**Title** : A controlled prospective real-world implementation study of a 12-gene pharmacogenetic panel to prevent adverse drug reactions in 6,944 patients in seven European countries.

**Authors:** Jesse J Swen, PhD1, Cathelijne H van der Wouden, PhD1\*, Lisanne EN Manson, PharmD1\*, Heshu Abdullah-Koolmees, PhD2, Kathrin Blagec, PhD3, Tanja Blagus, Bsc4, Stefan Böhringer, PhD1,5 , Prof Anne Cambon-Thomsen, PhD6, Erika Cecchin, PharmD7, Ka-Chun Cheung, PhD8, Vera HM Deneer, PhD2,9, Mathilde Dupui, PhD10, Prof Magnus Ingelman-Sundberg, PhD11, Siv Jonsson, PhD12, Candace Joefield-Roka, Bsc13, Katja S Just, MD14, Prof Mats O Karlsson, PhD12, Lidija Konta, PhD15, Rudolf Koopmann, PhD15,16, Marjolein Kriek, MD17, Prof Thorsten Lehr, PharmD18, Christina Mitropoulou, PhD19,20, Emmanuelle Rial-Sebbag, PhD21, Victoria Rollinson, PhD22, Matthias Samwald, PhD3, Elke Schaeffeler, PhD23, 24, Maria Skokou, PhD25, Prof Matthias Schwab, MD23,24,26, Prof Daniela Steinberger, MD15,16, Prof Julia C Stingl, MD14, Roman Tremmel, PhD23, Richard M Turner, PhD22, Mandy H van Rhenen, PharmD8, Cristina L Dávila Fajardo, PhD27, Prof Vita Dolžan, MD4, Prof George P Patrinos, PhD20,25,28,29, Prof Munir Pirmohamed, MD22, Prof Gere Sunder-Plassmann, MD13, Prof Giuseppe Toffoli, MD7, Prof Henk-Jan Guchelaar, PharmD1 , on behalf of the Ubiquitous Pharmacogenomics Consortium

\*Contributed equally

**Affiliations:**

1. Department of Clinical Pharmacy & Toxicology, Leiden University Medical Center, Leiden, The Netherlands.

2. Division Laboratories, Pharmacy and Biomedical Genetics, Hospital Pharmacy

University Medical Center Utrecht, Utrecht, The Netherlands

3. Medical University of Vienna, Center for Medical Statistics, Informatics and Intelligent Systems, Institute of Artificial Intelligence, Vienna, Austria.

4. Pharmacogenetics Laboratory, Institute of Biochemistry and Molecular Genetics, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia.

5. Department of Biomedical Data Sciences, Leiden University Medical Center, Leiden, The Netherlands.

6. CNRS, Center for Epidemiology and Research in POPulation health (CERPOP), Université de Toulouse, Inserm, UPS, Joint Unit 1295, Toulouse, France.

7. Experimental and Clinical Pharmacology Unit, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy

8. Medicines Information Centre, Royal Dutch Pharmacists Association (KNMP), The Hague, The Netherlands.

9. Division of Pharmacoepidemiology and Clinical Pharmacology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, The Netherlands.

10. Service de pharmacologie médicale et clinique, CEIP-addictovigilance de Toulouse, faculté de médecine, CHU, 31000 Toulouse, France.

11. Department of Physiology and Pharmacology, Karolinska Institutet, Biomedicum, Stockholm, Sweden

12. Department of Pharmacy, Uppsala University, Uppsala, Sweden

13. Department of Medicine III, Division of Nephrology and Dialysis, Medical University of Vienna, Vienna, Austria.

14. Institute of Clinical Pharmacology, University Hospital RWTH Aachen, Aachen, Germany.

15. Bio.logis digital health GmbH, Frankfurt am Main, Germany.

16. Diagnosticum Center for Humangenetics, Frankfurt am Main, Germany.

17. Department of Clinical Genetics, Leiden University Medical Center, Leiden, The Netherlands.

18. Clinical Pharmacy, Saarland University, Saarbrücken, Germany.

19. The Golden Helix Foundation, London, UK

20. United Arab Emirates University, College of Medicine and Health Sciences, Department of Genetics and Genomics, Al-Ain, Abu Dhabi, United Arab Emirates

21. UMR Inserm U1027 and Universite de Toulouse III Paul Sabatier, Toulouse, France

22. Department of Pharmacology and Therapeutics, Wolfson Centre for Personalised Medicine, The University of Liverpool.

23. Dr. Margarete Fischer-Bosch-Institute of Clinical Pharmacology, Stuttgart, Germany

24. iFIT Cluster of Excellence (EXC2180) “Image Guided and Functionally Instructed Tumor Therapies”, University of Tuebingen, Tuebingen, Germany

25. University of Patras School of Health Sciences, Department of Pharmacy, Division of Pharmacology and Biosciences, Laboratory of Pharmacogenomics and Individualized Therapy, Patras, Greece

26. Departments of Clinical Pharmacology, and of Pharmacy and Biochemistry, University of Tuebingen, Tuebingen, Germany

27. Clinical Pharmacy Department, Hospital Universitario Virgen de las Nieves, Instituto de Investigacio´n Biosanitaria Granada (Ibs.Granada), Granada,Spain

28. United Arab Emirates University, Zayed Center for Health Sciences, Al-Ain, Abu Dhabi, United Arab Emirates

29. Erasmus University Medical Center, Faculty of Medicine and Health Sciences, Department of Pathology – Clinical Bioinformatics Unit, Rotterdam, the Netherlands

**Correspondence to:**

Henk-Jan Guchelaar, PharmD

Department of Clinical Pharmacy & Toxicology

Leiden University Medical Center

P.O. Box 9600

NL 2300 RC Leiden, The Netherlands

Email: h.j.guchelaar@lumc.nl

**Summary**

**Background**

The benefit of pharmacogenetic testing prior to starting drug therapy has been well documented for a number of single gene-drug combinations. However, the clinical utility of a pre-emptive genotyping strategy using a pharmacogenetic panel has not been rigorously assessed.

