Integrating pharmacogenetics into clinical practice: Opportunities and challenges for the pharmaceutical care of patients with major depression

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"Science and everyday life cannot and should not be separated.
Science, for me, gives a partial explanation of life.
In so far as it goes, it is based on fact, experience and experiment."

Rosalind Franklin, 1940

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ABBREVIATIONS

A ABC, ATP binding cassette; AD, antidepressant; ADME, absorption distribution metabolism elimination; ADR, adverse drug reaction ATC, Anatomical Therapeutical Chemical

B BBB, blood–brain barrier; BDI, Beck Depression Inventory

C CNV, copy number variation; CPIC, Clinical Pharmacogenetics Implementation Consortium; Css, steady state plasma concentration; CYP, cytochrome P450

D DALY, disability adjusted life years; DDI, drug–drug interaction; DDGI, drug–drug–gene interaction; DGI, drug–gene interaction; DGGI, drug–gene–gene interaction; DPWG, Dutch Pharmacogenetics Working Group; DRP, drug-related problem

E EM, extensive metabolizer

H HAM-D, Hamilton Depression Rating Scale; 5-HTTLPR, serotonin transporter linked promotor region polymorphism

I ICD, International Statistical Classification of Diseases and Related Health Problems; IM, intermediate metabolizer

M MDD, major depressive disorder

N NGS, next generation sequencing; NHS, National Health Service; NM, normal metabolizer

P PCNE, Pharmaceutical Care Network Europe; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PGx, pharmacogenetics; PharmGKB, Pharmacogenomics Knowledge Base; PM, poor metabolizer

R RM, rapid metabolizer; RR, relative risk

S SERT, serotonin transporter; SGAD, Swiss Society of Anxiety and Depression; SNP, single nucleotide polymorphism; SSRI, selective serotonin reuptake inhibitor;

T TF, trial failure

U UM, ultrarapid metabolizer

V VNTR, variable number tandem repeat

W WGS, whole genome sequencing

1 Abstract

An individual's genetic makeup can affect the pharmacokinetic and pharmacodynamic behavior of a drug, which may have a clinically relevant impact on the drug's efficacy and tolerability. Pharmacogenetics aims to identify patients who are susceptible to therapy failure, adverse drug reactions or severe toxicities, as a result of their genetic predisposition. Such genetic information may be used to individualize pharmacotherapy to increase effectiveness and minimize adverse drug reactions. Multiple international consortia are translating pharmacogenetic (PGx) findings from research into recommendations for clinical practice, to support the use of genetic information in optimizing pharmacotherapy in terms of drug selection and dosing. Such recommendations exist for several antidepressants commonly used in Switzerland, including the selective serotonin reuptake inhibitors (e.g., citalopram, escitalopram, paroxetine and sertraline). Pharmacotherapy with antidepressants is an important pillar in the treatment of patients with major depressive disorder (MDD). However, it is known that about half of these patients do not respond sufficiently to a first-line treatment. As mentioned before, genetic predisposition is one factor affecting antidepressant efficacy and tolerability. Still, clinicians in Switzerland do not routinely consider PGx information in the pharmacotherapeutic management of patients with MDD. This thesis investigates the integration of PGx into clinical practice and evaluates opportunities and challenges for the pharmaceutical care of MDD patients in this context. The thesis presented here consists of four parts (A–D):

Project A: In a prospective, observational case study we collected and analyzed individual patient cases from primary and secondary care, where PGx information was used by pharmacists to elucidate histories of therapy failure and adverse drug reactions,

as well as to elaborate recommendations for further therapy optimization. This thesis gives an insight into five exemplary patient cases related to antidepressant treatment in secondary care. The application of individual PGx information to real-world, depressive-disorder patient cases did not always prove to be straightforward. Despite the availability of PGx dosing guidelines for certain drug-gene pairs, evidence for precise PGx-based drug selection and dosing is still fragmentary. Moreover, the integration of PGx information required consideration and evaluation of additional individual factors, including non-genetic factors such as the patients' comedication and comorbidities.

Project B: Pharmacists already consider several interindividual factors when analyzing a patient's medicines to propose interventions for therapy optimization and are an important point of contact for patients and healthcare professionals concerning drug-related problems. Accordingly, owing to the identified complexity of applying PGx information in individual patient cases (Project A) and the lack of education being described as a major barrier to the adoption of PGx in clinical practice, we developed and conducted a continuing education program. The aim of this training program was to prepare Swiss pharmacists for the application of PGx information in clinical practice. After attending the program, participants showed measurable improvement in both knowledge and skills to apply PGx information in providing pharmaceutical care to patients. However, the actual implementation of a PGx service presented several challenges for the participating pharmacists. One major challenge appeared to be the lack of interprofessional networks and physician support for such a PGx service.

Project C: In order for the PGx information processed by pharmacists to be taken into account in the treatment of patients, close collaboration with other healthcare professionals, especially the treating physician, is of importance. Based on our working

experience with over 140 patient cases in the aforementioned observational case study, we defined a six-step-approach for the implementation of a pharmacist-led PGx testing and counseling service (PGx service) for primary and secondary care settings. In this approach, pharmacists play a key role in enabling an individual and comprehensive evaluation of the patients' PGx profile by integrating this information into a medication review. In this way, non-genetic factors that may enhance or compensate for the genetic predisposition are also taken into account.

Project D: To evaluate the impact of the proposed pharmacist-led PGx service (Project C) on patient outcomes, we developed a clinical trial addressing antidepressant therapy in MDD patients. The PrePGx study is a multi-center, open-label, randomized controlled, parallel three-arm trial. We compare pharmacist-guided preemptive PGx testing for the selection and dosing of an antidepressant (intervention arm) to the current standard approach (control arm), where the psychiatrist prescribes the antidepressant without information on the patient's PGx profile and without a consultation with a pharmacist. We anticipate that this trial will have a direct impact on the application and handling of PGx information in routine psychiatric and pharmacy practice.

2 Introduction

People vary not only in appearance and preferences, but also in their inter-individual responses to drugs. When taking the same drug, some may achieve a sufficient effect, while others do not respond, suffer from unwanted side effects or even experience severe toxicities. Among other things, differences in systemic drug exposure can cause inter-individual drug reactions, leading to either toxicity in the case of supra-therapeutic drug plasma concentration or inefficacy due to sub-therapeutic drug plasma concentration [1]. In addition to modifiable factors such as drug–drug interactions (DDI) and medication adherence, drug plasma concentrations may also be affected by certain predispositions, including renal or hepatic function, and, notably, genetics [2,3].

The effect of individual genetic predisposition on drug response and treatment outcome is studied in the field of pharmacogenetics (PGx) [4]. Genetic variation can affect the function of enzymes and transporters involved in drug absorption, distribution, metabolism, or excretion (ADME), causing inter-individual differences in the pharmacokinetic behavior of substrate drugs. Moreover, genetic variation can affect the expression and/or the structure of receptors, enzymes and other drug targets, potentially altering the pharmacodynamic behavior of a compound. Both pharmacokinetics- and pharmacodynamics-related genetic variants can affect drug response [1,4,5]. Such effects may result in an increased risk of adverse drug reactions (ADR) or therapy failure (TF) in certain individuals. Pharmacogenetics aims to identify patients who are susceptible to TF, ADR or severe toxicities, due to their genetic predisposition. As a consequence, genetic information may be used to individualize pharmacotherapy in terms of drug selection and dosing to increase effectiveness and minimize adverse drug reactions [1,4,5].

Multiple consortia are aiming to translate pharmacogenetic findings from research into recommendations for clinical practice, in supporting the use of genetic information to optimize pharmacotherapy. In particular, the Clinical Pharmacogenetics Implementation Consortium (CPIC) [6] and the Dutch Pharmacogenetics Working Group (DPWG) [7], have published guidelines for PGx-guided drug selection and dosing. Today, their recommendations cover over 70 drug-gene pairs [8].

In addition to guidelines, healthcare professionals consult drug labels for information on drug selection and dosing. PGx information is also included on these labels and is approved by drug regulatory agencies, including Switzerland's Swissmedic [9]. PGx information on drug labels is of a diverse nature, ranging from references to specific PGx testing requirements to purely informative content about potential drug–gene interactions. The Pharmacogenomics Knowledge Base (PharmGKB, www.pharmgkb.org) [10] proposes a classification into four levels of PGx information, according to the indicated action: (i) "testing required", genetic testing is needed before drug usage; (ii) "testing recommended", genetic testing is recommended before drug usage; (iii) "actionable PGx", information on the impact of genetic variation on drug effectiveness or tolerability, without suggesting PGx testing; (iv) "informative PGx", genetic variation does not affect a drug or is not clinically relevant [11]. A recent analysis of Swiss drug labels found that 167 approved compounds contain PGx information in their drug label. However, over 55% of the PGx information is classified as "informative PGx", with only around 8% of the annotated information referencing specific genetic testing recommendations or requirements ("testing required" or "testing recommended") [9].

2.1 Pharmacogenetics and antidepressants

The vast majority of PGx information on Swiss drug labels was found to be attributed to drugs of the anatomical group "N – nervous system" [9], as defined by the Anatomical Therapeutic Chemical (ATC) classification system [12]. The anatomical group "N" includes antidepressants, such as selective serotonin reuptake inhibitors (SSRI), for which the CPIC and the DPWG have published specific PGx-guided dosing recommendations [13,14]. Multiple clinically used antidepressants are metabolized by polymorph cytochrome P450 (CYP) enzymes, notably, CYP2D6 and CYP2C19 (Table 1).

Table 1. Overview of antidepressants marketed in Switzerland and the cytochrome P450 isoforms involved in the respective phase I biotransformation. Adapted from Crisafulli *et al.* [15].

Antidepressant	**Enzymes involved in Phase I biotransformation**
Agomelatine	CYP1A2
Bupropion	CYP2B6
(Es-)Citalopram	CYP2C19, CYP2D6, CYP3A4
Duloxetine	CYP2D6, CYP1A2
Fluoxetine	CYP2D6, CYP2C9, CYP2C19, CYP3A4
Fluvoxamine	CYP1A2, CYP2D6
Mirtazapine	CYP2D6, CYP1A2, CYP3A4
Paroxetine	CYP2D6, CYP3A4
Reboxetine	CYP3A4
Sertraline	CYP2C9, CYP2C19, CYP2D6, CYP3A4
Trazodone	CYP3A4
Tricyclic antidepressants	CYP2C19, CYP2D6, CYP1A2, CYP3A4
Venlafaxine	CYP2D6, CYP3A4

For these polymorphic enzymes, individuals can present a broad range of metabolic capacities, which are generally divided into four main phenotypes: normal metabolizer (normal function, NM), ultra-rapid/rapid metabolizer (increased function, UM/RM), intermediate metabolizer (decreased function, IM) and poor metabolizer (no function, PM) [16,17]. These phenotypes are predisposed by the individual's genetic make-up, and therefore can be predicted by genotyping nucleotide polymorphisms (e.g. single nucleotide polymorphism, SNP) or in the case of *CYP2D6* also by complete gene deletions or duplications (copy number variations, CNV) [18]. The highly polymorphic CYP2D6 for instance is involved in the major biotransformation of over 20% of marketed compounds (Figure 1), including commonly prescribed antidepressants and other psychotropic drugs [2]. In fact, it was reported that over 50% of psychiatric patients are using at least one CYP2D6 substrate drug [19].

