Precision medicine using pharmacogenomic panel testing

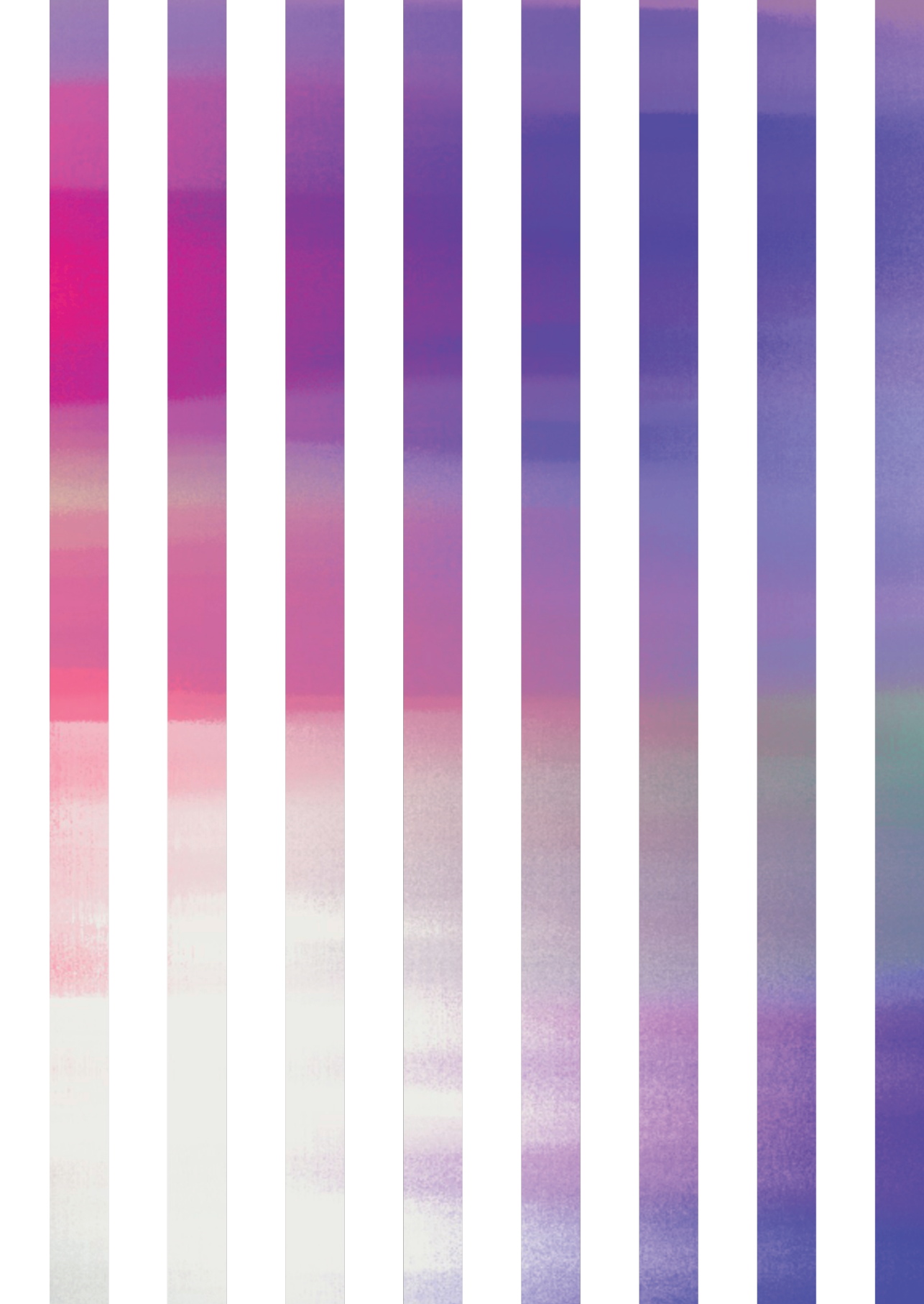
Wouden, C.H. van der

GENERAL DISCUSSION AND SUMMARIES

Chapter 10:

General Discussion and Future Perspectives

Adapted from Advances in Molecular Pathology. 2020; accepted for publication



The work of this thesis aims to support implementation of precision medicine using pharmacogenomic (PGx) panel testing. In provision of this aim, it has reported on generating evidence for PGx panel testing (**Part I**) and the development of tools facilitating implementation (**Part II**). Furthermore, it evaluated the implementation process utilizing these tools (**Part III**) and quantified the impact of PGx implementation on patient outcomes and cost-effectiveness (**Part IV**). The following sections reflect on findings from each part and present future perspectives. An overview of the general discussion and future perspectives is given in **Figure 1**.

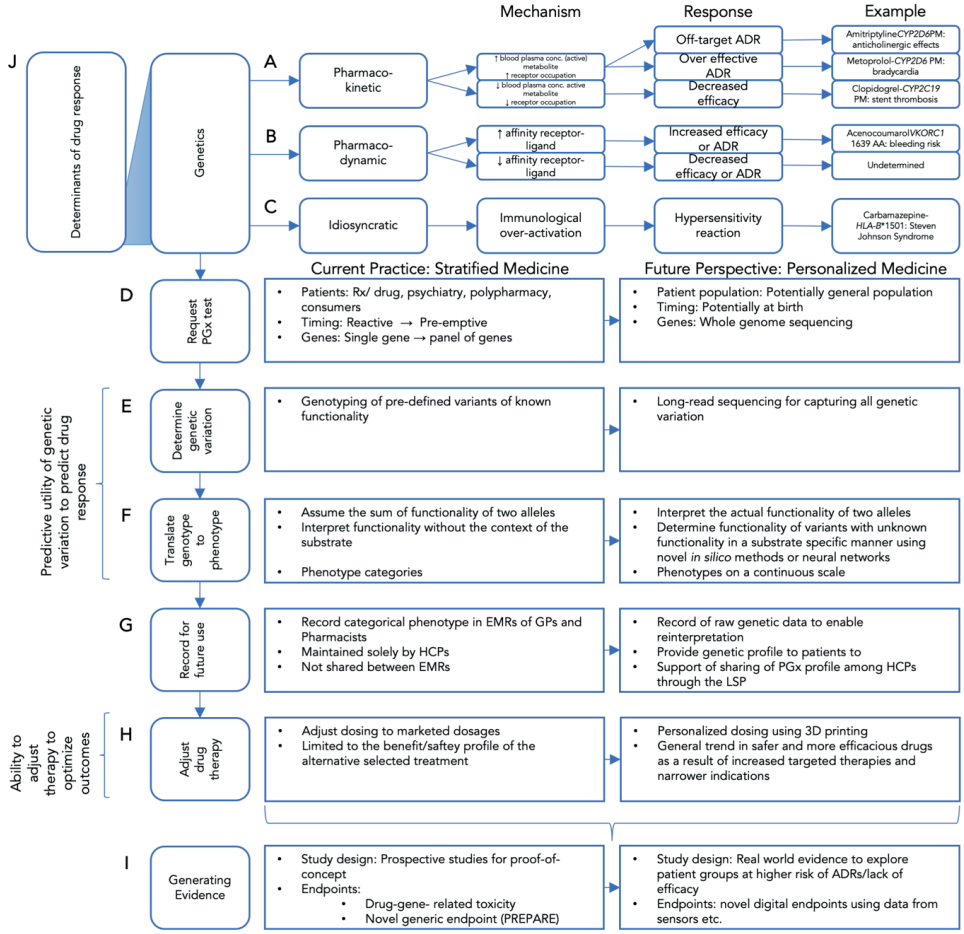


Figure 1 Overview of general discussion and future perspectives

Part I: Generating Evidence for Pharmacogenomic Panel Testing

Several of the reported hurdles obstructing the implementation of PGx are currently being addressed by various initiatives, both in the United States and the European Union. A compact overview of these initiatives is was provided in **Chapter 2**. From this overview, a significant research gap was identified: the absence of evidence presenting the collective clinical utility of a panel of PGx-markers for pre-emptive PGx testing. Although several randomized controlled trials (RCTs) support the clinical utility of individual gene-drug pairs, delivered in a single gene reactive approach, to either optimize dosing (1-4) or drug selection (5, 6); evidence supporting clinical utility of the remaining drug-gene interactions (DGIs) for which recommendations are available when delivered in a pre-emptive panel approach is lacking. Significant debate persists regarding both the nature and strength of evidence required for the clinical application of PGx. Some argue that gold-standard evidence is required for each individual DGI before clinical implementation is substantiated (7). Others argue that a mandatory requirement for prospective evidence to support the clinical validity for each PGx interaction is incongruous and excessive (8-11). As discussed in **Chapter 3**, we support the latter view. Generating gold-standard evidence for each of the 51 individual DGI for which we currently have Dutch Pharmacogenetics Working Group (DPWG) guidelines separately would require unrealistically large amounts of funds. However, extrapolating efficacy of all 51 DGIs based on the conclusions of the previously mentioned RCTs is not substantiated. Reasons for this being: the diversity in underlying pharmacology of the interactions, the predictive utility of genetic variation to predict drug response and the ability to reduce the risk of unwanted effects by adjusting pharmacotherapy. Nonetheless, the Ubiquitous Pharmacogenomics (U-PGx) consortium aims to fill this identified evidence gap to ultimately support implementation.

