a nationwide survey among 667 Dutch pharmacists in the context of a national CDS system are described. In this survey participants were asked a total of 41 questions divided among 7 themes. The results show that virtually all pharmacists (99.7%) believe in the concept of (partially) heritable drug response and have high expectations of PGx in relation to pharmacotherapy. In contrast, the adoption of PGx among the surveyed pharmacists was low as only 14.7% had ordered or recommended a PGx test in the previous 6 months. Additionally, 14.1% of the participants felt adequately informed about PGx testing and 88.8% would like additional training on the subject. Moreover, being aware of the existence of the therapeutic recommendations created by the DPWG or the incorporation of the DPWG guidelines in electronic medication surveillance systems did not have any significant effect on knowledge how to apply PGx or adoption of PGx-testing. From the results of this study we concluded that Dutch pharmacists are generally very positive toward PGx, but perceive a lack in knowledge on

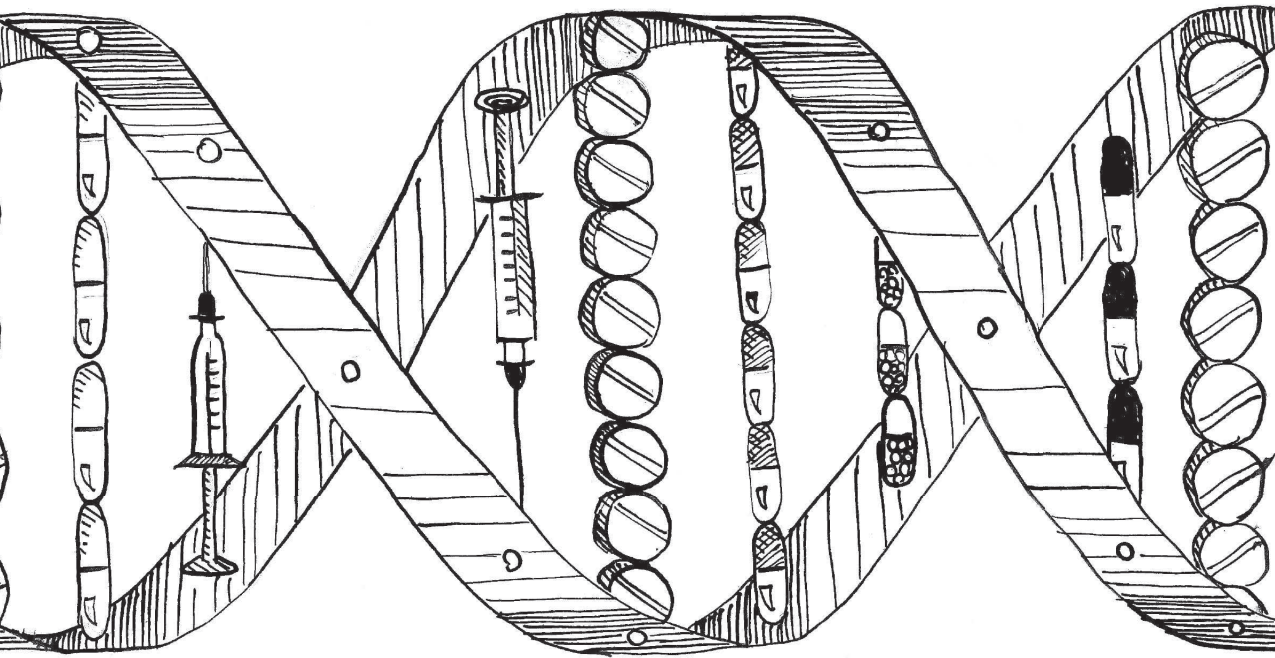
the subject and are need of extra training. **Chapter 9** provides insight in the knowledge and perceived abilities of pharmacy students regarding PGx testing. A survey consisting of 28 questions performed among 148 pharmacy students revealed that like their practicing colleagues they all believe in the concept of PGx and have high expectations that PGx will lead to safer and more effective pharmacotherapy. Virtually all surveyed students had received some sort of education on PGx, but only 12.8% felt adequately informed on how to apply PGx in pharmacotherapy indicating a knowledge gap on this subject among future pharmacists. A comparison with the cohort of 667 surveyed pharmacists using a multivariate analysis revealed that there are significant differences between the students and their practicing colleagues. Compared to pharmacist, the students feel more qualified to recommend PGx testing to predict drug efficacy, would use extra information to support the use of PGx testing in pharmacotherapy more often and other kind of sources of information to support the use of PGx within pharmacotherapy and to support changes in dose and drug when a patients' genotype is known. Additional research should investigate which type of education can reduce the PGx knowledge gap among pharmacy students.