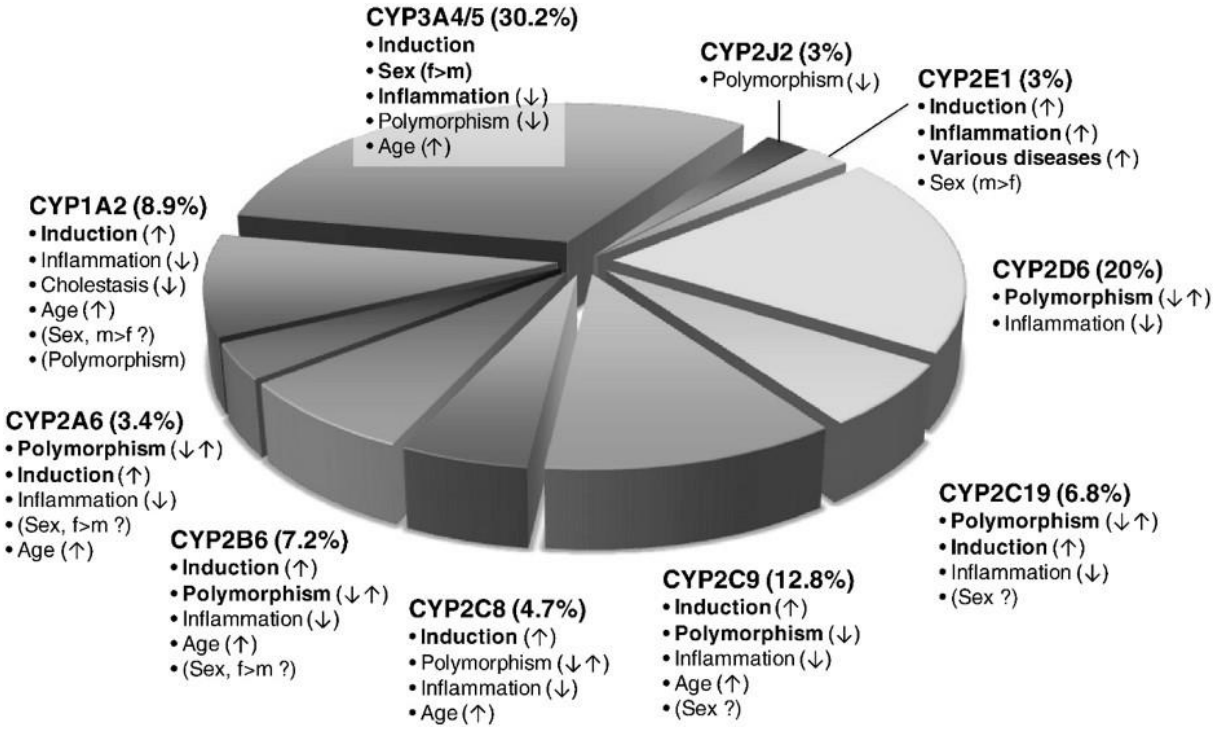

Figure 1. Proportions of cytochrome P450 (CYP) drug substrates used in clinical practice by their major metabolic pathways. CYP enzyme activity can be increased (↑) and/or decreased (↓) by multiple factors, the most important of which are depicted in bold. These factors include genetic polymorphism of CYP enzymes involved in antidepressant biotransformation (e.g. CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6 and CYP3A; compare Table 1). Adapted from Zanger and Schwab [2].

As an illustration, a relevant gene–drug pair is *CYP2D6* and the SSRI paroxetine. Paroxetine undergoes extensive first pass metabolism via CYP2D6, which forms an inactive metabolite in terms of serotonin reuptake inhibition [20]. Genetic variation of *CYP2D6* has repeatedly been associated with altered paroxetine exposure. This was also the case in two pharmacokinetic studies of patients taking a recommended daily dosage of 20 mg paroxetine (n = 108) by Gex-Fabry *et al.* and Charlier *et al*. On the one hand, patients identified by pharmacogenotyping as CYP2D6 UMs (n = 5) had significantly lower paroxetine steady-state plasma concentrations (Css) compared with CYP2D6 NMs. In fact, paroxetine plasma concentrations were below or just at the limit of detection for all CYP2D6 UMs. On the other hand, patients identified by pharmacogenotyping as CYP2D6 PMs (n = 8) had significantly higher paroxetine Css (127–346%) compared with CYP2D6 NMs [21,22]. In addition, Gex-Fabry *et al*. assessed therapy response (specified as a ≥ 50% reduction in the Montgomery-Asberg Depression Rating Scale) after four weeks of treatment with paroxetine. Notably, all the CYP2D6 UMs with sub-therapeutic paroxetine plasma concentrations (n = 4) did not achieve a persistent therapy response [21]. However, there are currently no conclusive reports directly linking CYP2D6 PMs with an increased risk of experiencing adverse reactions to paroxetine [14]. Still, it is conceivable that the reported significantly increased Css in CYP2D6 PMs may affect paroxetine tolerability. Based on the available data a CPIC guideline was published, which recommends the choice of an antidepressant other than paroxetine, which is not extensively metabolized via CYP2D6, for both CYP2D6 ultra-rapid and poor metabolizers [13]. This recommendation applies to a substantial, non-negligible part of the population. In fact, genetic CYP2D6 UMs and PMs are present in over 3% and 5% respectively of the European population [16].

Another pharmacokinetics-related genetic marker that has been associated with antidepressant response is *ABCB1*, which encodes the efflux transporter P-glycoprotein (P-gp). At the blood–brain barrier (BBB) P-gp has a protective function by extruding xenobiotics and drug molecules, including certain antidepressants [23]. Homozygous carriers of the *ABCB1* reference allele (wild type) are assumed to be less likely to respond to antidepressants that are P-gp substrates due to a reduced permeability of their BBB, limiting the antidepressants' concentration at their site of action [24]. This hypothesis is based on a very limited number of clinical studies linking two intronic *ABCB1* single nucleotide polymorphisms (rs2235015 and rs2032583) to antidepressant treatment outcome [24-26]. One of these studies is an analysis of 443 inpatients under antidepressive pharmacotherapy, where homozygous carriers of the rs2032583 reference T-allele had a significantly higher risk of therapy failure (depression non-remission) compared to carriers of the variant C-allele (62% vs. 25%) after six weeks of treatment with a P-gp substrate antidepressant (amitriptyline, paroxetine, venlafaxine, or citalopram) [24]. Although there are currently no mechanistic studies supporting the role of the aforementioned *ABCB1* polymorphisms in antidepressant efficacy, the Swiss Society for Anxiety and Depression (SGAD) suggests genotyping the P-gp polymorphisms rs2235015 and rs2032583 after antidepressant treatment failure [27].

In addition to the aforementioned genetic variants affecting the pharmacokinetic behavior of certain antidepressants, there is evidence that polymorphisms in pharmacodynamically relevant genes may affect antidepressant response [28]. These include the genes encoding proteins involved in serotonin signaling, such as the tryptophan hydroxylase (*TPH*) [29], the serotonin receptors (e.g. *5-HT1A*, and *-2A*) [30,31], and the serotonin transporter (*SLC6A4*) [32]. However, there is still ongoing debate

about whether genetic variants of antidepressant targets should be considered in clinical practice. To date, PGx guidelines and recommendations for the selection and dosing of antidepressants are not based on any pharmacodynamics-related gene variants [28]. Nevertheless, it is plausible that variants in both pharmacokinetics- as well as pharmacodynamics-related genes jointly influence the efficacy and tolerability of antidepressants (Figure 2).

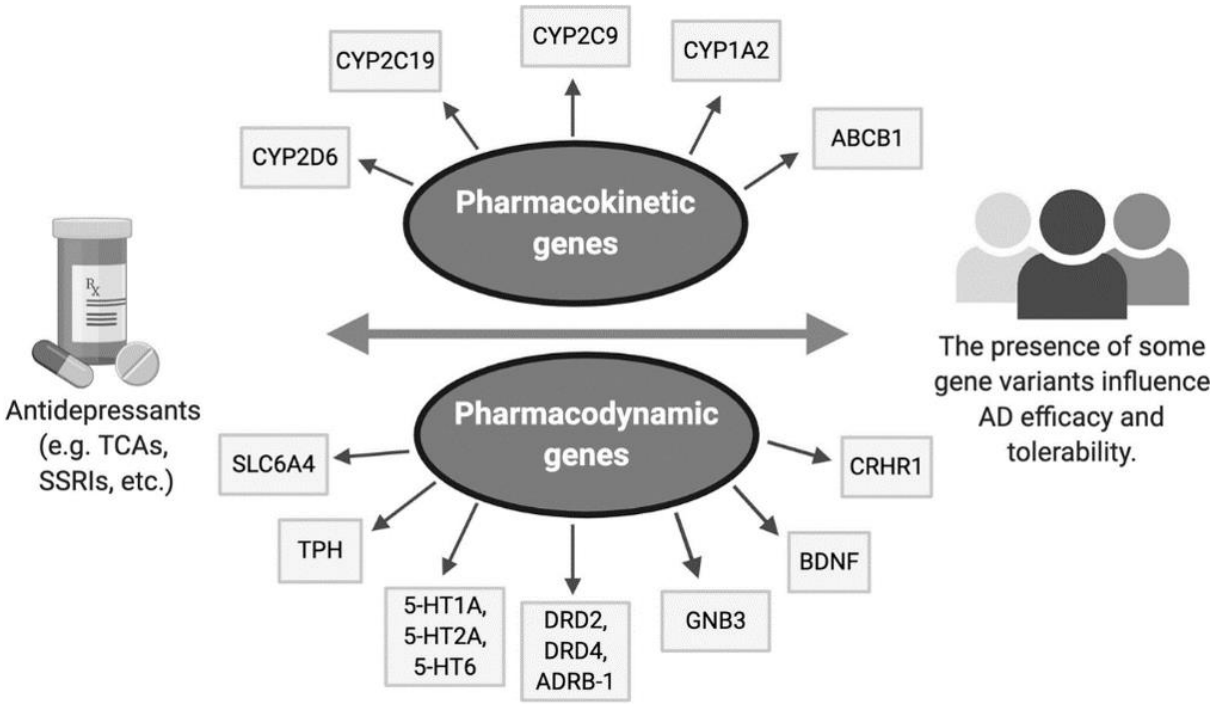

Figure 2. Overview of candidate genes associated with antidepressant (AD) efficacy and tolerability, e.g., selective serotonin reuptake inhibitors (SSRIs) and tricyclic antidepressants (TCAs). Pharmacokinetic candidate genes encode cytochrome P450 enzymes (*CYP*) and the P-glycoprotein transporter (*ABCB1*). Pharmacodynamic candidate genes encode the serotonin transporter (*SLC6A4*), the tryptophan hydroxylase (*TPH*), serotonin receptors (*5-HT*), dopamine receptors (*DRD*), the adrenoceptor (*ADR*), the guanine nucleotide-binding protein (*GNB3*), the brain-derived neurotrophic factor (*BDNF*) and the corticotropin-releasing hormone receptor (*CRHR1*). Used with permission of Springer Singapore from Islam et al. [28].

Depression is a common affective disorder affecting an estimated 5% of adults worldwide [33]. In Switzerland, the prevalence of major depressive disorder (MDD) was estimated at 8 to 10% of all people over 15 years of age in 2017 [34]. Recently, the situation surrounding the COVID-19 pandemic has additionally impacted mental health. In fact,

it is estimated that the COVID-19 pandemic triggered an increase of over 25% in MDD cases worldwide in 2020 [35].