As discussed in **Chapter 3**, a number of strategies may be deployed to generate evidence for the collective clinical utility of a panel of PGx-markers for pre-emptive PGx testing. In the context of precision medicine, several fundamental options for generating evidence have been suggested (12): 1) observational research designed to identify modifiers of the effectiveness of interventions received by patients in the course of health care delivery; 2) subgroup analyses and interaction testing in standard RCTs of intervention effectiveness; 3) dedicated precision medicine RCTs that directly compare targeted vs untargeted intervention approaches. When generating evidence for PGx effectiveness, we may envision an observational study wherein the available guidelines are implemented prospectively and compare a defined outcome with a historical control group, as recently performed for the *DPYD*-fluoropyrimidine interaction (13). However, historical controls may likely only be feasible for patient populations who are closely monitored, such as those on high-risk drugs as fluoropyrimidines. Additionally, it is not considered ethical to prospectively recruit a control group for DGIs where there is sufficient evidence for clinical implementation, again as is the case for *DPYD*-fluoropyrimidines. However, many drugs included in the DPWG

guidelines are low-risk primary care drugs for which close monitoring not routinely performed. Therefore, these studies are prone to many forms of bias. Alternatively, an RCT can be executed to generate evidence. Several RCTs have provided gold-standard evidence showing the clinical utility of individual DGIs to guide dosing (1-4) and drug selection (5, 6). However, the DPWG has recommendations for 51 DGIs, most of which have been devised in the absence of RCTs. It may not be feasible to conduct RCTs for specific DGIs of which the anticipated efficacy can only be observed after a long follow-up. For example, the improved efficacy of adjuvant tamoxifen by guiding dose on *CYP2D6* genotype may only be observed at an estimated 10-year follow-up (14). It is important to note that non-PGx interventions, such as dose adjustment of renally excreted drug in response to kidney function, have been widely implemented in the absence of RCTs validating its effectiveness for each individual drug. Genetic exceptionalism has been held responsible for this double standard (15).

Regardless of the inconvenience, there is still a demand for evidence substantiating patient benefit and cost-effectiveness, to enable stakeholders to practice evidence-based medicine. Therefore, the U-PGx consortium had decided upon an alternative model of evaluating the (cost-) effectiveness of PGx-guided pharmacotherapy. As an alternative to conducting 51 separate RCTs (one for each DPWG guideline), the consortium set out to quantify the collective clinical utility of a panel of PGx-markers (50 variants in 13 pharmacogenes) within one trial (the PREPARE study) as a proof-of-concept across multiple potentially clinically relevant DGIs, as described in **Chapter 2**. The PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions (PREPARE) Study (ClinicalTrials.gov: NCT03093818), aims to quantify the collective clinical utility of a panel of PGx-markers to guide dose and drug selection in reducing the risk of clinically relevant adverse drug reactions (ADRs) (16, 17). Additional outcomes include cost-effectiveness, process indicators for implementation and provider adoption of PGx.

Although PREPARE presents an unconventional and practical solution to enable quantification of the collective clinical utility of a panel of PGx-markers, it may be potentially underestimating the true effect. Reasons for this being: the inability of the PGx panel to determine all of an individual's genetic variation, the delayed initiation of PGx-guided dose or drug as a result of the turn-around-time, and limiting the primary endpoint to ADRs caused by the drug of enrolment within a 12-week follow-up. In addition, due to its limited 12-week follow-up, it does not enable quantification of the cost-effectiveness in patients encountering multiple DGIs over a longer time-horizon. This can be estimated using model-based methods to simulate long term (cost-)effectiveness. Nonetheless, PREPARE may generate proof-of-concept evidence for ubiquitous adoption of PGx-guided pharmacotherapy (18).

Future Perspectives: Generating Evidence Enabling Precision Medicine

Conventionally, evidence supporting novel interventions are generated within prospective studies. However, in an era where digitalization is driving data accumulation and

a concomitant increase in stratification of patient groups and a more precise diagnosis, we are moving towards the utilization of real-world data to support precision medicine. Several authors have pointed out that precision medicine, and genomic medicine, in particular, would benefit from a convergence of implementation science and a learning health system to measure outcomes and generate evidence across a large population (19, 20). However, this requires standardization of outcomes in Electronic Medical Records (EMRs) to enable aggregation of phenotype data across large populations for both discovery and outcomes assessment within a genomic medicine implementation (21). Many nationwide, large-scale initiatives are generating prospective longitudinal evidence supporting precision medicine approaches (22-24). In addition to the U-PGx consortium, a project specifically generating evidence for pharmacogenomics is the AllofUs project (25). Alternatively, pragmatic clinical trials offer researchers a means to study precision medicine interventions in real-world settings (26, 27). In contrast to traditional clinical trials that are performed in ideal conditions, these pragmatic trials are conducted in the context of usual care (27). Pragmatic clinical trials easily transition into existing healthcare infrastructures and therefore make them particularly appealing to comparative effectiveness research and the evidence-based mission of learning healthcare systems (28, 29). An example of such a pragmatic trial for generating evidence for pre-emptive PGx testing is the I-PICC study (30).

In any case, whether generating evidence for precision medicine through a prospective trial, pragmatic trial or real-world data approach, the timing and resources required to both understand and implement interventions should be taken into consideration. For example, in the case of understanding genetic variation to predict drug response, we argue this should ideally be performed before market authorization to maximize the benefit-risk ratio of drugs across the time in which they are used in patient care. However, since understanding germline PGx variation is not routinely included in drug development, it is mostly performed after market-authorization by investigator-initiated initiatives. The absence of this knowledge is a great limitation since the uptake of PGx may be limited by the increasing availability of alternative therapies which appear to be at least as effective without known major pharmacogenomic issues (31).

Evolving digital health technologies are driving data accumulation. Data collected by sensors (in smartphones, wearables, and ingestibles), mobile apps and social media can be processed by machine learning to support medical decision making (32). Raw sensor data can also be processed into digital biomarkers and endpoints (33). This development may be particularly useful for endpoint definition in disease areas where biological endpoints are lacking, as in psychiatry and neurology, to enable quantification of disease progression and drug response. For example, novel digital endpoints are being developed to stratify mental health conditions and predict remission using passively collected smartphone data (34). Another example is the development of a digital biomarker for Parkinson's disease using motor active tests and passive monitoring through a smartphone (35). For precision medicine,

in particular, we may also be more able to stratify patient groups into responders and non-responders with improved endpoint development in these disease areas. Increased stratification of patient groups on the basis of genetic, (digital) biomarker, phenotypic, of psychosocial characteristics will drive more precise diagnoses and pharmacotherapy optimization (36, 37). This trend will drive demand for innovations for more efficient study designs due to increasing numbers of indications, while resources to fund these trials remain constant (38). In the case of rare diseases, the ultimate form of generation of evidence for precision medicine is through "N-of-1" studies (39). Recently, a successful example of such a trial was presented. A paediatric patient was diagnosed with a rare, fatal neurodegenerative condition. The molecular diagnosis led to the rational design, testing, and manufacture of milasen, a splice-modulating antisense oligonucleotide drug tailored to this particular patient. Proof-of-concept experiments in cell lines from the patient served as the basis for launching an "N-of-1" study of milasen within 1 year after the first contact with the patient, resulting in a safe and effective therapy (40).

Part II: Developing Tools Facilitating Implementation

The utility of precision medicine using PGx guided pharmacotherapy is dependent on two factors. Firstly, the predictive utility of genetic variation to predict drug response and secondly our ability to adjust pharmacotherapy to reduce the risk of unwanted effects among high-risk individuals. We will discuss the current status of both factors using **Chapters 4 and 5** to illustrate current strengths and limitations. In **Chapter 4**, we presented the development of a tool to determine genetic variation to predict drug response. The PGx-passport uses 58 variant alleles within 14 pharmacogenes (*CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, F5, HLA-B, NUDT15, SLCO1B1, TPMT, UGT1A1,* and *VKORC1)* to predict patient phenotypes and corresponding drug response when exposed to an interacting drug. In **Chapter 5** we presented the development of a DPWG guideline on how to use predicted *DPYD* gene activity score (GAS) to adjust starting dose of fluoropyrimidines to reduce the risk of severe, potentially fatal toxicity.

Predictive Utility of Genetic Variation to Predict Drug Response

The paradigm of PGx to enable precision medicine is to determine an individual's genetic variation in a given gene, to predict the corresponding phenotype, or functionality, of its gene product which in turn corresponds to a higher risk of a particular drug response. For intrinsic and pharmacokinetic dependant drug response (see **Figure 1 A**), we expect the predicted phenotype to correspond to the drug plasma level and receptor occupancy and therefore predictive of an ADR or effect. For intrinsic and pharmacodynamic dependant ADRs (see **Figure 1 B**), we expect the predicted phenotype to correspond to the affinity between receptor and ligand and therefore predictive of an ADR or effect. For idiosyncratic ADRs (see **Figure 1 C**), we expect the predicted phenotype to correspond to a, most commonly immunological, mechanism which causes an ADRs. In PGx testing, as it is performed today,

genetic variants are determined (see **Figure 1 D**) and interpreted to predict patient phenotype and corresponding drug response when exposed to an interacting drug. However, due to both technical constraints to determine all genetic variation and constraints in the interpretation of variants due to unknown downstream functionality, we have not yet reached maximum predictive utility of genetic variation.