**Methods**

We conducted an open, multi-center, controlled, cluster-randomized, cross-over implementation study of a 12-gene pharmacogenetic panel in seven European countries. Patients receiving a first prescription for a drug with a clinical recommendation in the guidelines of the Dutch Pharmacogenetics Working Group (referred to as the “index drug”) were genotyped for 50 germline variants in 12 genes. Patients in the study arm were treated based upon their pharmacogenetic test results according to the DPWG recommendations. Patients in the control arm received standard treatment. To prepare clinicians for pre-emptive pharmacogenetic testing, local teams were educated during a site initiation visit and on-line educational material was made available. The primary outcome was the occurrence of clinically relevant adverse drug reactions (ADRs) within 12 weeks of follow-up. Outcomes were compared between the study and control arm, for both patients with an actionable test result and for all patients, based on an intention-to-treat analysis. This study is registered with ClinicalTrials.gov, NCT03093818.

**Findings**

Between March 7, 2017, and June 30, 2020, 6,944 patients were enrolled and assigned to receive genotype-guided drug treatment (n=3,342) or standard of care (n=3,602). In patients with an actionable test result for the index drug (n=1,558), the incidence of developing a clinically relevant ADR was 0.21 and 0.28 in the study and control arms respectively (OR 0.70; 95% CI 0.54 – 0.91; p=0.0075). For all patients, incidences were 0.21 and 0.29 in the study and control arms respectively (OR 0.70; 95% CI 0.61 – 0.79; p <0.0001)

**Interpretation**

Genotype-guided treatment using a 12-gene pharmacogenetic panel significantly reduced the incidence of clinically relevant adverse drug reactions.

**Funding**

Funded by the European Community’s Horizon 2020 Programme under grant agreement no. 668353 (U-PGx)

**Introduction**

Genetic variation in genes encoding drug metabolizing enzymes, drug transporters and drug targets affects drug disposition and action, and therefore contributes to variability in drug response. Several studies including randomized controlled trials for a few drugs have shown that individualizing drug therapy based on pharmacogenetic testing leads to improved patient outcomes for specific drug-gene combinations. 1-5

Based on the evidence from the literature, consortia such as the Dutch Pharmacogenetics Working Group (DPWG) and the Clinical Pharmacogenetics Implementation Consortium (CPIC) have created guidelines including more than 100 gene-drug pairs.6,7 While the minor allele frequencies of specific variants in the genes is low and ranges from approximately 0.1-5%, testing for a panel consisting of multiple actionable variants in the 12 most important pharmacogenes, identifies at least one actionable genotype in 90-95% of individuals across multiple populations. 8 Therefore, a panel based pharmacogenetic testing strategy appears most efficient. Indeed, a small number of pilot studies have investigated the feasibility of a pharmacogenetic panel test. 9-11 These studies reported a decrease in hospitalizations, emergency department visits and healthcare costs indicating a potential favorable outcome of this approach. However, while these results are encouraging, convincing data for the clinical utility of genotype-guided drug therapy using a pharmacogenetic panel is lacking. 12

Therefore, the Ubiquitous Pharmacogenomics Consortium (U-PGx) conducted the PREemptive Pharmacogenomic testing for Preventing Adverse drug Reactions (PREPARE) study. The PREPARE study is the first, large scale, prospective clinical study investigating the effect of a genotype-guided drug prescribing strategy using a pre-emptive 12-gene pharmacogenetic panel approach across different health-care setting in seven European countries.

**Methods**

**Study design**

The PREPARE study was an investigator-initiated open-label, multi-center, cluster-randomized, cross-over implementation study conducted in seven European countries (Austria, Greece, Italy, The Netherlands, Slovenia, Spain, United Kingdom) investigating the clinical utility of a pre-emptive genotyping strategy with a pharmacogenetic panel. The study design has been outlined in detail previously.13 Countries were block randomized to either start with genotype-guided drug prescribing (study arm) or standard clinical care (control arm). After 19 months, countries switched to the alternate arm. The study was funded by the European Community's Horizon 2020 Programme under grant agreement No. 668353 (U‐PGx). The study protocol was approved by the ethics committee of the Leiden University Medical Center and the ethics committees of participating centers in each country. The trial was performed in accordance with the principles of the Declaration of Helsinki.

**Genetic Variant and Drug selection**

During the preparatory phase of the study, germline-variant alleles were systematically selected as described previously.14 In brief, five predefined criteria were used including minor allele population frequency ≥ 1% (MAF), established effect on protein functionality, and availability of a DPWG guideline with an actionable therapeutic recommendation associated with the variant (further details appendix S1). “Actionable” was defined as a result with a DPWG recommendation to a change to standard-of-care drug treatment. A list of actionable variants is provided in appendix S2. The global MAF was defined as the mean frequency across all populations, using 1000 Genomes project phase III allele frequencies. In addition, variant alleles that had a global MAF < 1%, but a MAF ≥ 1% among selected populations (European/Asian/African) were also included in the panel. Finally, variants with a MAF < 1% that were already tested for in routine clinical practice at one or more of the U-PGx sites such as for example *DPYD*\*13 were also added to the panel. As the DPWG continuously reviews literature and periodically updates guidelines, by design the panel was not static, and changes to the variant panel were allowed during the study and several changes occurred as to represent a real-world situation. The panel at the start of the study comprised 50 germline variant alleles, located within 12 genes (*CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A5, DPYD, F5, HLA-B, SLCO1B1, TPMT, UGT1A1*, *VKORC1*) and was designated as the PGx-Passport. Further details are in appendix S3.