A depressive episode is defined by at least two of three key symptoms, namely depressed mood, anhedonia and loss of energy. In addition, symptoms such as weight loss or -gain, sleep disorders, cognitive dysfunction, as well as suicidality and others, may be observed. A combination of the aforementioned symptoms needs to be present for at least two weeks in order to be classified as a depressive episode in accordance with the International Statistical Classification of Diseases and Related Health Problems (ICD-10, version 10) [36]. When referring to MDD, one commonly speaks about depressive disorder (single or recurrent episodes) with an at least moderate severity as defined by the ICD-10 (F32.1, F32.2, F33.1 or F33.2) [37]. For these patients, pharmacotherapy, in addition to psychotherapy, is a relevant pillar in their treatment [27,37]. Fortunately, clinicians and patients today can choose from a variety of antidepressants. However, treating MDD remains challenging, with around 50% of these patients responding inadequately to a first-line antidepressant [38,39].

Multiple depression rating scales are available to assess the course of depression and therapy outcome. One commonly used scale is the Hamilton Depression Rating Scale (HAM-D), which is scored by an external rater [40]. Additionally, there are also patient self-assessed scales, such as the Beck Depression Inventory (BDI) [41]. In general, a reduction of at least 50% in the scale baseline score is considered as therapy response [37].

Effective treatment is important because MDD imposes a high burden of disease on patients. Globally, depressive disorders rank 13th among the leading causes of disease burden [42]. More precisely, a cumulative annual health loss of 45.7 million disability

adjusted life years (DALYs) was estimated for MDD in 2019 [42]. DALYs are a quantitative indicator of health loss resulting from premature death and disability that make the burden of disease comparable between different conditions. As an illustration, MDD is estimated to have a similar global disease burden as lung cancer and malaria, which each accounted for about 1.8% of global DALYs in 2019 [42]. Moreover, the disorder challenges healthcare systems and society, particularly because of the costs incurred. Most of these costs are indirect and result from unemployment, sick leave and early retirement [43,44]. For Switzerland, it is estimated that an individual suffering from severe MDD generates annual direct and indirect costs of up to 32'800 euros. Cumulatively, the approximated annual cost of depression is over 8 billion euros, which corresponds to about 20% of the expenditure on healthcare in Switzerland [43]. Ineffective and intolerable antidepressant treatment can prolong the illness and interfere with continued medication adherence, increasing the burden on the patient, the healthcare system, and society. Since recent approvals of novel antidepressant drugs are scarce [45], it is conceivable that interventions to improve the response rates and tolerability of already available antidepressants are important. In particular, an early or even preemptive prediction of the antidepressant treatment outcome could be beneficial. Today, treatment response can only be assessed after about four weeks of antidepressant drug exposure [27], which can make a trial-and-error approach inefficient, in terms of both time and money. Therefore, the goal of several studies currently underway is to identify biomarkers for the prediction of antidepressant treatment outcome [e.g. 46,47]. However, as mentioned before, evidence is already being compiled on PGx affecting antidepressant response. Indeed, it is estimated that over

40% of interindividual differences in antidepressant response may be attributed to the effects of common genetic variation [48].

2.3 Pharmacogenetic testing to guide antidepressant therapy

At this point, it seems plausible that PGx testing may provide an opportunity to optimize drug selection and dosing in order to increase response rates and the tolerability of antidepressants. Today, commercial PGx tests that consider several polymorphic genes involved in antidepressant pharmacokinetic as well as pharmacodynamic behavior, so-called panel testing, are already offered and used [49]. Hitherto, however, only a limited number of mainly commercially sponsored, prospective clinical trials that test the influence of PGx-guided antidepressant therapy on patient outcomes have been conducted. The clinical trials available to date have shown some promising effects of PGx-guided treatment of MDD. Two recent meta-analyses pooled the risk ratios of (i) 6 (n = 799) and (ii) 4 (n = 1556) clinical studies, and found significantly improved antidepressant response rates for patients receiving combinatorial PGx panel testing compared to patients under treatment as usual that did not consider their genetic profile ((i) RR=1.36; (ii) RR=1.40) [50,51]. Nevertheless, PGx testing is not yet part of routine clinical practice when prescribing antidepressants in Switzerland.

Diverse barriers to the adoption of PGx have been described, which include lack of education among healthcare professionals, restricted reimbursement for PGx tests and the still limited evidence from prospective clinical trials [52]. Hitherto, in Switzerland, PGx testing could only be initiated by physicians. Only recently, a revision of the Swiss law on genetic investigations in humans was passed, allowing additional healthcare professionals, including pharmacists, to initiate PGx testing [53]. It can be surmised that

the expansion of pharmacists' competencies in PGx may enhance the accessibility of PGx testing for patients, improve the interprofessional collaboration between healthcare professionals in PGx and thereby support the further adoption of PGx in clinical practice. Still, to ensure coverage by health insurers, most PGx tests must be ordered by a specialized pharmacologist. This is particularly the case for genetic variants of *CYP2D6* and *CYP2C19*, for which there are recommendations for PGx-guided antidepressant selection and dosing [54].

As briefly mentioned in the beginning, other factors besides PGx can influence drug response. In particular, these may include non-genetic factors such as organ function, drug–drug interactions (DDI), food–drug interactions and medication adherence [2,3]. Thus, a patient identified as a CYP2D6 NM by genotyping, may become a phenotypic IM or even PM with concomitant administration of a CYP2D6-inhibiting agent. Indeed, for NMs it has been demonstrated that the antitussive and CYP2D6 model substrate dextromethorphan shows an increased metabolic ratio (dextromethorphan/dextrophan), comparable to CYP2D6 IMs or PMs, when the potent CYP2D6 inhibitor paroxetine was co-administered. This effect was more pronounced in individuals already carrying a non-fully functional *CYP2D6* allele (activity score < 2), leading to a significantly increased number of individuals converted to PMs (94% vs. 56%) [55]. This deviation from the genotype-predicted phenotype due to non-genetic factors is referred to as phenoconversion [56]. Today, PGx analyses often seem to focus on single drug–gene interactions (DGI) only. However, as previously described, several non-genetic factors can impact therapy response and may also modify genetically predisposed phenotypes [56]. Therefore, it is conceivable that an individualized evaluation of PGx information in the context of non-genetic factors such as co-

medication, renal and hepatic function, and others is of importance for a beneficial integration of PGx in clinical practices, such as the selection and dosing of antidepressants.

In pharmaceutical care, pharmacists aim to optimize the use of medicines and the health outcomes of individuals [57]. Pharmacist-led medication reviews are an established intervention in pharmaceutical care. An expert working group of the Pharmaceutical Care Network Europe (PCNE) recently proposed a definition of the term *medication review*: "Medication review is a structured evaluation of a patient's medicines with the aim of optimizing medicines use and improving health outcomes. This entails detecting drug-related problems and recommending interventions" [58]. The PCNE further describes three main sources of information when performing a medication review: (i) patient interview, (ii) medication history and (iii) clinical data [58]. In the evaluation of a patient's medication, pharmacists already consider a variety of accepted, inter-individual factors affecting drug response (e.g. DDI, age, weight, renal and hepatic function) [58]. Clinical data on PGx may provide supplementary information that allows for more comprehensive medication analysis and more individualized treatment recommendations. In order for the recommendations, developed by the pharmacist, to be considered in the treatment of the patient, close collaboration with the treating physician is important. The interprofessional collaboration between pharmacists and psychiatrists has been investigated before. In an inpatient psychiatry setting, pharmacist-led medication reviews and their subsequent interprofessional discussion significantly reduced the number of unsolved drug-related problems (DRP) by a factor of nearly 2, compared to a non-concurrent control group [59]. It was also reported that psychiatrists primarily seek the help of pharmacists in selecting medications [60]. As

described above, PGx information could make an important contribution to the selection of antidepressants. However, at least in Switzerland, no clear or formal structures are in place to support such advancements.

In summary, the effectiveness and tolerability of certain antidepressants has been linked to genetic predisposition [7,13,14,61]. So far, a limited number of clinical studies have shown promising effects when PGx information was available for patients with MDD under treatment with antidepressants [50,51]. In addition to genetic predisposition, other non-genetic factors can also affect the effectiveness and tolerability of antidepressants [56]. When reviewing medications, pharmacists are trained to consider a variety of factors that may affect drug response [58]. Hitherto, PGx has not been considered when prescribing antidepressants in routine clinical practice in Switzerland. The barriers to the adoption of PGx are manifold [52]. Involving pharmacists in PGx testing may create an opportunity to beneficially integrate PGx into routine antidepressant prescribing.

3 Aims of the thesis

The overall goal of the research presented in this thesis was to gain an understanding for PGx in the pharmaceutical care of MDD patients. This overall goal was addressed by the following aims:

- To study the role of PGx in patients who experienced inefficacy or adverse reactions to their antidepressant pharmacotherapy (addressed in Project A).
- To investigate the feasibility of integrating PGx into pharmaceutical care in clinical practice (addressed in Project B and C).
- To further assess the impact of the proposed pharmacist-led PGx service in MDD patients (addressed in Project D).

4 Thesis overview

The aforementioned aims of the thesis were approached with four overarching projects (A-D) which resulted in seven peer-reviewed publications (**Error! Not a valid bookmark self-reference.**).

Table 2. Overview of projects (A-D) and associated publications

Project A – PGx in patient cases with ADR and TF under antidepressant therapy	
A-1	Nonresponse to high-dose bupropion for depression in a patient carrying CYP2B6*6 and CYP2C19*17 variants: a case report Stäuble CK, Lampert ML, Mikoteit T, Hatzinger M, Hersberger KE, Meyer zu Schwabedissen HE
A-2	Severe adverse drug reactions to quetiapine in two patients carrying CYP2D6*4 variants: a case report Stäuble CK, Lampert ML, Mikoteit T, Hatzinger M, Hersberger KE, Meyer zu Schwabedissen HE
A-3	Case report: Non-response to fluoxetine in a homozygous 5-HTTLPR S-allele carrier of the serotonin transporter gene Stäuble CK, Meier R, Lampert ML, Mikoteit T, Hatzinger M, Allemann S, Hersberger KE, Meyer zu Schwabedissen HE
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D	Pharmacist-guided pre-emptive pharmacogenetic testing in antidepressant therapy (PrePGx): study protocol for an open-label, randomized controlled trial Stäuble CK, Lampert ML, Allemann S, Hatzinger M, Hersberger KE, Meyer zu Schwabedissen HE, Imboden C and Mikoteit T

5 Results

5.1 Project A

PGx in patient cases with ADR and TF under antidepressant therapy

Nonresponse to high-dose bupropion for depression in a patient carrying CYP2B6*6 and CYP2C19*17 variants: a case report [A-1]

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Case Report

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Pharmacogenomics

Nonresponse to high-dose bupropion for depression in a patient carrying *CYP2B6**6 and *CYP2C19**17 variants: a case report

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We report the case of a patient with major depression treated with high-dose bupropion due to prior detected subtherapeutic blood concentrations at standard dosing. Pharmacogenetic panel testing identified the patient as a carrier of the *CYP2B6**6 allele, which has been associated with reduced bupropion metabolism and decreased concentrations of the pharmacologically active metabolite hydroxybupropion. Interestingly, we also found the patient to be homozygous for the *CYP2C19**17 allele, predicting an ultra rapid metabolizer phenotype. We propose a combined effect of the detected *CYP2C19* and *CYP2B6* genetic variants on bupropion metabolism. This case underlines the potential benefit of pre-emptive pharmacogenotyping but also the yet still fragmentary evidence making precise pharmacogenotype guided antidepressant selection and dosing challenging.