Even though multiple variants have been discovered we currently restrict testing to a subset of these variants. Restricting testing to individual variants disregards untested or undiscovered variants that may also influence the functionality of the gene product. Therefore we are unable to fully predict the functionality of the gene product (see **Figure 1 E**). Reasons for restriction of testing are twofold. Firstly, technical limitations regarding the sequencing of complex loci prevent complete determination of both the gene of interest and other areas in the genome which may have an effect on the gene product. Determining genetic variation is specifically difficult in highly polymorphic genes such as the *HLA* genes or genes located near pseudogenes such as *CYP2D6*. Secondly, even if we were to determine all genetic variation, the downstream effect on protein functionality may be unknown and therefore impossible to interpret clinically (41). However, progress in the interpretation of functional consequences of such uncharacterized variations may support future interpretation *in silico* (42), *in vitro* or *in vivo* (43). Importantly, a study has shown that 92.9% of genetic variation in ADME genes are rare and 30-40% of functional variability in pharmacogenes can be attributed to these variants (44). In addition to the downstream functionality, the penetrance (i.e. the potential of a variant to accurately predict the genetic component of drug response) is also unknown. The penetrance is a function of both the variant's effect on protein functionality and the extent to which the protein functionality is associated with clinical outcome. Significant debate persists regarding both the nature and strength of evidence required for the clinical application of variant alleles of unknown functionality. Since the strength of these functions differs across genes and DGIs, we do not foresee a one-size-fits-all consensus regarding an evidence threshold across all DGIs, but rather a different evidence threshold per individual DGI based on the genetics and pharmacology of the interaction. For example, in the case of the *TPMT*-thiopurine interaction, the effect of *TPMT* variation on protein functionality has been firmly established since it exhibits behavior similar to monogenetic co-dominant traits (45). Therefore identified variants in *TPMT* (*3A/*3B/*3D) are considered to have sufficient evidence to be applied in the clinic, even in the absence of studies specifically investigating clinical effects in patients carrying these particular variants. On the other hand, clinically relevant variant alleles in *CYP2D6* are based on the pharmacology of the interaction. For example, the flecainide-*CYP2D6* interaction is based on the associations between decreasing *CYP2D6* activity leading to increasing flecainide plasma levels which in turn leads to increased risk for flecainide intoxication. Therefore, all identified variants in *CYP2D6*, have shown to have a significant effect on CYP2D6 enzyme activity are defined to have sufficient evidence to be applied in the clinic.

In summary, both the functional effects and the penetrance of many rare variants is yet unknown. As an additional complication, these may also differ across substrates and drug responses. Even more fundamentally, variants may impact each other's functionality and therefore individual variants may have different functionalities depending on the absence or presence of other variants. For example, a non-synonymous insertion causing a frameshift upstream of another variant will cause the functionality of the gene product to be different than in the absence of this insertion.

Another significant limitation, which is applicable to PGx testing and interpretation as it is performed today, is that we interpret predicted phenotypes as categories rather than continuous scores and assume the sum of both alleles equals total metabolic capacity (see **Figure 1 F**). For example, for *CYP2D6,* patients are categorized into normal metabolizers (NM), intermediate metabolizers (IM), poor metabolizers (PM) or ultra-rapid metabolizers (UM) based upon their genotype. However, the actual *CYP2D6* phenotype is likely normally distributed. Imposing categorization, as opposed to the interpretation of the actual genotype, therefore sacrifices information in order to simplify clinical interpretation. In the process, we interpret the functionality of each allele individually and assume that the sum of these activity scores equals the total activity of the diplotype, thereby abstracting from potential compensatory effects. Furthermore, these categorizations are currently substrate invariant, even though the effects on metabolic capacity may differ between substrates (46). However, categorization is currently justified due to the lack of evidence to devise pharmacotherapeutic recommendations per diplotype or per substrate. For example, the *CYP2D6* activity score is now set at 0.5 for *CYP2D6**10 for all substrates. However, in reality, the effect on activity scores may be different across substrates. As the field of PGx evolves we foresee that phenotypes will be predicted substrate specifically on a continuous scale, and pharmacotherapeutic recommendations are provided for each value.

In **Chapter 4,** we have selected 46 SNPs and therefore base our phenotype prediction on 0.00092% of all 5 million known SNPs (47) and 0.38% of all known 12,152 variants in 146 genes involved in drug pharmacokinetics (44). Although the presented PGx-Passport has not yet reached maximum predictive utility of genetic variation, due to reasons stated above, we have found a practical solution for determining and interpreting variation. Here we based variant selection on criteria regarding their effect on protein functionality, minor allele frequency (MAF) and association with drug response. We argue that only including variants with known effect on protein functionality is substantiated as including variants of unknown effect in the reported results would provide clinically ambiguous results (41). Additionally, we argued to limit included variants to those with a MAF>1% in at least one ethnicity. Here, we restrict testing to common variants to limit the number of tested variants from an economic perspective. Since many PGx variant alleles vary in frequency across ethnicities (48) and since self-reported ethnicity is not always in agreement with genetic ethnicity (49), it is of clinical importance that the PGx-Passport contains all variant alleles, which are considered common

in at least one defined ethnicity. Lastly, we argued to limit to variants for which association with drug response is determined, regardless of their MAF, as it may be useful to determine these alleles even though the frequency may be low. For example, the DPYD variant alleles *DPYD**2A (MAF<1%), *DPYD**13 (MAF<1%), *DPYD* c.2846A>T (MAF<1%) were selected regardless of their MAF since their association with fluoropyrimidine-induced toxicity has been well-established and adopted clinically. Since the selection was performed on a *-allele level, we would enable determination through sequencing platforms. However, in the case of the PREPARE study, it was not feasible to perform sequencing and therefore we resorted to performing genotyping. To operationalize the PGx-passport on a genotyping platform we, therefore, selected particular variants to represent haplotype blocks. However, one must take special consideration when selecting and interpreting tagging SNPs for *HLA* genotyping since frequencies as linkage disequilibrium (LD) patterns vary across ethnicities. For example, *HLA-B*57:01* may be tested by using tagging SNP rs2395029(T>G). However, while rs2395029(T>G) is in complete LD with *HLA-B*57:01* in Han Chinese, LD is lower in Southeast Asians (50-52). Therefore, this result should be interpreted with caution in certain populations. Further examples are tagging SNPs for *HLA-A*31:01* and *HLA-B*15:02* in Asian populations, which cannot be interpreted in Caucasians due to lower LD (53, 54). In summary, we consider the PGx-passport a minimal list of clinically relevant variant alleles. An advantage of the approach as described in **Chapter 4** is that the number of clinically interpretable results within their PGx-Passport is maximized, while costs remain reasonable.

Future Perspectives: Improving the Predictive Utility of Genetic Variation to Predict Drug Response

The predictive utility of genetics to identify those at risk for intrinsic ADRs is determined by the ability to determine an individual's genetic variation and, subsequently, the ability to accurately predict protein functionality.

Recent advances have been made to improve the ability to determine an individual's genetic variation. Technical limitations regarding the sequencing of complex loci may be overcome by advances in long-read sequencing technologies and synthetic long-read assembly (55). As a result, an increasing number of variants with unknown functionality will need to be interpreted. Due to the larger number of rare variants, it is impossible to determine functionality in expression systems. To overcome this challenge, advances have been made in the development of *in silico* methods to predict functionality. However, these methods are based on genes that are evolutionarily highly conserved. Since many ADME genes are only poorly conserved, steps have been taken to calibrate *in silico* models on datasets (56). Nonetheless, these models still do not enable prediction of the functionality of synonymous mutations, intronic variants or variants in non-coding regions of the genome. An exciting initiative has provided an alternative method for the interpretation of variants with unknown functionality using machine learning, more specifically with a neural network (57).

Here, the investigators trained a neural network model on the long-read sequencing profiles of *CYP2D6* of 561 patients and used the metabolic ratio between tamoxifen and endoxifen as an outcome measure. The model explains 79% of the interindividual variability in CYP2D6 activity compared to 55% with the conventional categorization approach. Additionally, this model is capable of assigning accurate enzyme activity to alleles containing previously uncharacterized combinations of variants.

Due to the different underlying pharmacology of intrinsic (pharmacokinetic and -dynamic) and idiosyncratic ADRs, approaches to translate variants of unknown functionality to usable clinical phenotypes may also be specific to the underlying pharmacology.

Intrinsic Pharmacokinetic ADRs

Currently, phenotypes are predicted in a categorical approach. However, we may expect enzyme activity to be normally distributed within a population and therefore better described by a continuous phenotype scale. We envision a future where phenotypes can be predicted more precisely by using all of an individual's genetic variation, as opposed to limiting our view only to those variants included in a tested panel. Following a further understanding of the effects of individual variants to inform phenotype prediction on a continuous scale, we can imagine that this phenotype prediction will ultimately become substrate-specific as opposed to simply gene-specific. More fundamentally, in PGx we currently limit our view to a single DGI, while multiple genes may be involved in the metabolism of drugs and their metabolites. If we were to expand our view to multiple genes involved to predict drug response, the predictive utility will further improve. To incorporate genetic variations of multiple genes polygenic risk scores may provide useful (58).