Part V: General discussion & Summary

This dissertation is concluded with a general discussion in **Chapter 10** on the implementation of pharmacogenetics in everyday clinical settings. The chapter provides an overview of the current players in the field who are involved with the integration of PGx into routine clinical care. Additionally, several challenges which currently still hinder the implementation are identified such as selecting of clinically relevant pharmacogenes, providing data on diagnostic criteria of PGx testing, guidelines directing the clinical use of PGx test results, the evidence supporting improve of clinical care, information on cost-effectiveness and cost-consequences of PGx testing and the improvement of acceptance of PGx testing. The chapter closes with the conclusion that clinical grade assays have been developed, guidelines with therapeutic recommendation for actionable gene-drug pairs have been created and several RCT's have delivered evidence for implementation. One remaining barrier still hindering implementation is currently the knowledge gap on PGx among healthcare professionals and future clinicians which can potentially be resolved by a change in the current curriculum and post-academic education.



Nederlandse samenvatting



Introductie

In dit proefschrift wordt wetenschappelijk onderzoek beschreven welke als doel had om de implementatie van farmacogenetica in de gezondheidszorg te bevorderen. **Hoofdstuk 1** beschrijft hoe in de laatste decennia duidelijk is geworden dat een uniforme aanpak van farmacotherapie niet bruikbaar is omdat er grote inter-patiënt variabiliteit bestaat in zowel de effectiviteit als de bijwerkingen van geneesmiddelen. Er is inmiddels aangetoond dat individuele verschillen in absorptie, distributie, metabolisme en excretie van geneesmiddelen (en endogene substraten) deels een erfelijke oorsprong hebben. Recente klinische studies hebben aangetoond dat de toepassing van farmacogenetica de werkzaamheid en veiligheid van geneesmiddelen kan vergroten. De Nederlandse werkgroep Farmacogenetica (DPWG) en het Clinical Pharmacogenetics Implementation Consortium (CPIC) hebben farmacogenetica adviezen gebaseerd op systematische review van de wetenschappelijke literatuur opgesteld. De adviezen van de DPWG zijn in Nederland geïntegreerd in de voorschrijf- en afleversystemen van arts en apotheker. Op dit moment wordt farmacogenetica vooral toegepast in de tweede- en derdelijns gezondheidszorg om farmacotherapie te optimaliseren. Hoewel de meeste geneesmiddelen beschreven in de richtlijnen van de DPWG en CPIC door huisartsen veelvuldig worden voorgeschreven, wordt farmacogenetica in de eerstelijnsgezondheidszorg nog nauwelijks toegepast. Diverse studies hebben laten zien dat, wanneer er tegelijkertijd meerdere farmacogenen worden getest worden (“panel-based”), er bij de meeste patiënten tenminste één afwijkend genotype wordt gevonden. De hoge prevalentie van deze genetische variaties en de goede beschikbaarheid van farmacogenetische zijn sterke argumenten voor het invoeren van farmacogenetische screening in de eerstelijnsgezondheidszorg.

In het eerste deel van het proefschrift worden een zestal veelvoorkomende vragen van klinici over farmacogenetica beantwoord. Het tweede deel beschrijft de implementatie van farmacogenetica in de praktijk en de geschatte landelijke impact van farmacogenetica in de eerstelijnsgezondheidszorg. Het derde deel van dit proefschrift heeft als onderwerp het harmoniseren van de farmacogenetische adviezen van de DPWG en CPIC. In het vierde deel worden de kennis, ervaring en attitudes ten aanzien van farmacogenetica van apothekers en farmaciestudenten beschreven. Het vijfde deel bevat een algemene discussie van de voorgaande hoofdstukken in dit proefschrift.

Deel I: Antwoorden op door klinici veel-gestelde vragen over farmacogenetica

In **Hoofdstuk 2** worden zes door artsen en apothekers vaak gestelde vragen behandeld over de implementatie van farmacogenetica.

Vraag 1: Zijn farmacogenetische markers nodig voor de verbetering van de farmacotherapie? Ja!

De werkzaamheid en veiligheid van geneesmiddelen blijken in de praktijk vaak aanzienlijk lager te zijn dan bij fase III klinische studies. Met het vaststellen van de farmacogenetische biomarkers aan het begin van de therapie kunnen klinici geneesmiddelen op een betere manier inzetten.

Vraag 2: Wat zijn bronnen van genetische variatie die geneesmiddelrespons kunnen beïnvloeden?

Farmacokinetiek: Genetische variatie komt vaak voor in de vorm van een polymorfisme van één enkele nucleotide, inserties of deleties van één of van meerdere nucleotiden. Deze variaties kunnen leiden tot een verandering in activiteit van een enzym met als gevolg dat de afbraak van geneesmiddelen verminderd is of geheel niet plaatsvindt. Verhoogde enzymatische activiteit, door duplicatie van het coderende gen, kan ook voorkomen.