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Keywords: antidepressant • bupropion • *CYP2B6* • *CYP2C19* • *CYP450* • depression • pharmacogenomics • psychiatry

Depressive disorders show interindividual courses of disease but often proceed in recurrent episodes. In fact, half of the affected patients suffer from at least one further episode after the first one [1] and up to 20 % even develop a chronic course of depression [2]. Consequently, the need for effective treatment is high. However, about 50% of treated unipolar depressive patients do not experience remission under a first-line antidepressant treatment [1]. Furthermore, clinical treatment response in an acute depressive episode can only be assessed after several weeks of drug exposure [3], potentially making finding an effective treatment time consuming and exhausting for the patient.

One of the compounds used in antidepressant therapy is the selective noradrenaline and dopamine reuptake inhibitor bupropion. Besides, its use as an antidepressant, bupropion is also approved for supportive therapy in smoking cessation. Furthermore, it is considered a probe substrate for CYP2B6 and is part of various phenotyping assays including the widely used Geneva cocktail [4].

The parent compound bupropion is extensively metabolized giving rise to three major metabolites namely hydroxy-, threohydro- and erythrohydrobupropion, which are all pharmacologically active [5]. Hydroxy- and threohydrobupropion are considered to play an important role in the antidepressant effect of bupropion [5]. However, the aforementioned hydroxybupropion is the most active metabolite, exhibiting 50% of the parent molecule's pharmacologic effect [6]. Hydroxybupropion is a product of cytochrome P450 mediated hydroxylation, predominantly catalyzed by CYP2B6 [7]. The stereoisomers threohydro- and erythrohydrobupropion are both formed by the carbonyl reductases HSD11B1 and the aldo-keto reductase AKR7 in the liver and intestine [8]. The aforementioned Phase I metabolites are then further glucuronidated or excreted directly via urine [9,10].

Besides these very well described steps in the metabolism of bupropion, there has recently been data published, proposing a relevant contribution of CYP2C19 in hydroxylation at the 4′ benzo position of bupropion,

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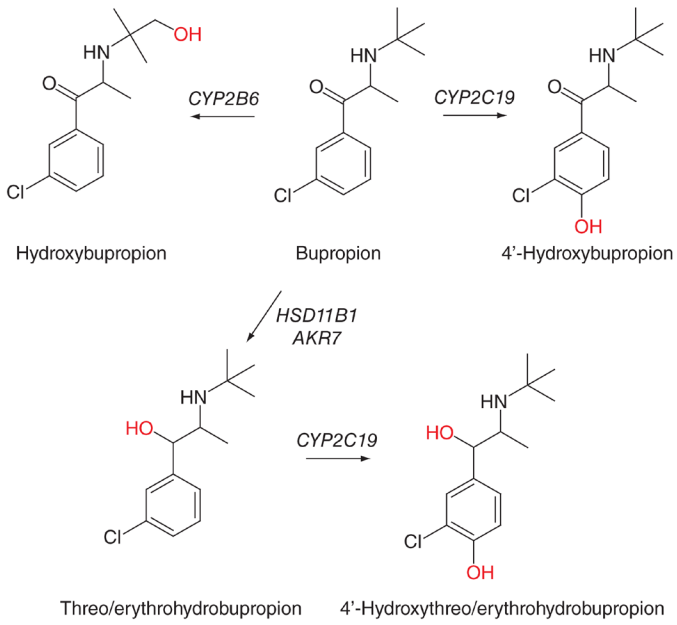

Figure 1. Illustration of the major steps in Phase I bupropion metabolism.

threohydro- and erythrohydrobupropion [11,12]. Hitherto, neither an antidepressant effect nor pharmacologic activity in general has been described for 4′-hydroxybupropion. However, the formation of the stereoisomers 4′-hydroxythreohydrobupropion and 4′-hydroxyerythrohydrobupropion via CYP2C19 was shown to be a major elimination pathway for the underlying active metabolites threohydro- and erythrohydrobupropion [11] (Figure 1).

Together with bupropion, current therapeutic drug monitoring (TDM) guidelines recommend assessment of the CYP2B6 product hydroxybupropion, as its serum concentration was found to be predictive for clinical response to bupropion in a psychiatric setting [13,14]. Moreover, bupropion's chemical instability is a relevant limitation for its use in TDM. Hence, TDM reference values of bupropion are primarily based on hydroxybupropion with only minor contribution of bupropion (850–1500 ng/ml) [13].

Case report

We herein report the case of a male, 38-year old patient, diagnosed with a recurrent depressive disorder (ICD-10 F33). He admitted himself to our hospital in December 2019 due to an acute deterioration of the current depressive episode with intercurrent visual and acoustic hallucinations. However, psychotic symptoms could not be clinically confirmed during the course of the hospitalization. He was diagnosed with a currently moderate depressive episode (ICD-10 F33.1) under treatment with a combination of bupropion (1050 mg/d), lithium (36.5 mmol/d) and trazodone (200 mg/d) at admission. Notably, the recommended maximum daily dosage of bupropion in depression treatment according to Swiss summary of product characteristics is 300 mg. Upon request, the prescribing outpatient psychiatrist explained to have performed this unusual dose escalation due to clinical ineffectiveness under regular doses of bupropion and based on TDM data (Table 1). Importantly, the patient has a medication history with inefficacy of various antidepressant pharmacotherapies since the age of 22. According to the patient, the so far unsuccessful antidepressant treatment attempts included escitalopram, sertraline, paroxetine, duloxetine, clomipramine, agomelatine, moclobemide, venlafaxine, fluvoxamine, fluoxetine, mirtazapine and reboxetine.

During the first 7 days of hospitalization bupropion dosage was steadily reduced to 300 mg per day. Resulting in subtherapeutic trough serum levels after reaching steady state (Table 1). Importantly, there was no evidence neither for a lack of adherence nor for drug–drug interactions explaining the low steady state serum concentration.

Table 1. Collected trough serum levels of total bupropion + hydroxybupropion with relevant therapeutic reference range of 850–1500 ng/ml, analyzed applying high-performance liquid chromatography-mass spectrometry/mass spectrometry.

Collection date	Bupropion dosage (mg/d)	Trough serum level (ng/ml)	Setting
07 November 2019	400	412	Outpatient
28 November 2019	1050	1003	Outpatient
17 December 2019	300	396	Inpatient

Table 2. Selected results of panel-pharmacogenotyping and phenotype interpretation.

Gene	Variant	Genotype	Predicted phenotype
CYP1A2	rs762881 g.75041917C>A (in *1F)	A/A	Increased inducibility (UM)
CYP2B6	rs3745274 c.516G>T (in *6)	G/T	Reduced function (IM)
CYP2C19	rs12248560 g.4195C>T (in *17)	T/T	Increased function (UM)
CYP2D6	rs3892097 c.506-1G>A (in *4)	G/A	Reduced function (IM)
CYP2D6	rs1065852 c.100C>T (in *4 and *10)	C/T	Reduced function (IM)
ABCB1	rs2032583 c.2685 + 49T>C	T/T	Wild type

IM: Intermediate metabolizer; UM: Ultra rapid metabolizer.

Meanwhile, adequate lithium serum levels (0.97 mmol/l, therapeutic reference range: 0.6–1.0 mmol/l) and normal range of hepatic – and kidney function laboratory values were detected (e.g., serum creatinine, total bilirubin, ALAT and ASAT).

Due to the patient's medication history with 16 years of difficulties in therapeutic management and the known involvement of CYP2B6 in metabolism of bupropion, a pharmacogenetic consultation by clinical pharmacists of the hospital was conducted after written informed consent of the patient. This intervention is part of an observational study approved by the local ethics committee. Panel-pharmacogenotyping was conducted applying the commercial service Stratipharm® offered by humatrix AG (Pfungstadt, Germany). In their laboratory, the polymorphisms are determined applying real-time PCR using the automated Life Technologies QuantStudio 12 k flex (Thermo Fisher, MA, USA) with the respective optimized and commercially available chemistry. Interpretation of the genotype identified the patient as CYP2C19 ultra rapid metabolizer (UM; *17 homozygous) and CYP2D6 intermediate metabolizer (IM; *4 heterozygous), while also exhibiting the genotypes resulting in reduced function of CYP2B6 (*6 heterozygous) and increased inducibility of CYP1A2 (*1F homozygous). Furthermore, the patient shows no variation at the analyzed *ABCB1* gene locus (Table 2). Especially the present CYP2C19 predicted ultra rapid metabolizer phenotype could very likely be an important cause for many of the previously ineffective antidepressant therapies (i.e., escitalopram, sertraline, moclobemide and clomipramine). Additional genetic markers might also have played a role in the patient's history of antidepressant therapy failure, including *CYP1A2**1F (i.e., agomelatine and duloxetine) and *ABCB1* wild type (i.e., escitalopram and venlafaxine).

Based on the patient's medication history, his genetic profile and the known contribution of CYP1A2, CYP2C19 and CYP2D6 to the metabolism as well as ABCB1 to the transport of various antidepressants, the following substances would have been recommended to be used in this patient: trazodone or mianserin. However, due to the long history of antidepressant treatment resistance and chronification of the depressive disorder, the patient refused further adaption of the antidepressant pharmacotherapy and was referred to a clinic specialized in electroconvulsive therapy for further treatment.

Discussion

Considering that both cytochromes CYP2B6 and CYP2C19, which are extensively involved in bupropion metabolism, are highly polymorphic, one might expect a relevant impact of genetic variability on interindividual differences of pharmacokinetics and eventually response to bupropion.

The impact of *CYP2B6* polymorphisms on bupropion pharmacokinetics has been studied before, associating *CYP2B6**6 allele carriers with reduced bupropion metabolism and decreased hydroxybupropion serum concentrations [15–17]. The pharmacogenomics knowledge for personalized medicine database rates this drug–gene interaction with moderate evidence and likely functional significance (level 2A) [18]. On the other hand, there is so far only

limited evidence linking *CYP2C19* genetic variation to altered bupropion pharmacokinetics. Hitherto, only a limited number of studies have been published, investigating the effect of *CYP2C19* polymorphisms on bupropion pharmacokinetics and therapeutic effectiveness [19,20]. The most conclusive study that was able to differentiate between metabolizer phenotypes, showed an impact of the predicted reduced activity variant (*CYP2C19**2) on bupropion, threohydro- and erythrohydrobupropion blood concentrations. However, in this trial the *17 variant did not reach statistical significance due to under-representation in a study population of 42 healthy volunteers undergoing smoking cessation [19]. Nevertheless, as mentioned before several studies were able to provide evidence for the impact of CYP2C19 on bupropion pharmacokinetics [11,12], supporting the hypothesis that *CYP2C19* genetic variation might play a role in modulating bupropion metabolism.