Subsequent to improved prediction of genetic variation and interpretation, the potential utility of correct phenotype prediction is determined by a number of factors including both the extent to which the enzyme determines drug exposure and the extent at which drug exposure is associated with a particular drug response. In current PGx research, blood plasma levels are often used as a surrogate endpoint for drug response. The underlying rationale here is that drug response is assumed to be a function of receptor occupancy in a specific tissue. However, receptors may be expressed in multiple tissues and may be expressed in varying densities within these tissues. Additionally, there may be interindividual differences in receptor expression across tissues, impacting the risk of intrinsic off-target ADRs, ADRs as a result of over efficacy and the lack of efficacy (see **Figure 1 A**). However, in current PGx research, we do not take into account interindividual differences in receptor expression across tissues. Therefore, it may be of interest to further clarify the interindividual relationship between blood plasma level, receptor occupancy across tissues and drug response to validate the utility of using blood plasma levels in PGx discovery. For example, *CYP2D6* phenotype may correctly predict plasma blood levels of endoxifen among patients treated with tamoxifen for adjuvant breast cancer. However, this does not seem to be

associated with clinical outcome (14). Therefore, the predictive utility of *CYP2D6*, in this case, is not substantiated.

Since we are not fully able to predict drug response with the PGx-passport as described in **Chapter 4**, we question ourselves to what extent the genetic component of drug response should be predictive of drug response to be useful. Theoretically, this is determined by the proportion of variation in drug response explained by genetics. For example, the genetic component of *CYP2D6* in metabolism on metoprolol pharmacokinetics is 91% (59). If we assume this is also the case for tamoxifen metabolism, then explaining 79% of the interindividual variability by using a neural network model (57) explains the majority of genetic variation. Whether explaining the remaining missing hereditability of 21% is clinically relevant will depend on a number of factors including the width of the therapeutic window, interpatient variability of plasma levels and the severity of the associated ADRs and therefore will potentially be determined per individual DGI.

Intrinsic Pharmacodynamic ADRs

In contrast to pharmacokinetic ADRs, pharmacodynamic ADRs often have a monogenetic association between receptor and ligand. Therefore, genetic variation underlying pharmacodynamic ADRs may be much easier to interpret. Potentially, a neural network could also be deployed to determine the functionality of variants. Here, one could use the ratio between bound and unbound ligand as a phenotype measured on a continuous scale.

Idiosyncratic ADRs

The biological mechanism underlying hypersensitivity reactions is yet undetermined. However, associations discovered until now are mono-variant and have a high effect size. Based on this, one could expect that future variants associated with hypersensitivity reactions will also be mono-variant and therefore do not follow a continuous phenotype scale, but rather a dichotomous scale.

Future Perspectives: Incorporating Predictive Utility of Other Determinants to Predict Drug Response

Although genetics is considered the causal anchor of biological processes (60), the biological mechanism underlying drug response may be downstream of a genetic variant. In these cases, genetics will have no predictive utility for drug response (see **Figure 1 J**). Therefore, incorporating processes downstream of the genome, such as the epigenome (61), transcriptome, microbiome (62), and metabolome (63), may further optimize our ability to predict drug response to enable more accurate stratification of patient populations. Combining these profiles in a systems medicine approach may have a synergistic effect.

Ability to Adjust Pharmacotherapy to Optimize Outcomes

In addition to the predictive utility of genetic variation to predict drug response, the second component determining the utility of precision medicine using PGx guided pharmacotherapy is our ability to adjust pharmacotherapy to reduce the risk of unwanted effects among these individuals. Currently, we have two tools available to reduce the risk of ADRs and lack of efficacy: 1) selecting another drug and 2) adjusting the dose (see **Figure 1 H**).

A successful example of choosing an alternative therapy to avoid an ADR is pre-emptive testing for *HLA-B 57:01* to guide drug selection for abacavir or another antiretroviral therapy that has an effect size of OR=0.03 95% CI [0.00-0.18] in reducing the risk of immunologically-confirmed hypersensitivity. Here, 0% of the prospectively screened group vs 2.7% of the control group experienced immunologically-confirmed hypersensitivity (5). In this example, the PGx intervention and subsequent adjustment completely eliminated the risk of hypersensitivity. This was due to the 100% sensitivity of the PGx test and the 100% efficacy of the pharmacotherapy adjustment. However, it is undetermined whether both the risk of ADRs other than hypersensitivity and the efficacy of the alternative antiretrovirals were similar. The safety and efficacy of the alternative dose or therapy should be kept in consideration when determining the utility of PGx testing.

An example of adjusting the dose to reduce the risk of ADRs is pre-emptive testing for *TPMT* to guide dose selection of thiopurines in reducing the risk of severe hematologic ADRs (4). In contrast to the previously stated example, this PGx intervention has a lower effect size of RR=0.11 95% CI [0.01-0.85]. Here, severe hematologic ADRs still occurred in 2.6% of TPMT variant carriers who received an adjusted dose based, compared to the 22.9% of TPMT variant carriers without an adjusted dose. Although dose adjustment prevented ~89% of severe hematologic ADRs, the remaining ~11% could not be prevented by this intervention. Indeed, this could be partially explained by the sensitivity of *TPMT* testing not reaching 100%, but could also due to the fact that dose reduction was not sufficient for avoiding this ADR. Furthermore, the incidence of severe hematological ADRs among non-carriers of TPMT variants was 7.3%. This indicates that other (genetic) factors may play a role in the risk of severe hematological ADRs. Opportunely, we have been able to improve dosing interventions to optimize outcomes. For example, a previous version of the DPWG guideline as described in **Chapter 5**, was updated to a further dose reduction for patients with *DPYD* GAS 1.5 to further decrease their risk of severe fluoropyrimidine-toxicity. Initially, those with a *DPYD* GAS 1.5 were to receive 75% of the normal fluoropyrimidine starting dose. However, a prospective study showed that among patients carrying c.2846A>T and c.1236G>A/HapB3 variants the 75% starting dose was inadequate to reduce the risk of toxicity. The updated to 50% was based on the median DPD enzyme activity and observed subsequent dose reductions in this prospective trial. With dose adjustments, one may superficially expect

under-dosing when decreasing a dose to prevent toxicity. This has been reported to lead to resistance to adhering to DPWG guidelines. Nevertheless, since the DPWG dose adjustments are calculated based on pharmacokinetic studies, the anticipated drug exposure among those with variant genotypes treated with a guideline-recommended lowered dose, are expected to have similar drug exposures to those with wildtype genotypes treated with normal doses. This was confirmed in a pharmacokinetic sub-study among patients carrying *DPYD* variants who were treated with a lower fluoropyrimidine dose (13). Still, one could argue that decreased efficacy is a significant outcome that should be measured, to place the results into context. Potentially, this could be inferred from surrogate endpoints, such as drug discontinuation, within the PREPARE study.

Future Perspectives: Ability to Adjust Pharmacotherapy to Optimize Outcomes

In the future, pharmacotherapy adjustment may be further improved by imminent technologies such as 3D-printing to enable personalized dosing and delivery (64). Currently, the DPWG calculates specific dose adjustments based on pharmacokinetic studies and rounds the recommended dose to the nearest corresponding marketed dose for clinical feasibility. The utilization of 3D-printing technologies may enable rapid compounding of tablets with a specific dose based on an individual's genetic profile. In any case, adjustment of the pharmacotherapy will always be limited by the safety profile of available drugs. Opportunely, over the last decades, newly developed drugs have been shifting from unspecific small molecules to more targeted drugs in the form of humanized monoclonal antibodies (65), cell therapies (66) and gene therapies (67). Therefore, as a general trend, on the one hand, the risk of off-target ADRs is decreasing thereby yielding safer drugs. On the other hand, the more personalized application of drugs in narrower diagnoses is yielding more efficacious drugs.

Part III: Evaluating the Implementation Process

Over the last years, a number of barriers preventing PGx implementation have been addressed. However, despite this progress, a number of remaining hurdles are hampering implementation. Previously surmounted barriers include the generation of gold-standard evidence demonstrating clinical utility or individual DGIs, the development of guidelines directing the use of PGx results to guide dose and drug selection (68-71) (**Chapter 4**), the incorporation of PGx information in drug labels (72), selection of clinically relevant variants (73-75) (**Chapter 5**) and the initiation of multiple implementation studies (16, 76) generating evidence and incorporating PGx results into clinical workflows (77-79). In **Chapter 8** we identified pharmacists reported remaining barriers of implementation. These included unclear procedures regarding timing and methodology of testing, inefficiencies in recording PGx results for future use, lacking health care provider (HCP) PGx knowledge and awareness and undetermined reimbursement for PGx test and consult. A number of these remaining

barriers and future perspectives on how these may be overcome will be discussed among the following sections.