Genotyping was performed in the laboratory at each local site with the SNPline workflow (LGC Group Middlesex, UK). To ensure quality and consistency of genotyping results, all laboratories participated in the quality assessment program for pharmacogenetics that was set up as a distinct proficiency test of the European Molecular Genetics Quality Network (https://www.emqn.org/).

The PREPARE study included all drugs for which an actionable drug-gene interaction was present in the DPWG recommendations with the exceptions of abacavir, omeprazole, esomeprazole, lansoprazole, pantoprazole, rabeprazole, and estrogen-containing drugs (appendix S3). Abacavir was excluded because HLA B\*57:01 is already routinely tested for in line with the mandatory testing requirements of the drug license. Proton pump inhibitors (PPI) were excluded because the DPWG recommendation focuses on increasing efficacy in CYP2C19 ultrarapid metabolizers (UM), and no PPI adverse drug reactions (ADRs) are associated with any of the other CYP2C19 genotype-predicted phenotypes. Estrogen containing drugs were only considered as subsequent drugs during study follow-up. Due to the implementation nature of this study, as with the genetic variant panel, changes to the drug panel were allowed. Further details regarding the changes to the drug and gene panel during the study are given in appendix (S4)

**Participants**

Patients were recruited in Austria (Medical University Vienna), Greece (University of Patras General Hospital, Psychiatric Clinic; ATTIKON University General Hospital, 2nd Psychiatric Clinic, Athens), Italy (3 sites of the Medical Oncology Department of the Centro di Riferimento Oncologico Aviano; San Filippo Neri Hospital, Dept. of Medical Oncology; Cà Foncello Hospital, Dept. of Medical Oncology, Treviso), The Netherlands (28 community pharmacies and the neurology department of the LUMC, further details are provided in the appendix S5), Slovenia (6 community health centres; 5 clinics and hospitals, further details are provided in the appendix S5), Spain (San Cecilio University Hospital, Granada; Hospital Universitario Virgen de las Nieves, Granada, Zaidín South Primary Care center, Granada and Zaidín Speciality Center, Granada), and the United Kingdom (The Royal Liverpool University Hospital; Vauxhall Primary Health Centre; Fulwood Green Medical Centre, all in Liverpool). Patients aged 18 years or older receiving a first prescription (defined as no prescription for the drug in the preceding 12 months) for a drug with an actionable recommendation in the DPWG [<https://www.knmp.nl/index.php/media/1058>] as part of routine care were eligible for inclusion. This drug we refer to as the “index drug”. Exclusion criteria included previous (direct-to-consumer, or clinical) genetic testing for a gene relevant to the index drug, planned duration of treatment less than seven consecutive days, and severe renal or liver insufficiency. Detailed inclusion and exclusion criteria are provided in the Supplementary appendix S6. All patients gave written informed consent before taking part in the study.

**Procedures**

To prepare clinicians, other healthcare professionals and patients for pre-emptive pharmacogenetic testing, a systematic survey on current knowledge about pharmacogenetics was performed.15 Based on this survey outcome, structured educational tools were developed that were provided for the study centers to assure equal level of knowledge and minimize inter-rater variability in the PREPARE-trial. For systematic education of the healthcare professionals active in the implementation of pharmacogenetics, an educational program was established during the study, including educational videos, brochures and an interactive educational game were created (www.upgx.eu).16 In addition, local participants were educated during a site initiation visit. A local study coordinator (Austria: GS, Greece: GP, Italy: GT, Netherlands: JS, Slovenia: VD, Spain CDF, United Kingdom MP) was responsible for execution of the study according to standard operating procedures provided by the U-PGx consortium.

At enrolment, a blood or saliva sample was obtained for DNA isolation. In the study arm, pharmacogenetic test results and DPWG recommendations related to the index drug were returned to the treating health-care provider within seven days of index drug initiation. Pharmacogenetic test results and DPWG recommendations for the other genes and drugs were returned to the health-care provider as soon as they were available through a standardized PGx decision support solution which has been described in detail previously.17 All patients received a Safety Code card (<https://safety-code.org/>) containing a quick response (QR) code that stores the patient's encoded pharmacogenetic test results and leads to a website that provides the relevant DPWG recommendations once the code is read with a standard smartphone or other device (appendix S7). The Safety Code card could be used to guide dose and drug selection for the index drug or any subsequent prescribed drugs. Adherence to DPWG guidelines was not mandatory, and was left to the discretion of the treating physicians and pharmacists. In the control group, patients received standard clinical care and a mock plastic card indicating their participation in the PREPARE study. Genotyping of patients in the control arm was performed after completion of follow up at which time the patients received their genetic test results. All patients were followed for at least 12 weeks up to a maximum of 18 months. T=0 was defined as the day the patient initiated the index drug. Patients were contacted at baseline (t=0) (± one week), four weeks (± two weeks), 12 weeks (± three weeks), and at the end of the time block (± four weeks) to go through a scripted questionnaire and to collect data on the occurrence and severity of adverse drug reactions (ADRs). First, an open question regarding the occurrence of any ADRs was asked, followed by various specific questions related to the patient’s answers. The full questionnaire can be found in appendix S8. In addition, patients were asked to complete a self-report online survey at two weeks and eight weeks after initiation of the index drug. To ensure a balanced inclusion of drugs in the study, inclusion of any given index drug was capped at 10% of all drugs in both the intervention and control arms. All clinical data were recorded in an electronic case report form (eCRF). Because of the nature of the study design, patients and investigators were not blinded to treatment.