The predicted CYP2C19 ultra rapid metabolizer status, overall, potentially causes increased metabolic degradation of the active substance via CYP2C19. Furthermore, the predicted reduced CYP2B6 activity might even enhance the alternative elimination pathway via CYP2C19. In conclusion, we attribute the striking subtherapeutic bupropion serum levels and observed unsatisfactory therapy response to a combined effect of the detected *CYP2C19* and *CYP2B6* genetic variants. Notably, we are reporting the case of a single patient in clinical routine care. Accordingly, this certainly limits the conclusions that can be or should be drawn from the observation. Another limitation is that due to the routine care setting, where a psychiatrist not linked to our clinic has also taken part in the patient's treatment, we are restricted to the data we are reporting. Importantly, we do not have access to any further blood samples, which could be used to determine the levels of any of the bupropion metabolites to strengthen our hypothesis.

There is yet more research needed to fully understand the possibly combined impact of *CYP2B6* and *CYP2C19* genotypes on bupropion metabolism and therapeutic response in depression therapy. According to pharmacogenomics knowledge for personalized medicine database, the average frequency of both *CYP2B6**6 and *CYP2C19**17 alleles is over 20 % in the European population [18], which emphasizes the potential clinical importance of these variants. Currently there are no guidelines available for genotype guided dosing and selection of bupropion.

It may be speculated, that with an early-panel pharmacogenotyping the patient may have been treated more adequately in terms of compound selection and dosing. However, there is still not enough evidence to formulate precise and definite recommendations for drug and dose selection in antidepressant pharmacotherapy. This case shows the need to perform further prospective studies to examine the use of pre-emptive pharmacogenotyping and thereof driven therapeutic recommendations.

Executive summary

Case report
- A male, 38-year old patient, diagnosed with a recurrent depressive disorder (ICD-10 F33), suffering from a moderate depressive episode (ICD-10 F33.1) at clinic admission.
- The patient shows a long history of unsatisfactory response to various antidepressants.
- Detection of subtherapeutic bupropion serum levels under recommended maximum daily dosage.

Selected results of panel-pharmacogenotyping
- *CYP2B6**6/*1 (reduced function and intermediate metabolizer), *CYP2C19**17/*17 (increased function and ultra rapid metabolizer).

Discussion
- The role of CYP2B6 and CYP2C19 in bupropion pharmacokinetics is well described.
- The evidence showing an impact of genetic variation on bupropion pharmacokinetics is still limited, especially for *CYP2C19*.
- We propose a combined effect of the detected *CYP2C19* and *CYP2B6* genetic variants on bupropion metabolism.

Conclusion
- There might be a potential benefit in early panel-pharmacogenotyping for antidepressant selection and dosing.
- The evidence to allow precise antidepressant selection and dosing based on results from pharmacogenotyping is still fragmentary.

Author contributions

CK Stauble, ML Lampert, KE Hersberger and HE Meyer zu Schwabedissen were involved in the study design. CK Stäuble, ML Lampert and HE Meyer zu Schwabedissen were responsible for the interpretation of the genotyping data. T Mikoteit and M Hatzinger were responsible for the psychiatric clinical assessments. CK Stauble wrote a first version of the manuscript and T Mikoteit,

M Hatzinger, ML Lampert, KE Hersberger and HE Meyer zu Schwabedissen contributed additional content and were responsible for critical revision.

Financial & competing interests disclosure

CK Stäuble receives funding from the Senglet Foundation (Stiftung zur Förderung des pharmazeutischen Nachwuchses in Basel) in Basel, Switzerland. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was involved in the preparation of the manuscript.

Ethical conduct of research

The local ethics committee approved the underlying observational study (2019-01452).

Informed consent disclosure

The authors state that they have obtained verbal and written informed consent from the patient/patients for the inclusion of their medical and treatment history within this case report.

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Severe adverse drug reactions to quetiapine in two patients carrying *CYP2D6**4 variants: A case report [A-2]

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Case Report

Severe Adverse Drug Reactions to Quetiapine in Two Patients Carrying *CYP2D6**4 Variants: A Case Report

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Abstract: We report two cases of patients who developed severe adverse drug reactions including persistent movement disorders, nausea, and vertigo during treatment with quetiapine at maximum daily doses ranging between 300 and 400 mg. The extensive hepatic metabolism of quetiapine is mainly attributed to cytochrome P450 3A4 (CYP3A4). However, there is recent evidence supporting the idea of CYP2D6 playing a role in the clearance of the quetiapine active metabolite norquetiapine. Interestingly, both patients we are reporting of are carriers of the *CYP2D6**4 variant, predicting an intermediate metabolizer phenotype. Additionally, co-medication with a known CYP2D6 inhibitor and renal impairment might have further affected quetiapine pharmacokinetics. The herein reported cases could spark a discussion on the potential impact of a patient's pharmacogenetic predisposition in the treatment with quetiapine. However, further studies are warranted to promote the adoption of pharmacogenetic testing for the prevention of drug-induced toxicities associated with quetiapine.

Keywords: pharmacogenetics; pharmaceutical care; psychiatry; depression; neuroleptics; antipsychotics; quetiapine; CYP2D6; CYP3A4; adverse drug reaction

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1. Background

Patients suffering from major psychiatric disorders often need long-term pharmacotherapy in order to reach remission and prevent relapse. Considering that, it seems even more important to select and prescribe safe and well-tolerated pharmacotherapies. However, interindividual variability in response to psychotropic drugs is well known, and adverse drug reactions (ADRs) are common. In fact, severe ADRs, requiring or prolonging hospitalization or limiting self-care and activities of daily life, are on average reported for 1–2% of psychiatric inpatients under treatment with psychotropic drugs [1–3]. In particular, excessive systemic drug exposure may increase the risk of experiencing unwanted side effects and toxicity. Apart from dosing errors, increased systemic drug exposure can also occur under regular dosing and may be attributed to drug–drug or food–drug interactions, impaired renal or hepatic elimination, and notably, individual genetic predisposition. The latter is of relevance when polymorphisms affect the expression and/or activity of genes encoding enzymes and transporters involved in drug absorption, distribution, metabolism, or excretion (ADME). As an illustration, cytochrome P450 2D6 (CYP2D6), which is highly polymorphic, has several known genetic variants translating into increased, reduced, or even lacking enzyme activity. Accordingly, phenotypes are termed ultrarapid, intermediate, or poor metabolizers, respectively. Systemic exposure of active drug molecules that are extensively metabolized by CYP2D6 may be elevated in individuals carrying genetic

variants translated into CYP2D6 enzymes with reduced or no activity [4]. In the case of several antipsychotics, namely, aripiprazole, haloperidol, risperidone, and zuclopenthixol, a gene–drug interaction with CYP2D6 has been rated as actionable, meaning that there is clinical evidence for dose adaptation to the respective geno- or phenotype. Accordingly, to prevent toxicities, a dose reduction is recommended for patients with predicted reduced CYP2D6 activity [5], and drug labels draw attention to possible risks [6]. Currently, no such pharmacogenetic recommendations are available for the widely prescribed atypical antipsychotic quetiapine.

Quetiapine is indicated and approved for the treatment of schizophrenia and bipolar disorder but also as a supplementary treatment for depressive episodes in patients inadequately responding to antidepressant monotherapy [7]. It is known that, in contrast to typical antipsychotics, quetiapine and its main active metabolite norquetiapine show increased selectivity for the serotonin receptor 2A (*HTR2A*) over the dopamine receptor (*DRD2*) [8,9] and are therefore associated with a limited risk of extrapyramidal symptoms [10]. Quetiapine exhibits an antagonistic mechanism of action at the aforementioned receptors, which is assumed to be responsible for its antipsychotic effect [11]. Moreover, quetiapine is also effective as an augmentation in the treatment of depressive episodes [7], which is attributed to its active metabolite norquetiapine and its high affinity for both the noradrenaline transporter (*SLC6A2*) and the serotonin receptor 1A (*HTR1A*), towards which it was shown to exhibit inhibitory and partial-agonistic activity, respectively [12,13]. Apart from the abovementioned targets, responsible for the therapeutic effect of quetiapine, there are also several known off-target interactions assumed to be linked to some of the frequently reported side effects in the treatment with quetiapine [14]. Both quetiapine and norquetiapine, for example, show relevant affinity for and antagonistic activity upon binding to the histaminergic (*HRH1*) and the adrenergic alpha 1 (*ADRA1*) receptors [12], which may cause symptoms like sedation and hypotension [7]. Furthermore, norquetiapine also binds to muscarinic (*CHRM1*) receptors [12], which may cause the often-observed anticholinergic side effects including dry mouth, constipation, and tachycardia [7]. Following absorption, quetiapine undergoes extensive hepatic metabolism mainly catalyzed by CYP3A4, which, inter alia via *N*-dealkylation, gives rise to the main active metabolite norquetiapine (*N*-desalkyl-quetiapine) (Figure 1) [15]. Even if less than 5% of the unaltered mother substance is renally eliminated, over 70% of quetiapine metabolites are excreted via urine [7]. Currently, dose adaptation is not recommended for renally impaired patients [7].

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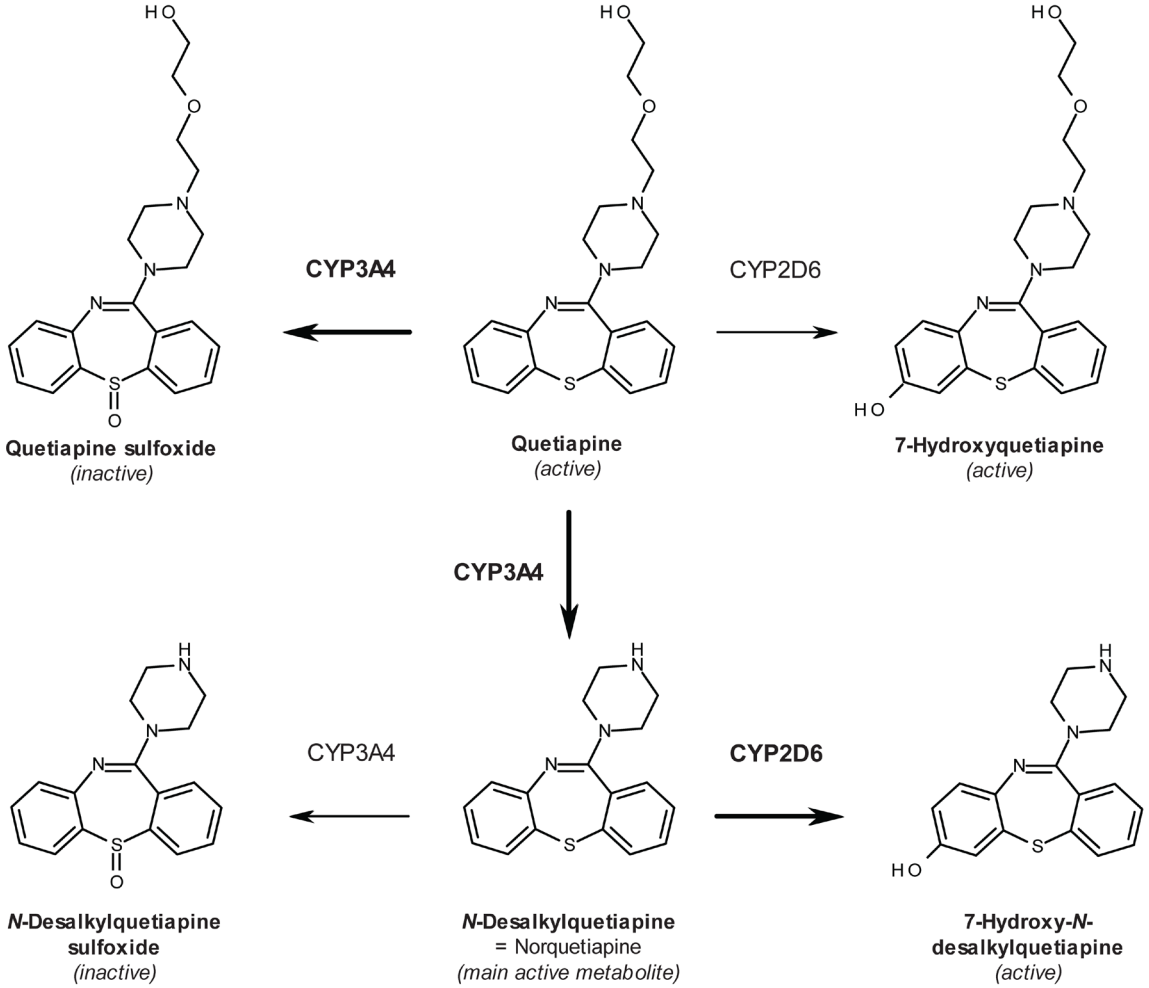

Figure 1. Illustration of major steps in phase I metabolism of quetiapine.