Optimal Timing and Methodology

Significant debate persists regarding the optimal timing and methodology of testing for delivering PGx testing in clinical care (76) (see **Figure 1 D**). Some support a pre-therapeutic single gene-drug approach, in which a PGx test of a single relevant gene is ordered once a target drug is prescribed; while others advocate for a pre-emptive panel-based strategy in which multiple genes are tested simultaneously and saved for later use, in preparation of future prescriptions throughout a patient's lifetime (80). When combined with a clinical decision support system (CDSS), the corresponding PGx guideline can be deployed by the CDSS at the point of care, thereby providing clinicians with the necessary information to optimize drug prescribing, when a target drug is prescribed. This is deemed useful since patients will receive multiple drug prescriptions with potential DGIs within their lifetime (76, 81). It has been estimated that half of the patients above 65 years will use at least one of the drugs for which PGx guidelines are available during a four year period, and one fourth to one third, will use two or more of these drugs (82). Logistics and cost-effectiveness are therefore optimized when delivered in a pre-emptive panel-based approach; pharmacotherapy does not have to be delayed, in awaiting single gene testing results and costs for genotyping are minimized, as marginal acquisition costs of testing and interpreting additional pharmacogenes is near-zero (18). However, the clinical utility of panel-based PGx testing must be demonstrated before wide-spread implementation is substantiated. In the meantime, sufficient evidence supports the implementation of a number of individual DGIs (83, 84). To support the uptake of these reactive tests, we present the clinical implication score (85) (**Chapter 4**), which directs the testing of "essential" genes before initiation of an interacting drug.

The timing at which panel-based testing should be performed is also yet undetermined. A panel test could be performed when no drug initiation is indicated, in preparation of future drug prescriptions. In this case, PGx testing would be a waste of resources if no drug would be initiated in the near future. Alternatively, panel testing could be performed once a patient plans to initiate a drug for which PGx testing may be useful and re-use these results when future DGIs are encountered. This model was deployed in **Chapter 7**, where pharmacists requested a PGx panel test when patients planned to initiate one of ten drugs for which PGx guidelines are available. Here, 97% of patients to (re)used PGx-panel results for at least one, and 33% for up to four newly initiated prescriptions with possible DGIs within a 2.5-year follow-up. In this case, 24% were actionable DGIs, requiring pharmacotherapy adjustment. This high rate of re-use indicates this may be a promising model for delivering PGx panel-based testing. As an alternative model, another initiative has

utilized a prediction model to select patients who may benefit from PGx testing in the near future algorithmically and using prescription data (86, 87).

In addition to undetermined timing and methodology, the most optimal target group for testing is also yet undetermined. Current studies have identified potential patient sub-groups for which pre-emptive PGx panel testing may be most useful. Some initiatives have selected patients with particular indications in psychiatry (88-91). Others have selected patients with particular characteristics such as polypharmacy and elderly patients (78, 92). In Chapter 7, we performed an exploratory analysis to identify patient characteristics associated with increased use of PGx panel results. It seemed that the number of newly initiated prescriptions increased with age, number of comorbidities and number of comedications, but this could not be statistically concluded. A larger sample size and a threshold defining usefulness would be required to identify subgroups based on these baseline characteristics.

Alternatively, consumers who have an interest in their PGx profile may also obtain their PGx test results outside the realm of healthcare and without the intervention of an HCP. In 2018, direct-to-consumer (DTC) PGx testing was approved by the US Food and Drug Administration (FDA). However, in contrast to DTC tests provided before 2013, the FDA has approved only a limited scope of 33 variants in 8 genes, and providers have mandated the need to retest. Concerns of DTC PGx testing have been reported to relate to patient actions (e.g., to stop taking a prescribed medication or adjusting the regimen based on genotype without consultation with a health provider) (93). However, a longitudinal study of DTC consumers showed that only 5.6% of consumers reported changing a medication they were taking or starting a new medication due to their PGx results. Of these, 45 (83.3%) reported consulting with an HCP regarding the change (94). Nonetheless, the involvement of HCPs will optimize the use of PGx results when delivered in a DTC setting. In the same longitudinal study, (Chapter 6) we found that 63% of consumers planned to share their results with a primary care provider. However, at 6-month follow-up, only 27% reported having done so, and 8% reported sharing with another HCP. Among participants who discussed results with their PCP, 35% were very satisfied with the encounter, and 18% were not at all satisfied. These results indicate that PGx testing in a DTC model may be a safe model for obtaining PGx testing.

Future Perspectives: Optimal Timing and Methodology

Consensus regarding who should be tested, and when it is most cost-effective to perform pre-emptive panel testing, remains undetermined (18). Moreover, the most cost-effective technique to determine the PGx profile is also undetermined. As novel DGIs are discovered, it may be more efficient to determine whole-genomes using sequencing techniques, to avoid testing of additional variants through genotyping over time. Clinically relevant PGx variants are currently able to be extracted from sequencing data using

bioinformatics pipelines (95, 96). As the cost of sequencing techniques decrease, genotype-based testing will become obsolete. In this case, it may be more cost-effective to perform population-wide sequencing at birth, to ensure the maximization of instances in which a PGx result is available when a DGI is encountered. Initially, however, sequencing may be indicated by other medical indications and objectives (97, 98). If this is to come into fruition, the determining the cost-effectiveness of implementing PGx testing will become redundant, as the information on PGx variants become secondary findings, free of additional costs.

Recording PGx Panel Results for Future Use

To enable pre-emptive PGx testing, it is imperative that the PGx test results are recorded in the EMR for future use (see **Figure 1 G**). In **Chapter 7** we found that both pharmacists and GPs are very able to record PGx results in their EMRs as contra-indications (96% and 33% of pharmacists and GPs, respectively); enabling the deployment of relevant guidelines by the CDSS when a DGI is encountered both at prescribing and dispensing. An advantage of recording PGx results in both pharmacy and GP EMRs is the minimization of the risk of missing a DGI. As a result, it is not disastrous that GPs also recorded them in other formats, thereby not enabling the CDSS at prescribing, in 35% of cases. In contrast, a recent study showed that genotyping results were sparsely communicated and recorded correctly; only 3.1% and 5.9% of reported genotyping results were recorded by GPs and pharmacists, respectively, within a similar follow-up time of 2.36 years (99). Surprisingly, 1.5% of PGx results were not recorded by pharmacists because they did not include actionable genotypes. However, it is still of importance to document these results to avoid unnecessary re-testing of the patient. Finally, the fact that discrepancies between reported results and the recorded result were only observed in 1% of pharmacy EMR cases, indicates that the current manual system of recording is error-prone. Regardless of the low error rate, PGx results are static and therefore life-long. It is therefore imperative that errors in the recording of PGx results are avoided.

Future Perspectives: Recording PGx Panel Results for Future Use

Future initiatives should focus on the development of automated sharing of PGx results across EMRs. In the Netherlands, such an initiative has been launched but requires patient consent before it can be utilized. The National Exchange Point ("Landelijk Schakel Punt" (LSP)) is a nationwide secured EMR infrastructure to which nearly HCPs access (100). Only when a patient has provided written consent for the LSP, can a professional summary of the local pharmacy or GP EMR, including PGx results, be downloaded by another treating HCP in the same region; unless the patient chose to shield this information. Alternatively, providing the PGx results directly to patients may resolve the issue in terms of communicating and recording PGx results; for example, utilizing the MSC safety-code card as utilized in the PREPARE study (101, 102).

Because the implications and potential actionability of PGx results will often apply to multiple drugs across a patient's lifetime, it is essential to have a system capable of updating interpretations of PGx variants based on the most state of the art literature (103). However, a consensus on how to incorporate such a system into EMRs is undetermined (104). As we shift from determining PGx profile through panel-based testing to genome sequencing and thereby shift from utilizing variants of known significance to variants of unknown significance to determine phenotypes, it may become of utmost importance to record uninterpreted variants in the EMR as opposed to only recording predicted phenotypes.

Part IV: Quantifying the Impact on Patient Outcomes and Cost-Effectiveness

Several small randomized and observational studies indicate the (cost-)effectiveness of PGx panel-based testing in psychiatry and polypharmacy (78, 88, 90, 92), where observed cost savings ranged from $218 (92) to $2,778 (105) per patient. Others have modeled the cost-effectiveness of one-time genetic testing to minimize a lifetime of adverse drug reactions and concluded an incremental cost-effectiveness ratio (ICER) of $43,165 per additional life-year and $53,680 per additional quality-adjusted life-year, and therefore cost-effective (106). However, cost-effectiveness may vary across ethnic populations, as a result of varying in allele frequencies; the target population, as a result of varying prescription patterns; and the healthcare setting, as a result of varying healthcare costs and ICER cost-effectiveness thresholds. Although these studies indicate the potential benefit of PGx panel-based testing, a sufficiently powered prospective study assessing the clinical and cost-effectiveness of pre-emptive PGx-testing is yet to be executed (7).