**Outcomes**

The primary outcome was the incidence of causal (definite, probable or possible), clinically relevant (classified as National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) grade 2, 3, 4, or 5) adverse drug reactions (ADRs) reported for the index drug within 12 weeks of follow-up. For oncology patients receiving 5-fluorouracil, capecitabine, tegafur or irinotecan, only hematological toxicities of NCI-CTCAE grade 4-5 and non-hematological toxicities of NCI-CTCAE grade 3-5 were considered clinically relevant. All collected ADRs during the follow-up period were assessed regarding severity and causality. Severity was assessed using the CTCAE (version 4.0) classification scale. Causality assessment was performed with the Liverpool Causality Assessment Tool (LCAT).18 If a patient reported multiple ADRs within the 12 weeks of follow-up, the most severe causal ADR was used for the primary analysis. A random 10% sample of severity and causality assessments was independently re-assessed by trained assessors from the Netherlands Pharmacovigilance Center Lareb who were blinded to the patients’ study arm allocation. Agreement was evaluated using Cohen’s kappa. This evaluation indicated that there were no significant differences between study and Lareb assessments (appendix S9). Adherence to DPWG guidelines among physicians and pharmacists was systematically collected and recorded in the eCRF.

**Statistical Analysis**

The study was designed to have 80% power to detect a 30% difference in incidence of

clinically relevant ADRs within 12 weeks of follow-up between the study arms.

Based on the European Medicines Agency (EMA) frequency classification of ADRs in drug labels, the incidence of clinically relevant adverse drug reactions was estimated to range between 0.04 and 0.10 in patients with an actionable genotype. Further details are provided in appendix S10. Data from a previous pilot study among 200 patients indicated approximately 30% of patients carry an actionable genotype for the index drug.19 With these assumptions, the sample size calculation led to a required sample size of 8,100 patients. Due to a two-month delayed start, a lower enrolment rate in the first time block and a higher than expected number of reported ADRs, the protocol was amended with a delayed crossover date (October 1st 2018). Due to the COVID-19 pandemic the second time-block was extended by a 3-month enrolment period to June 30 2020. Baseline characteristics were compared between the treatment groups with the use of chi square and Wilcoxon tests. The primary outcome was analyzed using a mixed logistic regression with country as a factor. A random center-level center-by-country interaction was included as well as covariates representing confounding factors (age, number of drug allergies, number of comedications and global health score, which is a measure to assess an individual’s physical, mental, and social health. The primary analysis was performed using a gatekeeping analysis. First the actionable sub-populations in the genotype-guided prescribing arm and control arm were compared. Only when this analysis was statistically significant, a second analysis with all patients included in the study was performed. All analyses were performed in the intention-to-treat population. A two-sided P value of 0.05 was considered to indicate statistical significance. All analyses were performed with the use of software R 4.1.1. Specific packages versions are given in R-markdown output (<https://github.com/KFTleiden/PREPARE>). Unblinding of the study arm allocation to the statistician was only performed after data-lock. This study is registered with ClinicalTrials.gov, NCT03093818.

**Role of the funding source**

The funder had no influence on the design or conduct of the trial and was not involved in data collection or analysis, in the writing of the manuscript, or the decision to submit for publication.

**Results**

Between March 7, 2017, and June 30, 2020, a total of 6,944 patients were enrolled of whom 3,342 (48.1%) in the genotype-guided arm and 3,602 (51.9%) in the control arm. Spain, Greece and Slovenia were randomized to start with the genotype-guided arm, and Austria, Italy, The Netherlands, and the United Kingdom were assigned to start recruiting controls. On October 1, 2018 all sites crossed over to the alternate arm. Out of the 6,944 patients 3,581 were enrolled during the first block while 3,363 were recruited during the second block (figure 1, appendix S11). Of these patients, 47 (1.3%) patients in the control arm and 52 (1.6%) in the study arm withdrew consent. Age, sex, and number of drug allergies were similar in the study and control patients (table 1). Self-reported ethnicity was Caucasian for 96% of the patients.

Small but statistically significant differences between study and control patients were observed in the global health score and number of comedications (table 1). Of the patients recruited, 5,675 (93.80%) carried at least one actionable variant; 375 (6.20%) carried no variants, while 1,091 (18.03%), 1,845 (30.50%), 1,593 (26.33%), 821 (13.57%), 264 (4.36%), 51 (0.84%), and 10 (0.17%) carried 1, 2, 3, 4, 5, 6 or 7 variants, respectively. The most common index drug was atorvastatin (n=716), followed by clopidogrel (n=619), and tacrolimus (n=472) (appendix S12). During the first block, the capping threshold was reached for atorvastatin and clopidogrel in the study arm, and for atorvastatin, capecitabine, codeine, flucloxacillin and tacrolimus in the control arm. In the second block capping was reached for atorvastatin and capecitabine in the study arm, and atorvastatin, clopidogrel and tramadol in the control arm. Of the patients, 25.2% carried an actionable variant for their index drug hereafter ”actionable patients”). *CYP2D6* resulted in the highest and *HLA-B\*57:01* in the lowest proportion of actionable patients with 44.6% and 4.1%, respectively (appendix S13). For the index drugs, the highest number of actionable patients was observed for atorvastatin 204 (28,5%), tramadol 183 (48.3%) and clopidogrel 168 (27.8%), and overall for drugs taken by more than 25 patients the highest extent of actionability was otherwise seen for venlafaxine, metoprolol, tamoxifen, codeine, oxycodone, amitriptyline, warfarin, simvastatin, sertraline, citalopram, and escitalopram (appendix S14). These percentages are consistent with the known actionable allele frequencies in Caucasian populations. Most patients completed the 12-week follow-up period ranging from 87% in the UK and Italy, to 98% in Greece. The median turnaround time of genotype results varied per site and ranged from 1-7 days (appendix S15). As expected, the number of reported ADRs varied per country ranging from 283 (1.1 per patient) to 4,811 (3.9 per patient) ADRs in Austria and Italy, respectively. The severity of ADRs also showed considerable variation between countries, in line with the types of medication prescribed (appendix S16). The highest incidence and most severe ADRs were reported in Italy where patients were recruited from a cancer clinic and mostly received cancer treatments at the maximum tolerated dose. By contrast, in the Netherland patients were recruited from primary care through community pharmacies, receiving substantially less toxic treatments. Adoption of the DPWG recommendations was high, and 69.9% of the provided recommendations were accepted by the physicians and pharmacists. As part of the intention-to-treat analysis all patient were included in the analysis irrespective of adherence to the DPWG guidelines. The primary outcome was the occurrence of causal clinically relevant adverse drug reactions (ADRs) within 12 weeks of follow-up. In total, 10,718 events were reported by 3,303 patients. After filtering for severity (NCI-CTCAE grade ≥2 with exception of oncology patients receiving 5-fluorouracil, capecitabine, tegafur or irinotecan, see methods section) and causality (LCAT score ≥ possible), 3,096 events reported by 1,563 patients remained (appendix S17).