Farmacodynamiek: Genetische variatie in de genen die coderen voor een receptor of enzymen die betrokken zijn bij de signaaltransductie, kan leiden tot veranderde signalen en/of veranderde processen in de cel.

Idiosyncratische geneesmiddelreacties: In sommige gevallen kan genetische variatie leiden tot een reactie op een geneesmiddel die niet gerelateerd is aan de dosis of concentratie werkzame stof. De hypothese is dat mutaties in de genoomregio's die coderen voor het Humaan Leukocyt Antigeen (HLA) de immuunrespons op het geneesmiddel moduleren.

Vraag 3: In welke mate wordt variabiliteit in geneesmiddelrespons verklaard door farmacogenetica? Variabel!

Met behulp van de verklaarde variantie (R^2) kan de bijdrage van een farmacogenetische biomarker op een eindpunt worden vergeleken met de conventionele klinische factoren die gebruikt worden voor het aanpassen van de ingestelde therapie. Afhankelijk van het bestudeerde fenotype kunnen farmacogenetische biomarkers met klinische factoren gecombineerd worden.

Vraag 4: Zijn farmacogenetische uitslagen “actionable”? Ja!

Hoewel de meerderheid van de geneesmiddelenbijsluiters nog geen aangepaste behandeladviezen ten behoeve van patiënten met afwijkende genotypes bevat, worden deze therapeutische adviezen steeds vaker opgenomen in bijsluiters. Als genetische biomarkers

door clinici gebruikt gaan worden in de zorg zullen resultaten van klinische studies vertaald moeten worden in therapeutische adviezen, scores of beslisbomen.

Vraag 5: Welke mate van bewijs is nodig voor implementatie? Hier is nog geen consensus over!

Wanneer gen-geneesmiddelinteracties op dezelfde manier wordt beschouwd als reguliere geneesmiddelinteracties of dosisaanpassingen voor klinische factoren (zoals verminderde nier- of leverfunctie), dan zou observationeel bewijs genoeg moeten zijn voor de implementatie van farmacogenetica. Positieve resultaten van enkele gerandomiseerde klinische studies, met als focus het onderzoeken van farmacogenetische markers, kunnen als bewijs dienen dat het concept van farmacogenetica werkt.

Vraag 6: Waar vind ik bronnen van up-to-date informatie met betrekking tot de interpretatie van farmacogenetica test uitslagen? PharmGKB!

De DPWG en CPIC hebben sinds 2008 resp. 2011 richtlijnen opgesteld voor doseeradviezen gebaseerd op een systematische review van de wetenschappelijke literatuur. Deze richtlijnen, terug te vinden op pharmgkb.org, vormen de handvatten voor artsen en apothekers om de farmacogenetische uitslagen van de patiënt te interpreteren en de therapie hierop aan te passen.

Deel II: Implementatie van farmacogenetica in de klinische setting en de impact op landelijk niveau

Om de implementatie van farmacogenetica in de zorg te laten slagen, moeten artsen en apothekers genotypes voor klinisch relevante genen kunnen laten bepalen. Omdat het gen *CYP2D6* betrokken is bij het metabolisme van 20-25% van de geneesmiddelen die momenteel op de markt zijn, is dit gen zeer relevant. Bij de start van dit promotieonderzoek was de Roche AmpliChip de gouden standaard voor het typeren van het *CYP2D6* gen. In **Hoofdstuk 3** wordt de vergelijking tussen de nieuwe GenoChip *CYP2D6* macroarray en de AmpliChip beschreven. De overeenkomst tussen de twee methoden is getest door, met de GenoChip *CYP2D6* macroarray, het DNA uit witte bloedcellen afkomstig van 200 monsters uit de CYPTAM-studie te genotypen voor het gen *CYP2D6*. De CYPTAM-studie is een prospectieve multicenter studie die is uitgevoerd in Nederland en België, waarin 671 vrouwen met borstkanker zijn getest met de Roche AmpliChip. De resultaten van de met de GenoChip 200 geteste monsters bleken voor 99% ($n = 198$) overeen te

komen met de resultaten van de AmpliChip. Bij twee monsters kwamen de uitslagen van de twee methoden niet overeen: de AmpliChip registreerde bij de twee monsters een *41 allel, terwijl met de GenoChip een *1 werd gevonden. Sanger sequencing van de twee afwijkende monsters toonde aan dat de 2988G>A mutatie niet aanwezig was. Deze mutatie wordt door de Humane Cytochroom P450 Allel Nomenclatuur Commissie gezien als de belangrijkste mutatie van het *41 allel. De resultaten van dit onderzoek geven aan dat de CYP2D6 GenoChip Macroarray een valide methode is voor het detecteren van 13 genetische varianten in het *CYP2D6* gen.