2. Case Presentation

We herein report two cases of severe ADRs related to quetiapine in patients who received a pharmacogenetic consultation by clinical pharmacists. Currently, this consultation is part of an observational study approved by the local ethics committee (EKNZ ID: 2019-01452), and the patients' consent was obtained prior to the intervention (ClinicalTrials.gov identifier: NCT04154553). Panel-pharmacogenotyping was conducted by using the commercial service Stratipharm® offered by humatrix AG (Pfungstadt, Germany). In their laboratory, polymorphisms are determined by applying real-time PCR using the automated Life Technologies QuantStudio 12 k flex (Thermo Fisher, Waltham, MA, USA) with the respective optimized and commercially available chemistry.

2.1. Case #1: Movement Disorder and Constipation

A 63-year-old male patient with a history of bipolar affective disorder (ICD-10 F31) type II, was admitted to our clinic for inpatient treatment due to an acute worsening of a depressive episode. Herein, he was diagnosed with a currently moderate depressive episode (ICD-10 F31.3) quantified by a rater-assessed 21-item Hamilton Rating Scale of Depression (HAM-D21) [16] with a score of 27 and a patient-assessed Beck Depression

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Inventory (BDI) [17] with a score of 31. Prior out-patient treatment attempts of the current depressive episode with trazodone 150 mg daily and later agomelatine 50 mg daily were ineffective. Furthermore, due to a previously diagnosed hypertensive and arrhythmogenic cardiomyopathy with an implanted cardiac pacemaker and a history of venous thrombosis, he was under co-medication with rivaroxaban (20 mg/d), eplerenon (25 mg/d), azilsartan (40 mg/d), and chlorthalidone (12.5 mg/d) (Table 1). Additionally, a history of congenital ureteral stenosis and thereafter unilateral nephrectomy caused a chronic renal insufficiency currently staged G3a, with measured eGFR CKD-EPI between 46 and 55 mL/min/1.73 m^2. As stated above, the goal of the current hospitalization was the adjustment of medications in order to treat the bipolar disorder currently presenting a moderate bipolar II depression. Therefore, vortioxetine was added to the already installed agomelatine and dosed up to 20 mg daily. Concomitantly, a treatment with quetiapine was started as first-line medication for bipolar depression and as an augmentation to the antidepressant treatment. For an optimal effect, the administration of quetiapine was split into two doses, an extended-release (XR) evening dose and a non-retarded night dose. Herein, quetiapine dosage was gradually increased over the course of three weeks to cumulative 400 mg daily (Table 1). Upon reaching this maximum dosage, the patient suddenly showed a strong sedation and severe movement disorders, which manifested as a persistent tremor. At the same time, the patient also complained of severe constipation. Thus, a laxative was prescribed, and quetiapine dosage was again reduced to 100–200 mg daily, which was well tolerated by the patient and led to remission of the aforementioned side effects. However, after one month in the clinic, the patient still showed no significant clinical improvement in depression. Therefore, the antidepressant treatment was again changed from vortioxetine to bupropion with a well-tolerated maximum dosage of 300 mg. Moreover, the patient was simultaneously referred to a consultation by clinical pharmacists of the hospital for an in-depth medication review including pharmacogenetic testing and counselling. Interpretation of the genotyping results identified the patient as a CYP2D6 intermediate metabolizer (IM, *4 heterozygous), CYP2C19 intermediate metabolizer (IM, *2 heterozygous), and CYP2B6 wildtype (WT, *1 homozygous) phenotype. Furthermore, the patient showed genetic variants resulting in increased inducibility of CYP1A2 (*1F homozygous) and no variation in the *ABCB1* polymorphism rs2032583 (Table 2). Based on these results, the switch to bupropion was considered appropriate, and no further antidepressant medication change was recommended. Indeed, the patient could finally be discharged after 9 weeks of hospitalization under remission, quantified by a HAM-D21 score of 6 and a BDI score of 11.

Table 1. Case #1 medication at the time of the reported severe ADRs.

Substance	Schedule
Quetiapine XR [1] 200 mg	0-0-1-0
Quetiapine 200 mg	0-0-0-1
Agomelatin 25 mg	0-0-0-2
Vortioxetine 10 mg	2-0-0-0
Lactitol 667 mg/mL	20-0-0-20 mL
Rivaroxaban 20 mg	1-0-0-0
Eplerenon 25 mg	1-0-0-0
Azilsartan/Chlorthalidone 20/12.5 mg	1-0-0-0

[1] XR: extended release.

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Table 2. Case #1 selected results of the panel-pharmacogenotyping and phenotype interpretation.

Gene	Variant *(Also Tested Variants in Gene Locus)*	Genotype	Predicted Phenotype
CYP1A2	rs762551 g.75041917C > A (in *1F) *(rs2069514)*	A/A	Increased inducibility
CYP2B6	*(rs8192709, rs28399499, rs3745274)*	WT [4], *1	Normal function (NM [1])
CYP2C19	rs4244285 c.681G > A (in *2) *(rs4986893, rs12248560, rs28399504)*	G/A	Decreased function (IM [2])
CYP2D6	rs3892097 c.506-1G > A (in *4) rs1065852 c.100C > T (in *4) *(CNV, rs35742686, rs5030655, rs5030867, rs5030865, rs5030656, rs201377835, rs28371706, rs59421388, rs28371725)*	G/A C/T	Decreased function (IM [2])
CYP3A4	rs2242480 c.1026+12G > A (in *1B) *(rs2740574)*	G/A	Substance specific function
CYP3A5	rs776746 c.219-237A > G (in *3)	G/G	No function (PM [3])
ABCB1	rs2032583 c.2685+49T > C *(rs1045642, rs1128503, rs2032582)*	T/T (WT [4])	Substance specific function

[1] NM: normal metabolizer; [2] IM: intermediate metabolizer; [3] PM: poor metabolizer; [4] WT: wild type.

2.2. Case #2: Emesis and Vertigo

A 26-year-old male was admitted to our clinic after a suicide attempt. Due to untreated, pre-existing arterial hypertension and tachycardia (diastolic pressure >100 mmHg and heart rate >100 bpm) at clinic entry, first of all, a treatment with lisinopril 7.5 mg daily was prescribed. In the further course of hospitalization, the patient was diagnosed with a moderate depressive episode (ICD-10 F32.1) based on clinical symptoms, predominantly sadness, anhedonia, amotivation, anxiety, pessimism, and insomnia. Subsequently, an antidepressant treatment with escitalopram 10 mg daily was initiated, with good tolerance. Meanwhile, due to pronounced circling thoughts and tension, an additive treatment with quetiapine at 50 mg daily was started. In the fourth week of hospitalization, the patient showed continuous tachycardia (heart rate >100 bpm), whereupon treatment with metoprolol 25 mg daily was started, and the dosage of the already established lisinopril was increased to 10 mg daily. At the same time, due to persistent sleeping disorder and circling thoughts, the dosage of quetiapine was increased to cumulative 300 mg daily over a period of 5 days (Table 3). Upon reaching the maximum quetiapine dosage, the patient suddenly developed massive and continuous emesis and vertigo with an unsteady gait. Due to lack of recovery after two days, the patient was transferred to the medical department for further evaluation. After cardiological and neurological assessment, the patient was diagnosed with a postural orthostatic tachycardia syndrome (normotonic, heart rate > 100 bpm). As a first intervention, quetiapine was slowly reduced and finally discontinued. Furthermore, as advised by internists and neurologists, lisinopril was stopped as well, and metoprolol dosage was increased to 75 mg, administered in two doses. Thereby, the aforementioned severe ADRs remitted. In the further course, escitalopram dosage was increased to 20 mg daily, and low-dose trazodone 100 mg daily plus pregabalin up to 150 mg daily, indicated for anxiety-related sleep-onset insomnia, were successfully established. Thus, the sleeping disorder and the depression improved markedly. Meanwhile, due to the aforementioned severe side effects, the patient was referred to a consultation by clinical pharmacists of the hospital for an in-depth medication review including pharmacogenetic testing and counselling. Interpretation of the genotyping results identified the patient as a CYP2D6 intermediate metabolizer (IM, *4 heterozygous), CYP2C19 intermediate metabolizer (IM, *2 heterozygous), and CYP2B6 wildtype (WT, *1 homozygous) phenotype. Furthermore, the patient showed genetic variants resulting in increased inducibility of CYP1A2 (*1F heterozygous), and no variation in the *ABCB1* polymorphism rs2032583 (Table 4). Based on

these results and the continuous clinical improvement of the patient, no further adjustments of the medication were necessary, and the patient was discharged in a stabilized condition after 12 weeks of inpatient treatment.

Table 3. Case #2 medication at the time of the reported severe ADRs.

Substance	Schedule
Quetiapine XR [1] 200 mg	0-0-1-0
Quetiapine 100 mg	0-0-0-1
Escitalopram 10 mg	1-0-0-0
Metoprolol DR [2] 25 mg	1-0-0-0
Lisinopril 10 mg	1-0-0-0

[1] XR: extended release; [2] DR: delayed release.

Table 4. Case #2 selected results of the panel-pharmacogenotyping and phenotype interpretation.

Gene	Variant *(Also Tested Variants in Gene Locus)*	Genotype	Predicted Phenotype
CYP1A2	rs762551 g.75041917C > A (in *1F) *(rs2069514)*	C/A	Increased inducibility
CYP2B6	*(rs8192709, rs28399499, rs3745274)*	WT [4], *1	Normal function (NM [1])
CYP2C19	rs4244285 c.681G > A (in *2) *(rs4986893, rs12248560, rs28399504)*	G/A	Decreased function (IM [2])
CYP2D6	rs3892097 c.506-1G > A (in *4) rs1065852 c.100C > T (in *4) *(CNV, rs35742686, rs5030655, rs5030867, rs5030865, rs5030656, rs201377835, rs28371706, rs59421388, rs28371725)*	G/A C/T	Decreased function (IM [2])
CYP3A4	*(rs2242480, rs2740574)*	WT [4], *1	Substance-specific function
CYP3A5	rs776746 c.219-237A > G (in *3)	G/G	No function (PM [3])
ABCB1	rs2032583 c.2685+49T > C *(rs1045642, rs1128503, rs2032582)*	T/T	Substance-specific function

[1] NM: normal metabolizer; [2] IM: intermediate metabolizer; [3] PM: poor metabolizer; [4] WT: wild type.