In the first gatekeeping analysis, out of the 1,753 patients with actionable variants, 195 did not complete 12 week follow up. Therefore 1,558 actionable patients were available (Figure 1). The incidence of developing a causal clinically relevant ADR in patients with an actionable test result was 0.21 and 0.28 in the study and control arm, respectively. The effect of the intervention significantly reduced ADR risk by 30% (OR of 0.70; 95% CI 0.54 – 0.91; p=0.0075). In the second gatekeeping analysis which included all patients, the incidence of developing a causal clinically relevant ADR was similar with 0.21 and 0.29 in the study and control arms, respectively, reducing the risk of an ADR by 30% (OR 0.70; 95% CI 0.61 – 0.79; p <0.0001) (figure 2). Pre-defined covariates were also associated with the risk of ADR. Patients with a better global health score or older age showed a decreased risk for ADR. By contrast, the incidence of ADRs increased with a higher reported number of drug allergies and a higher number of comedications (table 2).

The effect of the pharmacogenetic intervention varied per country (appendix S23). A lower incidence of clinically relevant ADRs was observed in the study versus control arm in Greece, Italy, Netherlands, Spain and the United Kingdom. No causal clinically relevant ADR was reported by any of the patients allocated to the control arm in Austria. Finally, a higher incidence of ADRs was observed in patients allocated to receive genotype-guided drug treatment in Slovenia.

In the study arm, pharmacogenetic test results could also be used to guide treatment for any consecutive drugs in addition to the index drug prescribed or dispensed during the follow-up period of the study. During follow up, out of the 6,944 patients, 953 (13.7%) patients received a second, 79 (1.2%) a third, 6 (0.1%) a fourth, and 1 (0.01%) a fifth prescription with an actionable recommendation based upon their genotype. Accounting for these prescriptions slightly increased the effect of the pharmacogenetic intervention (OR 0.69; 95% CI 0.61-0.78; p<0·0001).

When the primary analysis was repeated with all 6,944 included patients and all reported ADRs, without filtering for severity and causality, the effect of the pharmacogenetic intervention increased. Patients in the study arm showed a lower occurrence of ADRs compared to patients in the control arm (OR 0.55 95% CI 0.49 – 0.62; p<0.0001). The effect sizes of the other pre-defined covariates remained in the same order of magnitude except for the effect of country where a substantially increased number of ADRs was observed in Italy (appendix S18).

**Discussion**

This prospective real-world implementation study in 7 different European countries encompassing 6,944 patients showed that genotype-guided prescribing using a 12-gene pharmacogenetic panel significantly reduced the incidence of clinically relevant ADRs. Our results are the first to demonstrate the feasibility and clinical utility of the large scale implementation of a panel based pharmacogenetic testing strategy and underpin the benefits of implementing a standardized, validated and harmonized pharmacogenetic test system that supports pharmacogenetics guided decision making at point of care.

Few studies investigating the clinical implementation of pharmacogenetics have been initiated, many of which are US-based.8,13 These studies have addressed multiple barriers in the implementation of pharmacogenetics, and have focused on implementing either single drug-gene pairs one at a time or in the context of highly specialized care settings, rather than assessing the benefit of a pharmacogenetic testing strategy that focuses on a panel of pharmacogenes across various therapeutic areas and different health care systems. The few available panel based studies focusing on a panel based approach were mostly conducted in patients ≥65 years with polypharmacy and have limited power to demonstrate the benefit of intervention due to their observational design or limited sample size.9-11 More recently, a large retrospective analysis of the economic impact of the clinical implementation of a 23-gene pharmacogenetics panel in 5,288 patients of ≥65 years compared to 22,357 controls showed a reduction of ~$7,000 per patient in direct medical charges.20 These results are in line with our results, and support further clinical implementation of pharmacogenetic panel testing.

A major strength of our study is that it encompasses the diversity in national health system organizations within Europe, including a broad range of different diseases and drug therapies. This real-world design introduced several challenges. The gene panel, list of eligible drugs and recommendations were not static and changes resulting from updates of the DPWG guidelines were allowed. During the study, this resulted in changes including removal of oxycodone as none of the genotype was considered actionable anymore, and changes to the actionability of phenotypes for voriconazole, escitalopram, clomipramine, *CYP2B6*, and *DPYD* (appendix S4).

We estimated that a truly pre-emptive study investigating a genotyping strategy using a pharmacogenetic panel would require at least a 10-20 times larger samples size compared to our study, as many patients would not start an index drug within the timeframe of the study. Therefore, to increase efficiency, we enrolled patients receiving a first prescription for a drug with a recommendation in the DPWG guidelines. Pharmacogenetic test results and clinical recommendations were returned as per protocol within 7 days, and the medication was adjusted if needed. Results of turnaround time show that this was feasible for all participating centers. As some of the ADRs that may have occurred within these maximal 7 days could have been prevented if the pharmacogenetic testing would have been fully pre-emptive, our reported effect may be an underestimation of the real effect size in case of pre-emptive testing.