Naast de ontwikkeling van nieuwe hoogwaardige farmacogenetische testen is de praktische toepassing van deze nieuwe testen door artsen en apothekers in de kliniek van belang. Bij de start van dit promotieonderzoek werd farmacogenetica in toenemende mate in de tweedelijnsgezondheidszorg toegepast, maar de implementatie ervan in de eerstelijnsgezondheidszorg bleef achter. Om de toepassing van farmacogenetica in de eerstelijnsgezondheidszorg te verbeteren, werd het **Implementation of Pharmacogenetics in Primary care Project (IP3)** geïnitieerd. In **Hoofdstuk 4** wordt de IP3-studie beschreven: een studie waarin huisartsen en openbaar apothekers ervaring konden opdoen met farmacogenetica in hun eigen praktijk. Patiënten met een eerste uitgifte van een geneesmiddel met een bekende gen-geneesmiddel interactie (amitriptyline, atomoxetine, atorvastatine, (es)citalopram, clomipramine, doxepine, nortriptyline, simvastatine of venlafaxine) konden worden geïncludeerd. De patiënten werden vervolgens getest op 40 genetische varianten in de genen *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* en *VKORC1* met behulp van het DMET-platform. Om tevens variaties in het aantal kopieën van het *CYP2D6* gen te kunnen detecteren werd ook een kwantitatieve Polymerasekettingreactie (qPCR) Taqman methode toegepast. De genotypes werden vervolgens vertaald in voorspelde fenotypes en gecombineerd met adviezen van de DPWG in een rapport. Dit rapport werd vervolgens overgedragen aan de behandelend huisarts en openbaar apotheker met het verzoek om de resultaten in hun voorschrijf- en afleversystemen op te nemen om zo farmacogenetische medicatiebewaking te realiseren. In de periode van november 2014 tot en met juli 2017 zijn 200 patiënten geïncludeerd door de deelnemende zorgverleners. Van de patiënten die in aanmerking kwamen voor de studie werd uiteindelijk 18.0% geïncludeerd. De meerderheid van de patiënten werd geïncludeerd naar aanleiding van een eerste uitgifte van een statine, zijnde atorvastatine (57.5%) of simvastatine (14.5%). Het resterende deel van de patiënten (28.0%) werd geïncludeerd naar aanleiding van een eerste uitgifte voor een antidepressivum (amitriptyline, (es)citalopram, nortriptyline of venlafaxine). In 90% van alle patiënten werd tenminste één afwijkend genotype in de 8 genen aangetoond. Wanneer de informatie uit het farmacogenetisch profiel van de patiënt vervolgens gecombineerd werd met het voorgeschreven recept, bleek er in 31.0% van de patiënten sprake te zijn van een

afwijking in het gen waarvan een bekende gen-geneesmiddelinteractie is beschreven met het voorgeschreven geneesmiddel. Bij deze groep patiënten werd er vervolgens een advies op basis van de richtlijn van de DPWG uitgebracht aan de behandelend arts en apotheker om de behandeling aan te passen en/of de patiënt strenger te monitoren op effectiviteit of bijwerkingen. Deze studie toont aan dat de toepassing van farmacogenetica in de eerstelijnsgezondheidszorg mogelijk is en bevestigt de hoge prevalentie van afwijkende genotypen bij patiënten in het panel van de geselecteerde 8 genen. Verder maakte dit project het voor huisartsen en openbaar apothekers mogelijk om ervaring op te doen met farmacogenetica in hun eigen praktijk. In **Hoofdstuk 5** is een schatting gemaakt van de landelijke impact van het implementeren van een farmacogenetische screening bestaande uit een selectie van de genen *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A5*, *DPYD*, *SLCO1B1*, *TPMT* en *VKORC1* in de eerstelijnsgezondheidszorg in Nederland. Hierbij zijn de frequenties van de afwijkende genotypes van deze 8 genen, zoals verkregen uit de IP3-studie, gebruikt als een representatieve steekproef uit de Nederlandse bevolking. De frequenties van de genotypen werden gecombineerd met nationale prescriptiedata uit de eerste lijn afkomstig van de Stichting Farmaceutische Kengetallen (SFK). De hieruit berekende schatting laat zien dat voor de 45 geneesmiddelen die met de 8 genen geassocieerd zijn, er in 23.6% van de eerste uitgiftes ($n = 856.002$ uitgiftes/jaar), sprake is van een gen-geneesmiddel interactie zoals beschreven in de richtlijnen van de DPWG. Volgens de berekening zou er in één op de negentien (= 5.4%) eerste uitgiftes een verandering in de dosering of de keuze van het geneesmiddel moeten plaatsvinden bij start van de therapie. Duidelijk wordt dat in de nabije toekomst huisartsen en openbaar apothekers geconfronteerd worden met patiënten waarvan het farmacogenetische profiel bekend is en zij routinematig farmacotherapie zullen moeten aanpassen aan de hand van afwijkende genotypes.