In the meantime, to stimulate the adoption of single-gene PGx testing, we determined the cost-effectiveness of a sub-set of genes included in the PGx-Passport (**Chapter 9**) covering interactions that are classified as "Essential" according to the DPWG clinical implications score. These DGIs direct pre-therapeutic PGx testing for the relevant gene to guide initial dose and drug selection and concern high-risk drugs and intend to prevent severe clinical consequences such as gene-drug-related death. We found that 148,128 patients initiate at least one of seven drugs per year in the Netherlands. The corresponding PGx-testing of relevant single genes would cost €19.4 million. Of these drug initiators, 35,762 (24.1%) would have an actionable DGI, requiring an alternative dose or drug. HCP discussion of these actionable results would cost €586,000. The extra costs made to initiate a PGx-guided drug is €1.458 million. Overall, costs are therefore €21.4 million. Interestingly, PGx-guided drug costs are cost-saving for all DGIs (range per cost-saving DGI: 0.7%-4.6%), except the clopidogrel-*CYP2C19* interaction where the drug costs are €2.8 million (162%) higher than the standard of care. Remarkably, the costs of drugs saved in the PGx-guided group surmounts the cost of PGx testing and HCP interpretation combined for the irinotecan-*UGT1A1* interaction, making the intervention cost-minimizing and saving €481,000 on drug costs per year. The overall cost PGx-guided initial dose and drug selection

for "essential" DGIs would reduce the overall relative risk of gene-drug-related death with 10.6% (range per DGI: 8.1% – 14.5%) and prevent 419 (0.3% of initiators) deaths a year. The cost of preventing one gene-drug-related death is, therefore, €51,000 (range per DGI: €-752,000 – €633,000).

Future Perspectives: Quantifying the Impact on Patient Outcomes and Cost-Effectiveness

Clinical trials and prospective cohorts typically measure short-term benefits of PGx testing, while the time horizon for the benefit of PGx testing is over a lifetime and therefore unable to be captured within regular trials. As such, the life-long cost-effectiveness of one-time pre-emptive panel-based testing to prevent ADRs is yet undetermined. Other methodologies such as Markov models can be deployed to simulate effectiveness over longer time-horizons. This will be of interest to reimbursement policymakers, who require evidence that panel-based testing will yield downstream improved health outcomes at acceptable costs. Therefore, future research should model the cost-effectiveness of pre-emptive PGx testing to prevent a lifetime of adverse drug events. Optimally, such an analysis could be run on a longitudinal cohort of patients for which both prescription data and PGx results are available. Initial steps are being taken to obtain such data from the Doetinchem cohort. Furthermore, such a dataset could be used to explore the optimal timing and sub-group application of testing to optimize cost-effectiveness.

Conclusion: Precision Medicine Using Pharmacogenomic Panel Testing

The work of this thesis aims to support implementation of precision medicine using PGx panel testing. It reports on the development of tools facilitating implementation (**Part II**) such as a guideline for using PGx results to guide pharmacotherapy and a standardized panel of PGx variants. Subsequently, it evaluates of the implementation process utilizing these tools (**Part III**), underlining the importance of overcoming remaining implementation barriers, such as the generation of evidence supporting PGx panel testing, clarifying the most optimal timing and methodology for delivering and recording PGx results, improving acceptance and knowledge and acquiring reimbursement. Proof-of-concept evidence for precision medicine using PGx panel testing will be primarily generated through the PREPARE study (**Part I**) Currently, over 6,500 patients have been enrolled and the trial is aiming to report by the end of 2020. In the meantime, we have quantified the impact of PGx implementation on patient outcomes and cost-effectiveness for a subset of DGIs (**Part IV**). Depending on the trial results, this evidence set may help overcome the remaining implementation barriers to make precision medicine using PGx panel testing a reality for all European citizens and beyond.

In the coming years and decades, the utility of precision medicine using PGx guided pharmacotherapy will be further optimized as a result of technological developments improving the predictive utility of genetic variation to predict drug response and developments improving our ability to adjust pharmacotherapy to reduce risk of unwanted

effects among high-risk individuals. Additionally, the utility of precision medicine will be further improved by combining other determinants of drug response in prediction models. In parallel, implementation and evidence generation supporting improved precision medicine approaches can be generated concomitantly by combining digital medicine and innovative study designs within learning health care systems. In conclusion, these developments will revolutionize current stratified medicine to enable true personalized medicine.

REFERENCES

1. Pirmohamed M, Burnside G, Eriksson N, Jorgensen AL, Toh CH, Nicholson T, et al. A randomized trial of genotype-guided dosing of warfarin. The New England journal of medicine. 2013;369(24):2294-303.
2. Wu AH. Pharmacogenomic testing and response to warfarin. Lancet (London, England). 2015;385(9984).
3. Verhoef TI, Ragia G, de Boer A, Barallon R, Kolovou G, Kolovou V, et al. A randomized trial of genotype-guided dosing of acenocoumarol and phenprocoumon. The New England journal of medicine. 2013;369(24):2304-12.
4. Coenen MJ, de Jong DJ, van Marrewijk CJ, Derijks LJ, Vermeulen SH, Wong DR, et al. Identification of Patients With Variants in TPMT and Dose Reduction Reduces Hematologic Events During Thiopurine Treatment of Inflammatory Bowel Disease. Gastroenterology. 2015;149(4):907-17.e7.
5. Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, et al. HLA-B*5701 screening for hypersensitivity to abacavir. The New England journal of medicine. 2008;358(6).
6. Claassens DMF, Vos GJA, Bergmeijer TO, Hermanides RS, van 't Hof AWJ, van der Harst P, et al. A Genotype-Guided Strategy for Oral P2Y12 Inhibitors in Primary PCI. The New England journal of medicine. 2019.
7. Janssens AC, Deverka PA. Useless until proven effective: the clinical utility of preemptive pharmacogenetic testing. Clinical pharmacology and therapeutics. 2014;96(6):652-4.
8. Altman RB. Pharmacogenomics: "noninferiority" is sufficient for initial implementation. Clinical pharmacology and therapeutics. 2011;89(3):348-50.
9. van der Wouden CH, Swen JJ, Schwab M, Mitropoulou C, Schwab M, Guchelaar H-J. A brighter future for the implementation of pharmacogenomic testing. Eur J Hum Genet. 2016.
10. Pirmohamed M, Hughes DA. Pharmacogenetic tests: the need for a level playing field. Nature reviews Drug discovery. 2013;12(1):3-4.
11. Khoury MJ. Dealing with the evidence dilemma in genomics and personalized medicine. Clinical pharmacology and therapeutics. 2010;87(6):635-8.
12. Pletcher MJ, McCulloch CE. The Challenges of Generating Evidence to Support Precision Medicine. JAMA Internal Medicine. 2017.
13. Henricks LM, Lunenburg C, de Man FM, Meulendijks D, Frederix GWJ, Kienhuis E, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. The Lancet Oncology. 2018;19(11):1459-67.
14. Sanchez-Spitman A, Dezentje V, Swen J, Moes D, Bohringer S, Batman E, et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 2019;37(8):636-46.
15. Relling MV, Altman RB, Goetz MP, Evans WE. Clinical implementation of pharmacogenomics: overcoming genetic exceptionalism. The Lancet Oncology. 2010;11(6).
16. van der Wouden CH, Cambon-Thomsen A, Cecchin E, Cheung KC, Dávila-Fajardo CL, Deneer VH, et al. Implementing Pharmacogenomics in Europe: Design and Implementation Strategy of the Ubiquitous Pharmacogenomics Consortium. Clinical pharmacology and therapeutics. 2017;101(3):341-58.

17. Manson LE, van der Wouden CH, Swen JJ, Guchelaar H-JJ. The Ubiquitous Pharmacogenomics consortium: making effective treatment optimization accessible to every European citizen. Pharmacogenomics. 2017;18(11):1041-5.
18. Roden DM, Van Driest SL, Mosley JD, Wells QS, Robinson JR, Denny JC, et al. Benefit of Preemptive Pharmacogenetic Information on Clinical Outcome. Clinical pharmacology and therapeutics. 2018;103(5):787-94.
19. Chambers DA, Feero WG, Khoury MJ. Convergence of Implementation Science, Precision Medicine, and the Learning Health Care System: A New Model for Biomedical Research. Jama. 2016;315(18):1941-2.
20. Lu CY, Williams MS, Ginsburg GS, Toh S, Brown JS, Khoury MJ. A proposed approach to accelerate evidence generation for genomic-based technologies in the context of a learning health system. Genetics in medicine : official journal of the American College of Medical Genetics. 2018;20(4):390-6.
21. Peterson JF, Roden DM, Orlando LA, Ramirez AH, Mensah GA, Williams MS. Building evidence and measuring clinical outcomes for genomic medicine. Lancet (London, England). 2019;394(10198):604-10.
22. Turnbull C, Scott RH, Thomas E, Jones L, Murugaesu N, Pretty FB, et al. The 100 000 Genomes Project: bringing whole genome sequencing to the NHS. BMJ (Clinical research ed). 2018;361:k1687.
23. Gottesman O, Scott SA, Ellis SB, Overby CL, Ludtke A, Hulot JS, et al. The CLIPMERGE PGx Program: clinical implementation of personalized medicine through electronic health records and genomics-pharmacogenomics. Clinical pharmacology and therapeutics. 2013;94(2):214-7.
24. Leitsalu L, Haller T, Esko T, Tammesoo ML, Alavere H, Snieder H, et al. Cohort Profile: Estonian Biobank of the Estonian Genome Center, University of Tartu. International journal of epidemiology. 2015;44(4):1137-47.
25. Collins FS, Varmus H. A new initiative on precision medicine. The New England journal of medicine. 2015;372(9):793-5.
26. Khoury MJ, Rich EC, Randhawa G, Teutsch SM, Niederhuber J. Comparative effectiveness research and genomic medicine: an evolving partnership for 21st century medicine. Genetics in medicine : official journal of the American College of Medical Genetics. 2009;11(10):707-11.
27. Ford I, Norrie J. Pragmatic Trials. The New England journal of medicine. 2016;375(5):454-63.
28. Fiore LD, Lavori PW. Integrating Randomized Comparative Effectiveness Research with Patient Care. The New England journal of medicine. 2016;374(22):2152-8.
29. Weinfurt KP, Hernandez AF, Coronado GD, DeBar LL, Dember LM, Green BB, et al. Pragmatic clinical trials embedded in healthcare systems: generalizable lessons from the NIH Collaboratory. BMC Med Res Methodol. 2017;17(1):144-.
30. Brunette CA, Miller SJ, Majahalme N, Hau C, MacMullen L, Advani S, et al. Pragmatic Trials in Genomic Medicine: The Integrating Pharmacogenetics In Clinical Care (I-PICC) Study. Clinical and translational science. 2019:10.1111/cts.12723.
31. Roden DM, McLeod HL, Relling MV, Williams MS, Mensah GA, Peterson JF, et al. Pharmacogenomics. Lancet (London, England). 2019;394(10197):521-32.
32. Sim I. Mobile Devices and Health. The New England journal of medicine. 2019;381(10):956-68.