We used patient reported ADRs. These findings were collected during scheduled interviews with research nurses and not objectified with laboratory tests or physical examinations. Furthermore, we conducted causality analysis of the ADRs using a validated tool18, and this assessment was independently validated. We depended on the patient to re-contact the study team whenever a second drug was started during the study follow-up. Based on the available literature we had expected at least 1-2 additional pharmacogenetic guided adjustments per patient for ~30% of the patients.21 However, during our study only 946 (15.4%) patients reported the use of a secondary drug, indicating that our results may underestimate the true impact of our intervention. Importantly, the lower than expected number of patients with a secondary drug did not affect the primary endpoint of the study. Despite the considerable size of our study, for several drugs only very limited numbers of patients were accrued, including drugs with a high toxicity profile such as mercaptopurine, azathioprine and thioguanine. These thiopurines are metabolized by thiopurine methyltransferase for which highly penetrant variants are known and their absence in our study may therefore result in an underestimation of the potential of a PGx panel test. Of the included patients, 96% were of self-declared European ancestry. While our pharmacogenetic panel included specific variants with a MAF ≥ 1% in selected populations other than European (appendix S1), future studies will be required in patients of other ancestry groups to establish the global applicability of our findings.

The observed ~30% reduction of clinically relevant ADRs when analyzing all patients (second gatekeeping analysis) was similar to the effect size obtained in the actionable patients only (first gatekeeping analysis). Both drug capping and the addition of recruiting centers during the study to ensure sufficient patient enrolment might have led to differences in type of medications prescribed with respect to cross-over. For example, the addition of a center that prescribes more drugs with a high toxicity profile (capecitabine, tacrolimus) after cross-over to the control arm may result in an observed positive effect of the pharmacogenetic intervention in patients with a non-actionable test result when comparing the study arm to the control arm. Indeed some heterogeneity in the effect of the pharmacogenetic intervention between countries is present in our data. Particularly in Slovenia recruitment sites that prescribed different types of medications were added after the cross-over which might explain the increase in ADR in the intervention arm (appendix S23). A post-hoc exploratory analysis including index drug and an index drug-by-country interactions into the statistical model indeed indicate that changes in the case-mix are the main contributor to the observed comparable effect size in actionable and all-patients. We applied capping to prevent overrepresentation of a single drug-gene pair that would drive the effect simply as a result of prescribing patterns. A consequence of capping is that the distribution of index drugs does not fully represent natural prescription patterns

Patients with severe liver (stage Child-Pugh C) and kidney function (less than 15 ml/min per 1,73m2) were excluded. Other factors such as drug-drug interactions and polypharmacy reflect the real-world context of our study. Obviously these factors may also influence drug response but their influence on our primary endpoint is considered limited due to the cross-over design of our study.

Our study only investigated the effect of a pharmacogenetic panel test on the reduction of ADR. Potentially, the effect of such a test could even be larger if drug efficacy is also taken into account. However, while it was possible to design a composite endpoint that captures diverse toxicities, it is difficult to define an endpoint for efficacy for 39 drugs used to treat the multiple diseases covered in the PREPARE study. To assess the effect of pharmacogenetic testing on drug efficacy, well-designed prospective studies focusing on a specific drug and disease such as the recently completed TAILOR-PCI and POPular Genetics trials remain essential.4,23 We did not investigate the potential beneficial effect of the pharmacogenetic panel test for each of the specific drugs, settings and patient groups involved as our aim was to prospectively test a broad pharmacogenetics test panel which covered a large number of drugs. A panel based pre-emptive approach is likely to be the most cost-effective method for implementing pharmacogenetics. We are undertaking cost-effective analysis of the study, and this will be reported in a separate paper.

In conclusion, our study is the first study demonstrating the feasibility and benefits of a pharmacogenetic panel strategy across a diversity in European healthcare system organizations and settings, and provides evidence supporting large scale implementation of panel based pharmacogenetics testing to make drug therapy more safe.

**Contributors**

HJG was the scientific coordinator of the U-PGx consortium. HJG, JJS, MS, MS, and CM formed the executive board of the U-PGx consortium. JS was the PI of the PREPARE study. GSP, GPP, GT, VD, CLDF, and MP were PIs of the individual countries. HJG, JJS, MP, MS, MS, MIS, GP, and GT conceptualized the study design. JJS, SB, HJG, and LENM prepared the first draft of the manuscript. CvdW and SB wrote the statistical analysis plan. SB was the lead statistician. All authors critically reviewed the report and approved the final version before submission. All authors had full access to all the data in the study. The corresponding author had final responsibility for the decision to submit for publication.

**Acknowledgements**

The authors thank Dr. W. van Hemmen - van Veelen for her assistance during various stages of the study, Prof. R.B. Altman, MD; Prof. M. Eichelbaum, MD; D.U. Haerry; Prof. M.J. Ratain, MD; Dr. M.V. Relling, PharmD PhD; Prof. D.M. Roden, MD for their valuable support and advice as members of the Scientific Advisory Board of the U-PGx consortium. This study was funded by the European Community’s Horizon 2020 Programme under grant agreement no. 668353 (U-PGx)

**Declaration of interests**

M.P. has received partnership funding for the following: Medical Research Council (MRC) Clinical Pharmacology Training Scheme (co-funded by MRC and Roche, Union Chimique Belge [UCB] Pharma, Eli Lilly, and Novartis); and a PhD studentship jointly funded by Engineering and Physical Sciences Research Council and Astra Zeneca. He has also received unrestricted educational grant support for the UK Pharmacogenetics and Stratified Medicine Network from Bristol-Myers Squibb. He has developed a human leukocyte antigen genotyping panel with MC Diagnostics but does not benefit financially from this**.** None of these sources of funding were used for this study.

JCS has received speaker honoraria for lectures on CYP2C9 pharmacogenetics and siponimod metabolism by Novartis.

M.S., R.T and E.S were supported in part by the Robert Bosch Stiftung (Stuttgart, Germany) and M.S and E.S. by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) under Germany’s Excellence Strategy—EXC 2180—390900677). Independently from this work M.S. received support by Green Cross WellBeing Co. Ltd., Gilead Sciences Inc., Robert Bosch GmbH, CORAT Therapeutics GmbH, and Agena Bioscience.