Deel III: Harmonisatie van interpretatie van uitslagen en therapeutische adviezen

Een belangrijk doel is het vaststellen van een wereldwijde uniforme interpretatie van farmacogenetische testuitslagen en het standaardiseren van de daarbij passende behandeladviezen voor patiënten met afwijkende farmacogenen. **Hoofdstuk 6** geeft een reactie op een artikel van Magnani et al. In dit artikel wordt een prospectieve studie met 180 individuen beschreven waarin de onderzoekers de patiënten screende voor de *DPYD* IVS14+1G>A splice site variant (ofwel het *2A-allel) en routinematig evalueerde voor fluoropyrimidine-gerelateerde toxiciteit. Vier van de 180 gescreende patiënten bleken heterozygoot drager te zijn van het *DPYD* *2A allel. Aan de hand van deze uitslag werd in één patient afgezien van behandeling met fluoropyrimidines en werd bij drie patiënten een dosis reductie van

50% toegepast op basis van de richtlijnen van de DPWG. Ondanks de toegepaste dosis-reductie observeerden de onderzoekers ernstige toxiciteit bij de drie patiënten als gevolg van de fluoropyrimidine behandeling en concluderen dat er een verdere reductie van de startdosis van fluoropyrimidines nodig is voor patiënten die heterozygoot drager zijn van de *DPYD* IVS14+1G>A variant. In onze reactie, gebaseerd op uitgebreide review van de literatuur en een farmacokinetische studie van Deenen et al., wordt beredeneerd dat een reductie van 50% in de startdosis, gevolgd door een dosistitratie gebaseerd op toxiciteit, een betere benadering is voor het doseren van fluoropyrimidines in patiënten die heterozygoot drager zijn van de IVS14+1G>A variant.

Verschillen in farmacogenetische richtlijnen, gebaseerd op dezelfde onderliggende literatuur, kan het gevolg zijn van verschillen in interpretatie van de literatuur. **Hoofdstuk 7** beschrijft het initiatief om de richtlijnen van de DPWG en de CPIC te harmoniseren door de inventarisatie van bestaande verschillen in interpretatie van uitslagen en therapeutische adviezen. Hiervoor zijn de methodologieën van consortia systematisch beoordeeld en is een vergelijking gedaan van zowel interpretatie van het genotype, als de therapeutische adviezen voor gen-geneesmiddel interacties die beide consortia richtlijnen zijn opgesteld. De vergelijking laat een grote overeenkomst zien tussen de therapeutische adviezen opgesteld door de twee consortia. De verschillen in doseeradviezen en therapeutische strategieën kunnen verklaard worden door de verschillen in de gebruikte methodes, het verschil in de tijdstippen van het raadplegen van de literatuur of verschillen in de klinische praktijk tussen diverse landen of continenten.

Een belangrijk verschil tussen de richtlijnen van de twee consortia is de vertaling van het genotype naar fenotype bij het gen *CYP2D6*. CPIC classificeert namelijk de combinatie van een normaal *CYP2D6* allel met een nul allel als een normale metaboliseerder, terwijl de DPWG deze combinatie als een intermediäre metaboliseerder beschouwt. Momenteel werkt een internationale groep van experts, waarin ook leden van CPIC en de DPWG in vertegenwoordigd zijn, aan een standaardvertaling van *CYP2D6* genotypen.

Deel IV: Kennis, ervaring en attitudes ten opzichte van farmacogenetica onder apothekers en farmacistudenten

In het vierde deel van dit proefschrift wordt gekeken naar de attitudes van (toekomstige) zorgprofessionals ten opzichte van farmacogenetica. Naast klinisch bewijs, is het beeld dat klinici van een beschikbare moleculaire laboratoriumdiagnostiek hebben ook een belangrijke factor bij implementatie in de gezondheidszorg. Studies onder Amerikaanse artsen en Canadese apothekers tonen aan dat deze professionals zich niet voorbereid