33. Coravos A, Khozin S, Mandl KD. Developing and adopting safe and effective digital biomarkers to improve patient outcomes. NPJ Digit Med. 2019;2(1):14.
34. Mindstrong Health and Takeda Partner to Explore Development of Digital Biomarkers for Mental Health Conditions 2018 [Available from: https://www.prnewswire.com/news-releases/mindstrong-health-and-takeda-partner-to-explore-development-of-digital-biomarkers-for-mental-health-conditions-300604553.html].
35. Lipsmeier F, Taylor KI, Kilchenmann T, Wolf D, Scotland A, Schjodt-Eriksen J, et al. Evaluation of smartphone-based testing to generate exploratory outcome measures in a phase 1 Parkinson's disease clinical trial. Mov Disord. 2018;33(8):1287-97.
36. Clay I. Impact of Digital Technologies on Novel Endpoint Capture in Clinical Trials. Clinical pharmacology and therapeutics. 2017;102(6):912-3.
37. Haendel MA, Chute CG, Robinson PN. Classification, Ontology, and Precision Medicine. The New England journal of medicine. 2018;379(15):1452-62.
38. Miksad RA, Samant MK, Sarkar S, Abernethy AP. Small But Mighty: The Use of Real-World Evidence to Inform Precision Medicine. Clinical pharmacology and therapeutics. 2019;106(1):87-90.
39. Schork NJ. Personalized medicine: Time for one-person trials. Nature. 2015;520(7549):609-11.
40. Kim J, Hu C, Moufawad El Achkar C, Black LE, Douville J, Larson A, et al. Patient-Customized Oligonucleotide Therapy for a Rare Genetic Disease. The New England journal of medicine. 2019;381(17):1644-52.
41. Drogemoller BI, Wright GE, Warnich L. Considerations for rare variants in drug metabolism genes and the clinical implications. Expert opinion on drug metabolism & toxicology. 2014;10(6):873-84.
42. Li B, Seligman C, Thusberg J, Miller JL, Auer J, Whirl-Carrillo M, et al. In silico comparative characterization of pharmacogenomic missense variants. BMC genomics. 2014;15 Suppl 4:S4.
43. Zhou Y, Mkrtchian S, Kumondai M, Hiratsuka M, Lauschke VM. An optimized prediction framework to assess the functional impact of pharmacogenetic variants. The pharmacogenomics journal. 2018.
44. Kozyra M, Ingelman-Sundberg M, Lauschke VM. Rare genetic variants in cellular transporters, metabolic enzymes, and nuclear receptors can be important determinants of interindividual differences in drug response. Genetics in medicine : official journal of the American College of Medical Genetics. 2016.
45. Weinshilboum RM, Sladek SL. Mercaptopurine pharmacogenetics: monogenic inheritance of erythrocyte thiopurine methyltransferase activity. American journal of human genetics. 1980;32(5):651-62.
46. Hicks JK, Swen JJ, Gaedigk A. Challenges in CYP2D6 phenotype assignment from genotype data: a critical assessment and call for standardization. Current drug metabolism. 2014;15(2):218-32.
47. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, et al. A global reference for human genetic variation. Nature. 2015;526(7571):68-74.
48. Zhou Y, Ingelman–Sundberg M, Lauschke VM. Worldwide Distribution of Cytochrome P450 Alleles: A Meta-analysis of Population-scale Sequencing Projects. Clinical Pharmacology & Therapeutics. 2017.

49. Shraga R, Yarnall S, Elango S, Manoharan A, Rodriguez SA, Bristow SL, et al. Evaluating genetic ancestry and self-reported ethnicity in the context of carrier screening. BMC genetics. 2017;18(1):99.
50. Melis R, Lewis T, Millson A, Lyon E, McMillin GA, Slev PR, et al. Copy number variation and incomplete linkage disequilibrium interfere with the HCP5 genotyping assay for abacavir hypersensitivity. Genetic testing and molecular biomarkers. 2012;16(9):1111-4.
51. Rodriguez-Novoa S, Cuenca L, Morello J, Cordoba M, Blanco F, Jimenez-Nacher I, et al. Use of the HCP5 single nucleotide polymorphism to predict hypersensitivity reactions to abacavir: correlation with HLA-B*5701. The Journal of antimicrobial chemotherapy. 2010;65(8):1567-9.
52. Badulli C, Sestini R, Sbarsi I, Baroncelli M, Pizzochero C, Martinetti M, et al. Tag SNPs of the ancestral haplotype 57.1 do not substitute HLA-B*57:01 typing for eligibility to abacavir treatment in the Italian population. Pharmacogenomics. 2012;13(3):247-9.
53. de Bakker PI, McVean G, Sabeti PC, Miretti MM, Green T, Marchini J, et al. A high-resolution HLA and SNP haplotype map for disease association studies in the extended human MHC. Nature genetics. 2006;38(10):1166-72.
54. Amstutz U, Ross CJ, Castro-Pastrana LI, Rieder MJ, Shear NH, Hayden MR, et al. HLA-A 31:01 and HLA-B 15:02 as genetic markers for carbamazepine hypersensitivity in children. Clinical pharmacology and therapeutics. 2013;94(1):142-9.
55. Lauschke VM, Ingelman-Sundberg M. How to Consider Rare Genetic Variants in Personalized Drug Therapy. Clinical pharmacology and therapeutics. 2018;103(5):745-8.
56. Zhou Y, Mkrtchian S, Kumondai M, Hiratsuka M, Lauschke VM. An optimized prediction framework to assess the functional impact of pharmacogenetic variants. The pharmacogenomics journal. 2019;19(2):115-26.
57. van der Lee M, Allard WG, Vossen RHAM, Baak-Pablo RF, Menafra R, Deiman BALM, et al. A unifying model to predict variable drug response for personalised medicine. 2020:2020.03.02.967554.
58. Gibson G. On the utilization of polygenic risk scores for therapeutic targeting. PLoS Genet. 2019;15(4):e1008060-e.
59. Matthaei J, Brockmoller J, Tzvetkov MV, Sehrt D, Sachse-Seeboth C, Hjelmborg JB, et al. Heritability of metoprolol and torsemide pharmacokinetics. Clinical pharmacology and therapeutics. 2015;98(6):611-21.
60. Watson JD, Crick FH. Genetical implications of the structure of deoxyribonucleic acid. Nature. 1953;171(4361):964-7.
61. Lauschke VM, Zhou Y, Ingelman-Sundberg M. Novel genetic an epigenetic factors of importance for inter-individual differences in drug disposition, response and toxicity. Pharmacology & therapeutics. 2019.
62. Sun L, Xie C, Wang G, Wu Y, Wu Q, Wang X, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. Nature medicine. 2018;24(12):1919-29.
63. Kaddurah-Daouk R, Weinshilboum R. Metabolomic Signatures for Drug Response Phenotypes: Pharmacometabolomics Enables Precision Medicine. Clinical pharmacology and therapeutics. 2015;98(1):71-5.
64. Afsana, Jain V, Haider N, Jain K. 3D Printing in Personalized Drug Delivery. Current pharmaceutical design. 2018;24(42):5062-71.
65. Hansel TT, Kropshofer H, Singer T, Mitchell JA, George AJT. The safety and side effects of monoclonal antibodies. Nature reviews Drug discovery. 2010;9(4):325-38.