M.K. has received research funding from Bayer and Roche, educational grants from Novartis and Servier and consultancy fees from Pharmetheus.

SJ has received consultancy fees from Pharmetheus.

**Data Sharing**

Data from the PREPARE study are not publicly available but are planned to be made available in the future. A complete deidentified dataset will be made accessible, together with a data dictionary. Requests for access to the data can be made by sending an email together with a research plan to the corresponding author.

**Research in context**

**Evidence before this study**

The benefit of pharmacogenetic testing prior to starting drug treatment has been well documented for a number of single gene-drug pairs. However, the clinical utility of large scale implementation of a pre-emptive genotyping strategy with a pharmacogenetic panel remains to be elucidated. Several studies investigating the implementation of pharmacogenetics are available, many of which are US-based. These studies focused on implementing either single drug-gene pairs one at a time and were done in highly specialized care settings. We searched PubMed for trials published in English before July 1 2022 which investigated the implementation of pre-emptive pharmacogenetic panel testing with the search terms "pharmacogenetics", "clinical utility", “implementation”, “prospective”, and "panel". There are no prospective studies that assessed the clinical utility of a pre-emptive genotyping strategy with a pharmacogenetic panel across multiple European countries and health care settings.

**Added value of this study**

Our study is the first to investigate the benefits of a pharmacogenetic panel strategy combined with the Dutch Pharmacogenetics Working Group guidelines across a diversity in European health system organizations and settings. Our results show that pharmacogenetic guided prescribing results in a 30 percent reduction of clinically relevant adverse drug reactions. Furthermore, our results underpin the benefits of implementing a standardized, validated and harmonized pharmacogenetic test system that supports pharmacogenetics guided decision making at point of care and demonstrate the value of an educational program to ascertain a similar knowledge base on personalized medicine and pharmacogenetic testing at the begin of the study.

**Implication of all the available evidence**

Together with the evidence from randomized clinical trials for a variety of single drug-gene combinations our results support a personalized medicine approach with pharmacogenetics guided drug prescribing to reduce the incidence of clinically relevant adverse drug reactions.

***Figure 1 Trial profile***



Patients were assessed for eligibility by the treating physician or pharmacist. In the Netherlands, community pharmacists used automated queries to identify potentially eligible patients as described previously.19 The queries included broad criteria resulting in a higher number of potentially eligible patients compared to the other countries. % indicate the percentage of subjects calculated of the total number per country.

***Figure 2 Frequency of causal clinically relevant adverse drug reactions in the study and control arm for patients with an actionable test result***



Event rates per study arm. Error bars represent confidence intervals for event rates. P-values are based on the mixed models used in the primary analysis. See Text.

**Table 1 Baseline characteristics of the patients**

|  |  |  |  |
| --- | --- | --- | --- |
|  | All Patients n=6,944  | Studyn=3,342 | Controln=3,602 |
| Sex, n (%) |  |  |  |
| Male | 3,375 (49) | 1,587 (47) | 1,801 (50) |
| Female | 3,569 (51) | 1,755 (53) | 1,801 (50) |
| Age, median (IQR)  | 58.0 (22.0) | 58.0 (22.0) | 59.0 (22.0) |
| Global Health Score, mean (SD)  | 0.69 (0.1) | 0.69 (0.1) | 0.70 (0.1) |
| Number of allergies, mean (SD)  | 0.38 (1.0) | 0.36 (1.0) | 0.40 (0.9) |
| Number of comedications, mean (SD) | 7.88 (6.6) | 6.85 (5.8) | 8.83 (7.1) |
| Country, n (%) |  |  |  |
| Austria | 269 (3.9) | 145 (4.3) | 124 (3.4) |
| Greece | 1,321 (19.0) | 684 (20.4) | 637 (17.7) |
| Italy | 1,232 (17.8) | 622 (18.6) | 610 (16.9) |
| Netherlands | 1,406 (20.2) | 643 (19.2) | 763 (21.2) |
| Slovenia | 716 (10.3) | 317 (9.5) | 399 (11.1) |
| Spain | 963 (13.9) | 489 (14.6) | 474 (13.1) |
| United Kingdom | 1037 (14.9) | 442 (13.2) | 595 (16.5) |

SD: standard deviation; IQR: interquartile range.

**Table 2 Results of the multivariable logistic regression analysis of the occurrence of causal clinically relevant adverse drug reactions within 12 weeks of follow-up.**

|  |  |  |
| --- | --- | --- |
| Factor | Patients with actionable variants | All patients |
| OR (95% CI) | p-value | OR (95% CI) | p-value |
| Intercept | 10.66(2.38 – 47.80) | **0.0020** | 8.15(2.28 – 29.2) | **0.0013** |
| Pharmacogenetic intervention | 0.70(0.54 – 0.91) | **0.0075** | 0.70(0.61 – 0.79) | **<0.0001** |
| Age (per year increase) | 0.98(0.97-0.99) | **<0.0001** | 0.98(0.98 – 0.99) | **<0.0001** |
| Global Health Score (per point increase) | 0.056(0.016 – 0.20) | **<0.0001** | 0.11(0.06 – 0.21) | **<0.0001** |
| Number of drug allergies | 1.15(1.01 – 1.31) | **0.029** | 1.09(1.03 – 1.16) | **0.0062** |
| Number of comedications | 1.04(1.02 – 1.07) | **0.0012** | 1.04(1.02 – 1.05) | **<0.0001** |
| Austria\* | 0.39(0.08 – 2.04) | 0.27 | 0.38(0.06 – 2.46) | 0.31 |
| Greece\* | 0.33(0.08 – 1.28) | 0.11 | 0.18(0.04 – 0.910 | **0.036** |
| Italy\* | 0.33(0.09 – 1.21) | 0.094 | 0.24(0.06 – 1.01) | 0.052 |
| Slovenia\* | 1.11(0.37 – 3.35) | 0.84 | 1.09(0.29 – 4.06) | 0.896 |
| Spain\* | 0.31(0.08 – 1.25) | 0.10 | 0.23(0.04 – 1.46) | 0.119 |
| United Kingdom\* | 0.56(0.16 – 1.87) | 0.34 | 0.46(0.11 – 2.01) | 0.304 |

\*ORs were calculated per country relatively to The Netherlands (OR=1)

P-values were calculated using a Wald test for each regression coefficient for the null hypothesis of the regression coefficient being zero on the log-scale.