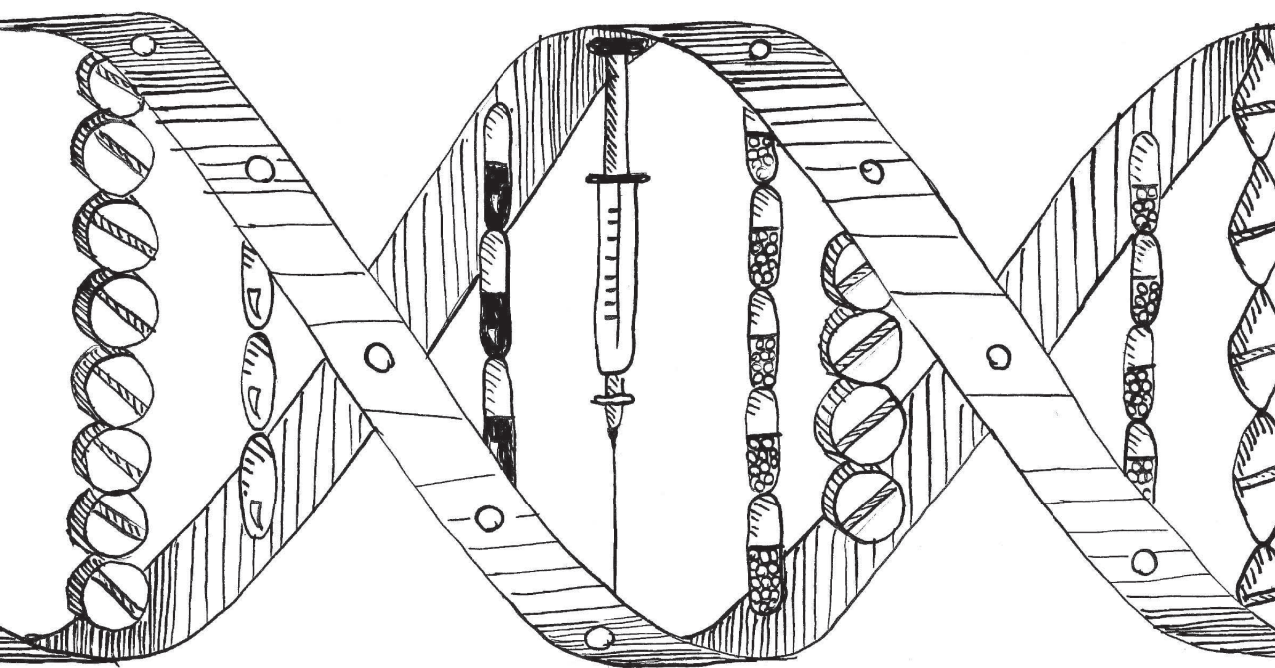
voelen op de snelle evolutie in (farmaco)genetisch onderzoek. De implementatie van farmacogenetica in de zorg kan stagneren als clinici niet overtuigd zijn van het concept van farmacogenetica. Dat wil zeggen dat geneesmiddelrespons (ten dele) wordt bepaald door erfelijkheid. Ook spelen factoren zoals het niet weten waar farmacogenetische testen aangevraagd kunnen worden, of het niet kunnen interpreteren van testuitslagen, een rol.

Hoofdstuk 8 beschrijft een landelijke enquête onder 667 Nederlandse apothekers over farmacogenetica in de context van beslissingsondersteuning beschikbaar in de kliniek. De enquête bestond uit 41 vragen verdeeld over zeven thema's. De resultaten laten zien dat bijna alle apothekers (99.7%) overtuigd zijn van het concept van farmacogenetica en dat ze er hoge verwachtingen van hebben. Echter, de mate waarin ze farmacogenetica toepassen is laag, slechts 14.7% van de ondervraagden had in de afgelopen zes maanden een farmacogenetische test aangevraagd of aanbevolen. Verder voelde slechts 14.1% van de deelnemers zich voldoende geïnformeerd over farmacogenetica en wilde 88.8% aanvullende scholing over dit onderwerp. Uit de analyse bleek dat er geen significante associatie was tussen het op de hoogte zijn van het bestaan van de therapeutische richtlijnen van de DPWG of van de integratie van de richtlijnen in de elektronische medicatiebewakings-systemen met 1) de kennis over hoe farmacogenetica te gebruiken in relatie tot therapie of 2) de adoptie van farmacogenetische testen. Uit de resultaten van deze studie kan worden geconcludeerd dat Nederlandse apothekers erg positief zijn over de mogelijkheden van farmacogenetica, maar een gebrek aan kennis over dit onderwerp ervaren en graag extra geschoold willen worden.

Hoofdstuk 9 geeft inzicht in de kennis en de attitudes van farmaciestudenten met betrekking tot farmacogenetica. Een enquête van 28 vragen afgenomen onder 148 farmaciestudenten toont aan dat zij, net als hun afgestudeerde collega's, geloven in het concept van erfelijke geneesmiddelrespons en hoge verwachtingen hebben in farmacogenetica als hulpmiddel voor veiliger en effectiever farmacotherapie. Bijna alle studenten hadden enige vorm van onderwijs over farmacogenetica genoten, maar slechts 12.8% van de ondervraagde studenten voelde zich voldoende geïnformeerd om de kennis toe te passen. Een multivariaat analyse laat zien dat er significante verschillen bestaan tussen de studenten en de praktiserende apothekers. In vergelijking met de apothekers voelen de farmaciestudenten zich meer gekwalificeerd om farmacogenetische testen aan te bevelen. De studenten zouden vaker extra informatie opzoeken en andere informatiebronnen gebruiken om de toepassing van farmacogenetica binnen farmacotherapie te onderbouwen. Daarnaast zouden studenten andere bronnen gebruiken om informatie over farmacogenetica te vinden. Aanvullend onderzoek moet uitwijzen welk type onderwijs het meest geschikt is om farmaciestudenten voor te bereiden voor het gebruik van farmacogenetische informatie in hun carrière als apotheker.