3. Discussion and Conclusions

We report on two patients experiencing pronounced adverse drug reactions. In the first case, the patient showed a sudden onset of severe movement disorders and constipation after increasing the quetiapine daily dose to 400 mg. In a second case, the patient developed persistent nausea and vertigo, diagnosed as a postural orthostatic tachycardia syndrome, when the daily dosage of quetiapine was increased to 300 mg. All of the aforementioned side effects are observed frequently (1–10%) to very frequently (>10%) in patients treated with quetiapine [7]. Due to the temporal relationship between the onset of strong symptoms and the increase of quetiapine dosage, an excessive, systemic exposure to quetiapine could be suspected. However, in both cases, quetiapine blood concentrations were not measured as part of the clinical routine. Rather, the treating physicians attempted a quetiapine dose reduction, which led to remission of the afore-described side effects in both cases and, as a result, may further support the hypothesis of dose-dependent induced adverse reactions to quetiapine. A closer look at the pharmacogenetic profiles revealed that both patients carry a *CYP2D6**4 variant, most likely translating into an enzyme with reduced activity and giving rise to the so-called intermediate metabolizer phenotype. Even if there are no recommendations on quetiapine use or dosing in patients genotyped for CYP2D6, we want to highlight that there are data supporting a role for this enzyme in quetiapine metabolism alongside with CYP3A4. More precisely, CYP2D6 was found to catalyze the 7′-hydroxylation of quetiapine and its active metabolite norquetiapine, leading

to the formation of active metabolites, namely, 7-hydroxyquetiapine and 7-hydroxy-N-desalkylquetiapine (Figure 1) [18,19]. However, 7′-hydroxylation via CYP2D6 might be an important route of clearance for the main active metabolite norquetiapine, as in vitro data showed a significantly higher affinity for CYP2D6 compared to CYP3A4 (Figure 1) [19]. This is further supported by clinical data showing that the intake of strong CYP3A4 inductors influences quetiapine but exhibits only a limited effect on norquetiapine serum concentration [20]. Moreover, *CYP2D6* polymorphisms with predicted reduced activity have been associated with increased norquetiapine serum concentrations by 22 and 30% for intermediate and poor metabolizers, respectively, compared to normal metabolizers [21]. It seems noteworthy in this context that clinical data showed serum concentrations of norquetiapine at steady state to be almost two-fold higher compared to those of quetiapine [20]. In addition, the elimination half-life of norquetiapine was reported to be of 12 h, which is notably longer compared to 7-h half-life reported for the mother substance quetiapine [7]. Distinct differences between quetiapine and norquetiapine can also be found in their pharmacologic profiles. Apart from the postulated norquetiapine antidepressant activity via interaction with the noradrenaline transporter (*SLC6A2*) and the serotonin receptor 1A (*5HTR1A*), a remarkably higher affinity for the histamine H1 (*HRH1*) and muscarinic M1 (*CHRM1*) receptors was detected, compared to quetiapine [12]. These histaminergic and muscarinic off-target effects may be associated with some of the known side effects under treatment with quetiapine [7,12] and may also be associated with the observed side effects in the herein reported cases, including drowsiness, nausea, sedation, constipation, and tachycardia.

We further found that, in both cases, additional factors might have influenced quetiapine clearance. In the first case, the patient exhibited a relevant renal impairment, which may have further slowed down drug clearance, as over 70% of the partly active quetiapine metabolites are excreted renally [7]. However, quetiapine dosage reduction is currently not recommended for renally impaired patients, and studies on the topic are sparse. It may be speculated that reduced CYP2D6 activity and renal impairment may have had an additive effect on the overall clearance of quetiapine and its metabolites. In the second case, the patient was co-medicated with escitalopram, a known CYP2D6 inhibitor [22,23]. Due to his genetic predisposition, with an already reduced CYP2D6 activity, this might have additively affected quetiapine clearance. Phenoconversion is the deviation from an individual's genotype-predicted phenotype and is caused by nongenetic factors such as comedication, comorbidities, or nutrition [24]. It is suspected that, especially in the case of genetic intermediate metabolizers, the addition of an enzyme inhibitor may lead to the phenotypic display of an actual poor metabolizer [24]. In the first case, switching to the known CYP2D6 inhibitor bupropion [25] was, however, well tolerated in combination with quetiapine at the already lowered dosage of 100–200 mg daily. This may point out the importance of pre-emptive measures such as dose reduction to support the prescription of safe and efficient therapies in cases like these. For the second case, we want to mention that it should certainly be realized that the reported ADRs may also be linked to the antihypertensive medication initiated at hospitalization. Indeed, side effects including nausea and vertigo are also reported for metoprolol and lisinopril. Additionally, metoprolol clearance may as well be affected by alterations in CYP2D6 activity [7]. However, after remission of the reported ADRs, the patient well tolerated an increase of metoprolol dosage from 25 to 75 mg daily. Still, the reported, pronounced adverse effects may be conclusively linked to quetiapine, taking into account additive factors, such as genetic predisposition, comedication, and renal function, likely affecting its pharmacokinetics.

At present, the impact of CYP2D6 and its genetic variants on overall quetiapine and, especially, norquetiapine clearance is still not well elucidated, and further research is needed to allow a recommendation for its management in clinical practice. On the one hand, cases like these, including our recently reported cases on antidepressants and tamoxifen [26,27], point out the complexity and the yet still fragmentary available evidence, making the integration of pharmacogenetic data into clinical practice challenging. On the

other hand, the consideration of pharmacogenetic predispositions may offer additional insights for a better understanding of adverse drug reactions as well as of non-response and create an opportunity for healthcare professionals to further enhance safety and effectiveness of marketed drugs.

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Institutional Review Board Statement: The underlying observational study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the local ethics committee of "Ethikkommission Nordwest- und Zentralschweiz" (2019-01452, 3 October 2019).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available for ethical and privacy reasons.

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Case report: Non-response to fluoxetine in a homozygous 5-HTTLPR S-allele carrier of the serotonin transporter gene [A-3]

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Case report: Non-response to fluoxetine in a homozygous 5-HTTLPR S-allele carrier of the serotonin transporter gene

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We report the case of a 50-year-old male with major depressive disorder (MDD) to illustrate the challenge of finding effective antidepressant pharmacotherapy and the role that the patient's genetic makeup may play. Recent treatment attempts before clinic admission included venlafaxine and fluoxetine. Venlafaxine was discontinued due to lack of response, and subsequently switched to fluoxetine based on pharmacogenotyping of the P-glycoprotein transporter (P-gp, encoded by *ABCB1*) by the outpatient psychiatrist. Despite steady state serum levels within the therapeutic range, the patient did not benefit from fluoxetine either, necessitating admission to our clinic. Here a clinical pharmacist-led medication review including additional pharmacogenetic (PGx) analysis resulted in the change of the antidepressant therapy to bupropion. Under the new regimen, established in the in-patient-setting, the patient remitted. However, based on the assessed pharmacokinetics-related gene variants, including *CYP*s and *ABCB1*, non-response to fluoxetine could not be conclusively explained. Therefore, we retrospectively selected the serotonin transporter (SERT1, encoded by *SLC6A4*) for further genetic analysis of pharmacodynamic variability. The patient presented to be a homozygous carrier of the short allele variant in the 5-HTTLPR (S/S) located within the *SLC6A4* promoter region, which has been associated with a reduced expression of the SERT1. This case points out the potential relevance of panel PGx testing considering polymorphisms in genes of pharmacokinetic as well as pharmacodynamic relevance.

KEYWORDS

pharmacogenetics, depression, pharmaceutical care, *SLC6A4*, 5-HTT, *ABCB1*, pharmacodynamics, venlafaxine

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Introduction

Major depressive disorder (MDD) is a common condition that imposes a high disease burden on the individual patient (1). However, not only the affected patients, but also the healthcare system and society are challenged by the disorder, in particular due to the resulting costs. The majority of the costs are of indirect kind and arise due to unemployment, sick leave and early retirement (2, 3). Therefore, it is important to effectively treat MDD. A relevant pillar in the treatment of MDD is pharmacotherapy. Fortunately, a wide range of marketed antidepressants is available today for clinicians and patients to choose from. Still, treatment of MDD remains challenging as it is known that up to 50% of unipolar depressed patients treated with antidepressants do not respond to their first-line treatment (4, 5). Ineffective antidepressant treatment may prolong the disease state, increasing the burden on the patient, the health care system, and society.

Multiple factors impact the response to antidepressants, including the patient's genetic makeup. On the one hand, genetic variation can alter the expression and/or activity of enzymes and transporters involved in drug absorption, distribution, metabolism, or excretion (ADME), causing interindividual differences in pharmacokinetics. On the other hand, genetic variation can affect the expression and/or structure of drug targets, potentially interfering with pharmacodynamics. Pharmacokinetic as well as pharmacodynamic alterations may impact both, tolerability and effectiveness of a drug (6).

The role of genetic predisposition in antidepressant response is extensively discussed in basic research as well as in clinical practice (7–9). So far, mainly pharmacokinetics-related genetic markers have found their way into clinical practice. In particular, compelling evidence on the impact of genetic variation of the enzyme cytochrome P450 (CYP) 2D6 and CYP2C19 has led to the publication of guidelines with recommendations for genotype-based selection and dosing of selective serotonin reuptake inhibitors (SSRI) and tricyclic antidepressants (10, 11). Both cytochromes, CYP2D6 and CYP2C19, are highly polymorphic which is reflected by the fact that over 60% of the general European population have a predicted phenotype that deviates from a normal metabolizer (extensive metabolizer, EM) (12). Moreover, the Swiss Society for Anxiety and Depression (SGAD) recommends genotyping of the P-glycoprotein (P-gp, encoded by *ABCB1*) after antidepressant treatment failure (13). P-gp is an efflux transporter which is also expressed in the blood-brain barrier (BBB), where it has an important gatekeeping role and extrudes various substances including certain antidepressants (14). It is hypothesized that carriers of the respective reference variant (wildtype) have restricted permeability of their BBB to antidepressants that are P-gp-substrates and therefore may only reach a limited concentration in the brain at their site of action (15). This theory is based on a limited number of clinical studies that associated certain *ABCB1* polymorphisms to antidepressant treatment response (15–17).

In addition to the afore described pharmacokinetics-related genetic variants, there is also evidence indicating effects of polymorphisms in pharmacodynamic-related genes on antidepressant efficacy and tolerability (18). It still remains controversial whether genetic variants in pharmacodynamically relevant antidepressant targets should be adopted in clinical practice. To date, there are no treatment recommendations based on any pharmacodynamic-related gene variants available for antidepressants. Extensive research is ongoing in this area, in particular studies on polymorphisms in the *SLC6A4* gene, encoding for the serotonin transporter (SERT1). The promoter region of the *SLC6A4* harbors a highly polymorphic region, named 5-HTTLPR (rs774676466), with a 44 base pair insertion-deletion (INDEL) variation (19). The short variant (S-allele) has a minor allele frequency of about 20% on a global average (20) and has been linked with reduced transcriptional activity and therefore limited expression of the encoded SERT1 (19). The SERT1 facilitates the reuptake of serotonin from the synaptic cleft into the presynapse and is a relevant target of various antidepressants, especially SSRIs (21). Hitherto, multiple studies linked the 5-HTTLPR variation with antidepressant therapy outcome (22, 23). However, it is difficult to apply these findings in practice, as there are currently no guidelines available associating *SLC6A4* genotypes with concrete recommendations for antidepressant selection and dosing. Herein we are reporting a case, where the *SLC6A4* 5-HTTLPR variation was likely causative in the tediously protracted search for an effective antidepressant.