**References**

1. Mallal S, Phillips E, Carosi G, et al. HLA-B\*5701 screening for hypersensitivity to abacavir. *N Engl J Med* 2008; **358**(6): 568-79.

2. Pirmohamed M, Burnside G, Eriksson N, et al. A randomized trial of genotype-guided dosing of warfarin. *N Engl J Med* 2013; **369**(24): 2294-303.

3. Coenen MJ, de Jong DJ, van Marrewijk CJ, 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.

4. Claassens DMF, Vos GJA, Bergmeijer TO, et al. A Genotype-Guided Strategy for Oral P2Y(12) Inhibitors in Primary PCI. *N Engl J Med* 2019; **381**(17): 1621-31.

5. Henricks LM, Lunenburg C, de Man FM, et al. DPYD genotype-guided dose individualisation of fluoropyrimidine therapy in patients with cancer: a prospective safety analysis. *Lancet Oncol* 2018; **19**(11): 1459-67.

6. Bank PCD, Caudle KE, Swen JJ, et al. Comparison of the Guidelines of the Clinical Pharmacogenetics Implementation Consortium and the Dutch Pharmacogenetics Working Group. *Clin Pharmacol Ther* 2018; **103**(4): 599-618.

7. Abdullah-Koolmees H, van Keulen AM, Nijenhuis M, Deneer VHM. Pharmacogenetics Guidelines: Overview and Comparison of the DPWG, CPIC, CPNDS, and RNPGx Guidelines. *Front Pharmacol* 2020; **11**: 595219.

8. Dunnenberger HM, Crews KR, Hoffman JM, et al. Preemptive clinical pharmacogenetics implementation: current programs in five US medical centers. *Annu Rev Pharmacol Toxicol* 2015; **55**: 89-106.

9. 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): e0170905.

10. Finkelstein J, Friedman C, Hripcsak G, Cabrera M. Pharmacogenetic polymorphism as an independent risk factor for frequent hospitalizations in older adults with polypharmacy: a pilot study. *Pharmgenomics Pers Med* 2016; **9**: 107-16.

11. Brixner D, Biltaji E, Bress A, 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. *J Med Econ* 2016; **19**(3): 213-28.

12. Weitzel KW, Cavallari LH, Lesko LJ. Preemptive Panel-Based Pharmacogenetic Testing: The Time is Now. *Pharm Res* 2017; **34**(8): 1551-5.

13. van der Wouden CH, Cambon-Thomsen A, Cecchin E, et al. Implementing Pharmacogenomics in Europe: Design and Implementation Strategy of the Ubiquitous Pharmacogenomics Consortium. *Clin Pharmacol Ther* 2017; **101**(3): 341-58.

14. van der Wouden CH, van Rhenen MH, Jama WOM, et al. Development of the PGx-Passport: A Panel of Actionable Germline Genetic Variants for Pre-Emptive Pharmacogenetic Testing. *Clin Pharmacol Ther* 2019; **106**(4): 866-73.

15. Just KS, Steffens M, Swen JJ, Patrinos GP, Guchelaar HJ, Stingl JC. Medical education in pharmacogenomics-results from a survey on pharmacogenetic knowledge in healthcare professionals within the European pharmacogenomics clinical implementation project Ubiquitous Pharmacogenomics (U-PGx). *Eur J Clin Pharmacol* 2017; **73**(10): 1247-52.

16. Just KS, Turner RM, Dolžan V, et al. Educating the Next Generation of Pharmacogenomics Experts: Global Educational Needs and Concepts. *Clin Pharmacol Ther* 2019; **106**(2): 313-6.

17. Blagec K, Swen JJ, Koopmann R, et al. Pharmacogenomics decision support in the U-PGx project: Results and advice from clinical implementation across seven European countries. *PLoS One* 2022; **17**(6): e0268534.

18. Gallagher RM, Kirkham JJ, Mason JR, et al. Development and inter-rater reliability of the Liverpool adverse drug reaction causality assessment tool. *PLoS One* 2011; **6**(12): e28096.

19. Bank PCD, Swen JJ, Schaap RD, Klootwijk DB, Baak-Pablo R, Guchelaar HJ. A pilot study of the implementation of pharmacogenomic pharmacist initiated pre-emptive testing in primary care. *Eur J Hum Genet* 2019; **27**(10): 1532-41.

20. Jarvis JP, Peter AP, Keogh M, et al. Real-World Impact of a Pharmacogenomics-Enriched Comprehensive Medication Management Program. *J Pers Med* 2022; **12**(3).

21. Samwald M, Xu H, Blagec K, 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): e0164972.

22. Li JH, Joy SV, Haga SB, et al. Genetically guided statin therapy on statin perceptions, adherence, and cholesterol lowering: a pilot implementation study in primary care patients. *J Pers Med* 2014; **4**(2): 147-62.

23. Pereira NL, Farkouh ME, So D, et al. Effect of Genotype-Guided Oral P2Y12 Inhibitor Selection vs Conventional Clopidogrel Therapy on Ischemic Outcomes After Percutaneous Coronary Intervention: The TAILOR-PCI Randomized Clinical Trial. *Jama* 2020; **324**(8): 761-71.