Deel V: Algemene discussie

Dit proefschrift eindigt in **Hoofdstuk 10** met een algemene discussie over de implementatie van farmacogenetica in de dagelijkse klinische praktijk. Het hoofdstuk geeft een overzicht van de belangrijkste spelers. Verder worden de volgende uitdagingen besproken die momenteel een rol spelen in de implementatie van farmacogenetica in de praktijk: het selecteren van klinisch relevante farmacogenen; data over de diagnostische criteria van farmacogenetica; de richtlijnen die het gebruik van farmacogenetica adviseren; het klinische bewijs dat deze testen tot verbetering van de therapie; informatie over kosteneffectiviteit en kostenconsequenties en de verbetering van de implementatie van farmacogenetische testen. Het hoofdstuk eindigt met de conclusie dat er hoogwaardige farmacogenetische testen beschikbaar zijn, er richtlijnen zijn voor gen-geneesmiddel interacties en enkele RCT's het klinisch bewijs hebben geleverd dat toepassing van farmacogenetica zinvol is. Een barrière die momenteel nog bestaat, is een kennishiaat over farmacogenetica onder huidige zorgprofessionals en toekomstige artsen en apothekers die wellicht opgelost kan worden door een aanpassing aan het huidige curriculum en postacademisch onderwijs.



Curriculum vitae

Paul Christiaan Dirk Bank was born on the 28th of June 1989 in Heerlen, The Netherlands. After completion of secondary school at the Christelijke Scholengemeenschap Walcheren in 2007 he started with the bachelor Pharmacy at the University of Utrecht. This bachelor was completed in 2010 after a research project entitled “Induction and inhibition of the metabolizing enzymes CYP3A4 and CYP2C9 by complementary & alternative medicine” at Division of Pharmacoepidemiology and Clinical Pharmacology under supervision of dr. ir. Irma Meijerman and was awarded the F.A.F.C Went award. In the same year Paul continued with his Pharm.D and in 2011 he started with a research internship on the robustness of the flow cytometric P-selectin platelet function assay at the St Antonius Center for Platelet Function Research, St. Antonius hospital (Nieuwegein) supervised by dr. H.J. Bouman, dr. T. Godschalk and Drs. Th. O. Bergmeijer. During his Pharm.D he worked at the St. Antonius Apotheek (Nieuwegein) and followed internships at the Slotervaartziekenhuis (Amsterdam), the Leiden University Medical Center (Leiden) and the Zuiderpark Apotheek (The Hague). In 2013, Paul obtained his Pharm.D and enrolled on a project to implement pharmacogenetic testing in healthcare centers in primary care coordinated from the department of Clinical Pharmacy & Toxicology of the Leiden University Medical Center. In 2014 a PhD was started under supervision of prof. dr. H.J. Guchelaar and dr. J.J. Swen entitled “Improving acceptance of pharmacogenetic testing in patient care”. For abstracts on the Implementation of Pharmacogenetics into Primary Care Project (IP3) and the nation-wide impact of implementing a preemptive pharmacogenetic panel approach to guide drug prescribing in primary care in The Netherlands Paul received the American Society for Clinical Pharmacology & Therapeutics (ASCPT) Presidential Trainee Award 2017 and ASCPT Top Abstract Award 2018 respectively. In August 2018 Paul started as a pharmacist at the hospital pharmacy of Noordwest Ziekenhuis (NWZ) in Alkmaar to aid with the implementation of Healthcare Information X-change and in April 2018 he enrolled in a residency program to become a hospital pharmacist at NWZ. Paul has formed a civil union with Maud Ten Koppel and live together in Hoofddorp.