66. Jackson HJ, Rafiq S, Brentjens RJ. Driving CAR T-cells forward. Nature reviews Clinical oncology. 2016;13(6):370-83.
67. Naldini L. Gene therapy returns to centre stage. Nature. 2015;526(7573):351-60.
68. Swen JJ, Nijenhuis M, de Boer A, Grandia L, der Zee M-vAH, Mulder H, et al. Pharmacogenetics: from bench to byte--an update of guidelines. Clinical pharmacology and therapeutics.89(5).
69. Swen JJ, Wilting I, de Goede AL, Grandia L, Mulder H, Touw DJ, et al. Pharmacogenetics: from bench to byte. Clinical pharmacology and therapeutics.83(5).
70. Relling MV, Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the Pharmacogenomics Research Network. Clinical pharmacology and therapeutics. 2011;89(3).
71. Relling MV, Klein TE, Gammal RS, Whirl-Carrillo M, Hoffman JM, Caudle KE. The Clinical Pharmacogenetics Implementation Consortium: 10 Years Later. Clinical pharmacology and therapeutics. 2020;107(1):171-5.
72. Ehmann F, Caneva L, Prasad K, Paulmichl M, Maliepaard M, Llerena A, et al. Pharmacogenomic information in drug labels: European Medicines Agency perspective. The pharmacogenomics journal. 2015;15(3):201-10.
73. van der Wouden CH, van Rhenen MH, Jama WOM, Ingelman-Sundberg M, Lauschke VM, Konta L, et al. Development of the PGx-Passport: A Panel of Actionable Germline Genetic Variants for Pre-Emptive Pharmacogenetic Testing. Clinical pharmacology and therapeutics. 2019.
74. Caudle KE, Keeling NJ, Klein TE, Whirl-Carrillo M, Pratt VM, Hoffman JM. Standardization can accelerate the adoption of pharmacogenomics: current status and the path forward. Pharmacogenomics. 2018;19(10):847-60.
75. Pratt VM, Del Tredici AL, Hachad H, Ji Y, Kalman LV, Scott SA, et al. Recommendations for Clinical CYP2C19 Genotyping Allele Selection: A Report of the Association for Molecular Pathology. The Journal of molecular diagnostics : JMD. 2018;20(3):269-76.
76. Dunnenberger HM, Crews KR, Hoffman JM, Caudle KE, Broeckel U, Howard SC, et al. Preemptive clinical pharmacogenetics implementation: current programs in five US medical centers. Annual review of pharmacology and toxicology. 2015;55:89-106.
77. O'Donnell PH, Wadhwa N, Danahey K, Borden BA, Lee SM, Hall JP, et al. Pharmacogenomics-Based Point-of-Care Clinical Decision Support Significantly Alters Drug Prescribing. Clinical pharmacology and therapeutics. 2017;102(5):859-69.
78. Elliott LS, Henderson JC, Neradilek MB, Moyer NA, Ashcraft KC, Thirumaran RK. Clinical impact of pharmacogenetic profiling with a clinical decision support tool in polypharmacy home health patients: A prospective pilot randomized controlled trial. PLOS ONE. 2017;12(2).
79. Van Driest SL, Shi Y, Bowton EA, Schildcrout JS, Peterson JF, Pulley J, et al. Clinically actionable genotypes among 10,000 patients with preemptive pharmacogenomic testing. Clinical pharmacology and therapeutics. 2014;95(4):423-31.
80. Weitzel KW, Cavallari LH, Lesko LJ. Preemptive Panel-Based Pharmacogenetic Testing: The Time is Now. Pharmaceutical Research. 2017;34(8):1551-5.
81. Driest VSL, Shi Y, Bowton EA, Schildcrout JS, Peterson JF, Pulley J, et al. Clinically actionable genotypes among 10,000 patients with preemptive pharmacogenomic testing. Clinical pharmacology and therapeutics.95(4).

82. Samwald M, Xu H, Blagec K, Empey PE, Malone DC, Ahmed SM, et al. Incidence of Exposure of Patients in the United States to Multiple Drugs for Which Pharmacogenomic Guidelines Are Available. PloS one. 2016;11(10).
83. Lunenburg CA, Henricks LM, Guchelaar H-JJ, Swen JJ, Deenen MJ, Schellens JH, et al. Prospective DPYD genotyping to reduce the risk of fluoropyrimidine-induced severe toxicity: Ready for prime time. European journal of cancer (Oxford, England : 1990). 2016;54:40-8.
84. Sanderson JD. TPMT Testing Before Starting Azathioprine or Mercaptopurine: Surely Just Do It? Gastroenterology. 2015;149(4):850-3.
85. Swen JJ, Nijenhuis M, van Rhenen M, de Boer-Veger NJ, Buunk A-MM, Houwink EJFJF, et al. Pharmacogenetic Information in Clinical Guidelines: The European Perspective. Clinical pharmacology and therapeutics. 2018;103(5):795-801.
86. Pulley JM, Denny JC, Peterson JF, Bernard GR, Jones CL, Ramirez AH, et al. Operational implementation of prospective genotyping for personalized medicine: the design of the Vanderbilt PREDICT project. Clinical Pharmacology & Therapeutics. 2012;92(1):87-95.
87. Grice GR, Seaton TL, Woodland AM, McLeod HL. Defining the opportunity for pharmacogenetic intervention in primary care. Pharmacogenomics. 2006;7(1):61-5.
88. Pérez V, Salavert A, Espadaler J, Tuson M, Saiz-Ruiz J, Sáez-Navarro C, et al. Efficacy of prospective pharmacogenetic testing in the treatment of major depressive disorder: results of a randomized, double-blind clinical trial. BMC psychiatry. 2017;17(1):250.
89. Bradley P, Shiekh M, Mehra V, Vrbicky K, Layle S, Olson MC, et al. Improved efficacy with targeted pharmacogenetic-guided treatment of patients with depression and anxiety: A randomized clinical trial demonstrating clinical utility. Journal of psychiatric research. 2018;96:100-7.
90. Espadaler J, Tuson M, Lopez-Ibor JM, Lopez-Ibor F, Lopez-Ibor MI. Pharmacogenetic testing for the guidance of psychiatric treatment: a multicenter retrospective analysis. CNS spectrums. 2017;22(4):315-24.
91. Walden LM, Brandl EJ, Tiwari AK, Cheema S, Freeman N, Braganza N, et al. Genetic testing for CYP2D6 and CYP2C19 suggests improved outcome for antidepressant and antipsychotic medication. Psychiatry research. 2018.
92. Brixner D, Biltaji E, Bress A, Unni S, Ye X, Mamiya T, et al. The effect of pharmacogenetic profiling with a clinical decision support tool on healthcare resource utilization and estimated costs in the elderly exposed to polypharmacy. Journal of medical economics. 2016;19(3):213-28.
93. Haga SB. Managing Increased Accessibility to Pharmacogenomic Data. Clinical pharmacology and therapeutics. 2019;106(5):922-4.
94. Carere DA, VanderWeele TJ, Vassy JL, van der Wouden CH, Roberts JS, Kraft P, et al. Prescription medication changes following direct-to-consumer personal genomic testing: findings from the Impact of Personal Genomics (PGen) Study. Genetics in medicine : official journal of the American College of Medical Genetics. 2017;19(5):537-45.
95. Yang W, Wu G, Broeckel U, Smith CA, Turner V, Haidar CE, et al. Comparison of genome sequencing and clinical genotyping for pharmacogenes. Clinical pharmacology and therapeutics. 2016;100(4).

96. van der Lee M, Allard WG, Bollen S, Santen GWE, Ruivenkamp CAL, Hoffer MJV, et al. Repurposing of Diagnostic Whole Exome Sequencing Data of 1,583 Individuals for Clinical Pharmacogenetics. Clinical pharmacology and therapeutics. 2019:10.1002/cpt.665.
97. Holm IA, Agrawal PB, Ceyhan-Birsoy O, Christensen KD, Fayer S, Frankel LA, et al. The BabySeq project: implementing genomic sequencing in newborns. BMC Pediatr. 2018;18(1):225-.
98. Kalia SS, Adelman K, Bale SJ, Chung WK, Eng C, Evans JP, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genetics in medicine : official journal of the American College of Medical Genetics. 2017;19(2):249-55.
99. Simoons M, Mulder H, Schoevers RA, Ruhe HG, van Roon EN. Availability of CYP2D6 genotyping results in general practitioner and community pharmacy medical records. Pharmacogenomics. 2017;18(9):843-51.
100. Track your own healthcare with 'Volgjezorg' 2019 [Available from: https://www.volgjezorg.nl/en].
101. Samwald M, Minarro-Giménez JAA, Blagec K, Adlassnig K-PP. Towards a global IT system for personalized medicine: the Medicine Safety Code initiative. Studies in health technology and informatics. 2014;205:261-5.
102. Blagec K, Koopmann R, Crommentuijn-van Rhenen M, Holsappel I, van der Wouden CH, Konta L, et al. Implementing pharmacogenomics decision support across seven European countries: The Ubiquitous Pharmacogenomics (U-PGx) project. Journal of the American Medical Informatics Association : JAMIA. 2018.
103. Haidar CE, Relling MV, Hoffman JM. Preemptively Precise: Returning and Updating Pharmacogenetic Test Results to Realize the Benefits of Preemptive Testing. Clinical pharmacology and therapeutics. 2019;106(5):942-4.
104. Collins T, Power K, McCallie D, Jr., Owings R. Finding a Place for Pharmacogenetics in the Electronic Health Record. Clinical pharmacology and therapeutics. 2019;106(2):295-7.
105. Winner JG, Carhart JM, Altar CA, Goldfarb S, Allen JD, Lavezzari G, et al. Combinatorial pharmacogenomic guidance for psychiatric medications reduces overall pharmacy costs in a 1 year prospective evaluation. Current medical research and opinion. 2015;31(9):1633-43.
106. Alagoz O, Durham D, Kasirajan K. Cost-effectiveness of one-time genetic testing to minimize lifetime adverse drug reactions. The pharmacogenomics journal. 2015;16(2):129-36.