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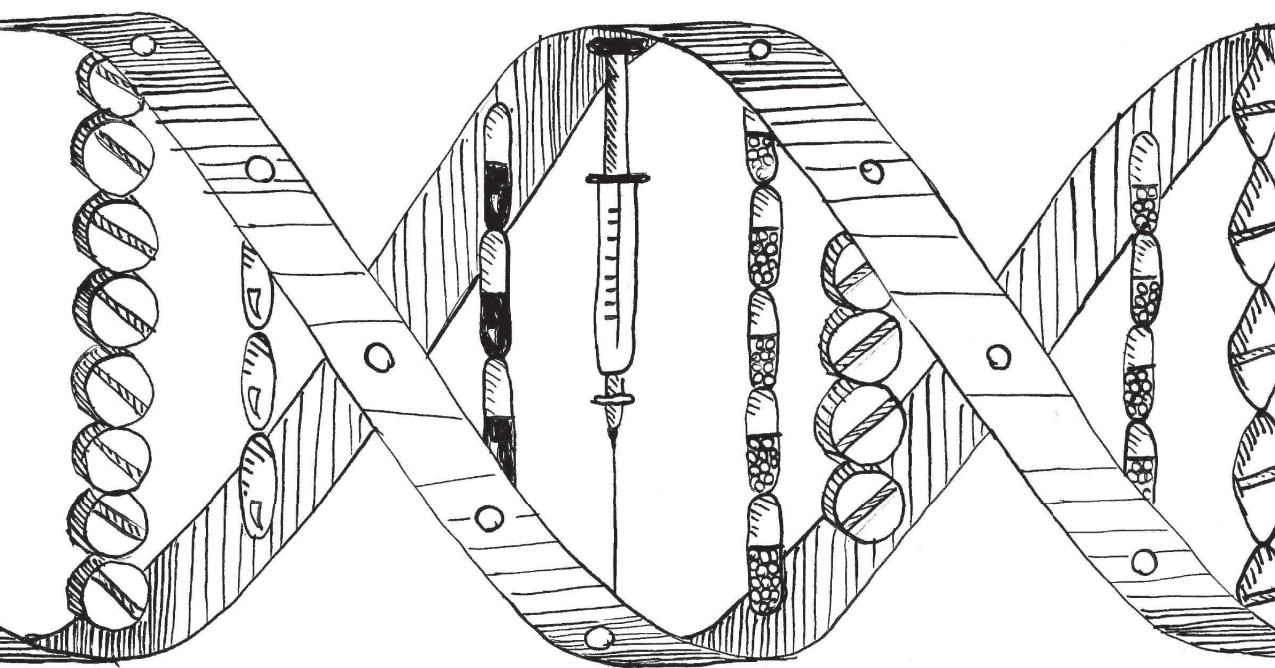
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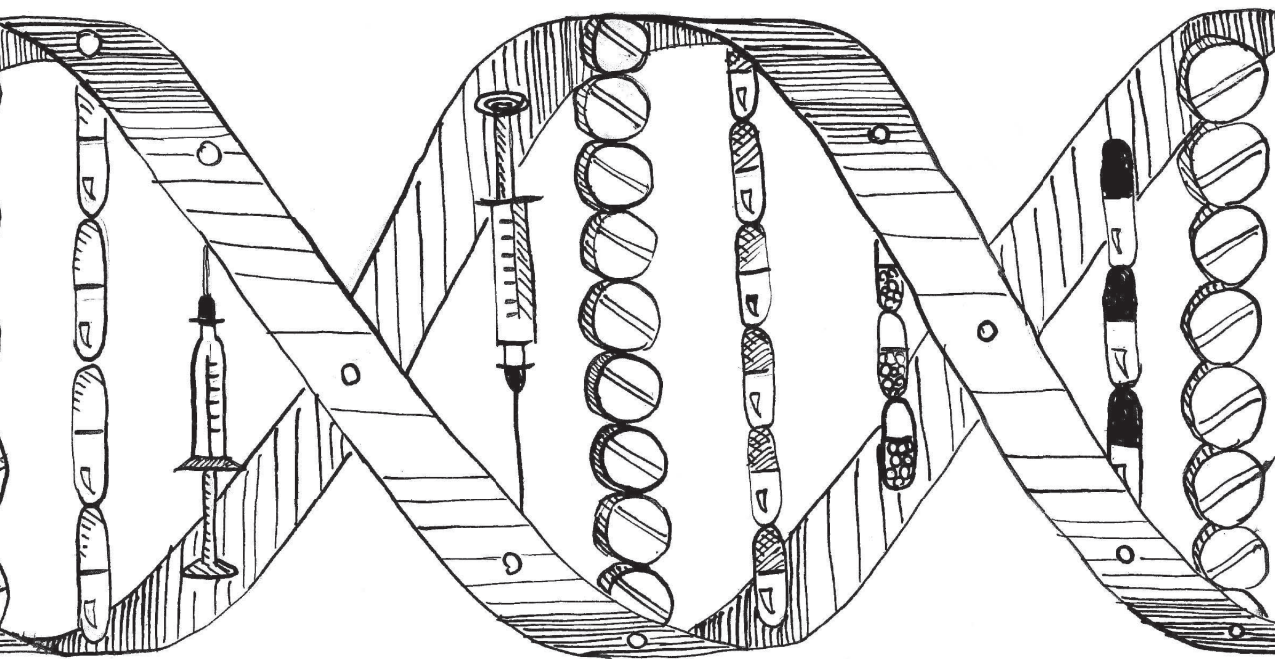
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Dankwoord



Na vijf jaar geduld en doorzetten is het proefschrift dan af. Graag wil ik iedereen bedanken die een bijdrage heeft geleverd aan dit promotieonderzoek.

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Verder wil ik de KNMP bedanken voor het mogelijk maken van het IP3 en diverse andere hoofdstukken.

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