Case presentation

Clinical case and medication history

A 50-year-old male with a long lasting history of recurrent MDD (ICD-10 F33), admitted himself to the medical emergency ward and was referred to our psychiatric crisis intervention unit. There he presented himself with sleeping disorders, rumination, anxiety, a lack of drive and recently increasing suicidal ideation. According to the patient, his current depressive episode started over 2 years ago with the loss of his employment and culminated in an acute deterioration a month prior to admission. At our clinic he was diagnosed with a moderate depressive episode (ICD-10 F33.1), reflected by a score of 19 on the 21-item Hamilton Rating Scale (HAMD-21) (24) and by a score of 26 on the patient-rated Beck Depression Inventory (BDI) (25).

At clinic entry, the patient was under treatment with a combination of low-dose trimipramine (50 mg/d) for sleep

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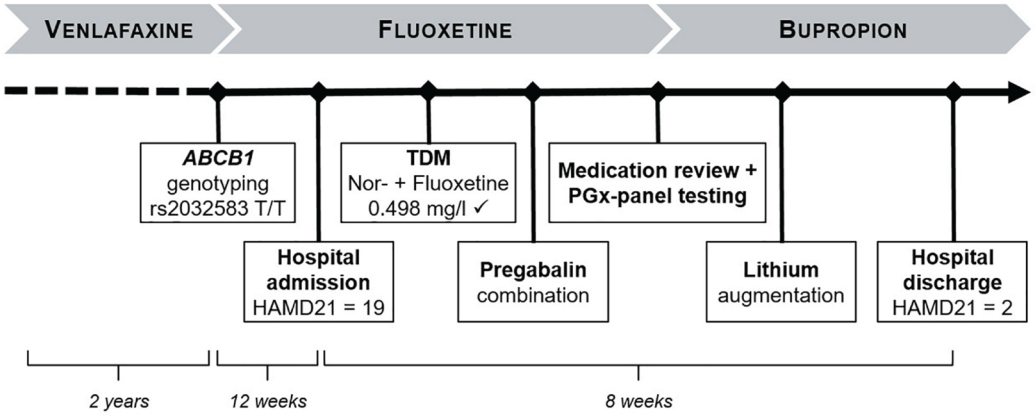

FIGURE 1
Overview case presentation. TDM, therapeutic drug monitoring; HAMD, Hamilton depression rating scale; PGx, pharmagocenetic.

TABLE 1 Selected results of the panel pharmacogenotyping and phenotype interpretation.

Gene	Variant (*also tested variants in gene locus*)	Genotype	Diplotype	Predicted phenotype
ABCB1	rs2032583, c.2685 + 49T > C, rs2235015, c.497-25G > T *(rs1045642, rs1128503, rs2032582)*	T/T (WT[a]), G/G (WT[a])	NA[a]	Substance specific function
CYP2B6	*(rs8192709, rs28399499, rs3745274)*	WT[a]	*1/*1	Normal function (NM[c])
CYP2C9	*(rs1799853, rs1057910, rs9332131, rs7900194, rs28371685)*	WT[a]	*1/*1	Normal function (NM[c])
CYP2C19	rs4244285 c.681G > A (in *2) *(rs4986893, rs12248560, rs28399504)*	G/A	*1/*2	Reduced function (IM[d])
CYP2D6	*(rs35742686, rs3892097, rs5030655, rs5030867, rs5030865, rs5030656, rs1065852, rs201377835, rs28371706, rs59421388, rs28371725)*	WT[a]	*1/*1	Normal function (NM[c])
HTR2A	rs7997012, c.614-2211T > C, rs9316233, g.47433355C > G *(rs6311, rs6313, rs6314)*	T/T, G/G	NA[b]	Substance specific function

[a]WT, wildtype; [b]NA, not applicable; [c]NM, normal metabolizer; [d]IM, intermediate metabolizer.

promotion, and fluoxetine (40 mg/d) for depression, which was established 3 months earlier by an outpatient psychiatrist (Figure 1). Before starting this treatment, a long-term treatment with venlafaxine was terminated by the outpatient psychiatrist due to ineffectiveness. His decision to switch to fluoxetine was based on two genetic markers of the *ABCB1* gene, encoding for P-glycoprotein (Table 1), determined in the laboratory of Viollier AG (Allschwil, Switzerland), as recommended by the SGAD (13).

Despite a daily dose of 40 mg fluoxetine and steady state trough serum levels within the therapeutic range [fluoxetine + norfluoxetine = 0.498 mg/l, ref. 0.120–0.500 mg/l (26)], the patient did not benefit from treatment with fluoxetine. Therapeutic efficacy did not improve in the in-patient-setting and in combination with pregabalin, which was initiated at the clinic due to restlessness and strain. Due to persisting non-response within the first month of hospitalization, a clinical pharmacist-led medication review including additional pharmacogenetic analysis was initiated. This clinical pharmacy service was part of an observational study approved by the local ethics committee (ClinicalTrials.gov identifier: NCT04154553). The patient gave written informed consent for panel pharmacogenotyping and health data retrieval. A buccal swab was collected to apply the commercial pharmacogenotyping service Stratipharm® offered by humatrix AG (Pfungstadt, Germany). In their laboratory, the polymorphisms are determined by applying real-time PCR using the automated Life Technologies QuantStudio 12 k flex (Thermo Fisher, MA, United States) with the respective optimized and commercially available chemistry. The applied commercial PGx panel test includes genetic variants frequently observed in the European population, including alleles discussed in the CPIC guidelines.[1] Interpretation of

1 www.cpicpgx.org

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the genotyping results identified the patient as a normal metabolizer (NM, *1 homozygous) for CYP2B6, CYP2C9 and CYP2D6 (Table 1). In addition, the patient's CYP2C19 phenotype was predicted as intermediate metabolizer (IM, *2 heterozygous) (Table 1). Based on these results and the patient's history of non-response to venlafaxine, a selective serotonin and norepinephrine reuptake inhibitor (SNRI), and fluoxetine, a selective serotonin reuptake inhibitor (SSRI), a switch to bupropion, a norepinephrine–dopamine reuptake inhibitor, was recommended by the clinical pharmacist. Bupropion is mainly metabolized *via* CYP2B6 and not a substrate of the P-gp transporter (27, 28). After the patient's medication was switched from fluoxetine to bupropion, a clinical improvement in drive and mood was observable within 1 week. For further improvement and maintenance treatment, an augmentation with lithium was added. Under this combined treatment regimen (Table 2), the patient remitted and was discharged to out-patient care within 4 weeks of treatment change to bupropion, and after a total of 8 weeks of in-patient care. Remission was quantified at discharge with a HAM-D21 score of 2 and a BDI score of 5, compared to 19, respectively, 26 at clinic admission. When followed up 8 weeks after discharge, the patient was still in remission.

Pharmacogenetic data interpretation and further analysis

Prior to the introduction of bupropion, which eventually proved to be effective, our patient had to endure insufficient antidepressant treatment over the course of more than 2 years. The initial non-response to venlafaxine was attributed to the patients *ABCB1* genotype, with no variation for the polymorphisms rs2032583 and rs2235015. Homozygous carriers of the respective wildtype alleles have been associated with a reduced likelihood of depression remission when treated with antidepressants that are P-gp-substrates. Since venlafaxine is a known P-gp-substrate (15, 29), the treating ambulant psychiatrist decided to switch to the SSRI fluoxetine, a non-relevant P-gp-substrate (30, 31). However, despite these considerations, the patient's depression deteriorated even further under fluoxetine, necessitating in-patient treatment. There, due to the known involvement of polymorph CYPs in the metabolism of venlafaxine and fluoxetine, further panel pharmacogenotyping was initiated. For venlafaxine, there are PGx-based dosing guidelines available, taking the predicted CYP2D6 phenotype into account (32). Fluoxetine is known to be mainly metabolized *via* the polymorph CYP2D6 and CYP2C9. Although there is no PGx-based dosing guideline available for fluoxetine, genetic variants of *CYP2D6* and *CYP2C9* have been associated with alterations in its pharmacokinetics (33, 34). However, based on the patient's genetic analysis (Table 1), both CYP2D6 and CYP2C9 are predicted to have normal activity, suggesting that there are no known drug-gene interactions. This is also reflected in the measured nor-/fluoxetine serum levels, which was within the therapeutic reference range at steady state with a common daily dosage of 40 mg.

TABLE 2 Medication at hospital admission vs. at hospital discharge.

Hospital admission		Hospital discharge	
Substance	**Schedule**	**Substance**	**Schedule**
Fluoxetine 20 mg	1-1-0-0	Bupropion 150 mg	1-0-0-0
Trimipramine 100 mg	0-0-0-0.5	Lithium 12 mmol	1-0-1-0
		Pregabalin 75 mg	1-1-0-0
		Pregabalin 100 mg	0-0-1-0
		Colecalciferol 1000 IU	1-0-0-0

The non-response to fluoxetine could not be conclusively explained by the assessed pharmacokinetics-related gene variations, including *CYPs* and *ABCB1*. The SERT1 2A (*HTR2A*), which is part of the commercial panel, was also inconspicuous in relation to fluoxetine (12). Therefore, we retrospectively selected the SERT1 (encoded by *SLC6A4*) for further genetic analysis of pharmacodynamic variability. We genotyped for the 5-HTTLPR polymorphism applying the protocol described elsewhere (35) and using gDNA isolated from the patient's whole blood sample using the QIACube® with the QIAamp® DNA Blood Mini Kit (Qiagen, Hilden, Germany). Herein, the patient presented to be a homozygous carrier of the minor short allele variant in the 5-HTTLPR polymorphism (S/S) of the *SLC6A4*. The 5-HTTLPR S-allele is assumed to cause reduced expression of the SERT1, the target of serotonin reuptake inhibitors including fluoxetine and venlafaxine (19). Several studies associated the 5-HTTLPR major variant, so called L-allele, with an increased likelihood of antidepressant response, especially in Caucasians (22). A recent meta-analysis further specified that the 5-HTTLPR L-allele predicts response specifically to SSRI's (23). It seems plausible that in the reported case, the present *SLC6A4* variant has indeed affected fluoxetine effectiveness. We hypothesize that this is a relevant reason why the patient clearly benefited from a switch to the noradrenaline and dopamine reuptake inhibitor bupropion, which does not target the genetically affected SERT1.

Conclusion and outlook

The patient's *SLC6A4* genotype (S/S) may likely explain why switching to fluoxetine proved ineffective and even led to an acute exacerbation of the depression. The SERT1 is selectively targeted by SSRIs, but its inhibition also contributes to the therapeutic effect of SNRIs and tricyclic antidepressants

(36). Consequently, an influence of *SLC6A4* genetic variants on the effect of other antidepressants binding the SERT1 seems plausible. However, the number of studies evaluating the effectiveness of non-SSRI antidepressants in context with *SLC6A4* polymorphisms is still very limited and recent meta-analyses were unable to detect corresponding effects (23, 37). It may be speculated that a pre-emptive approach in PGx testing of the 5-HTTLPR might have significantly reduced the patient's burden and even avoided hospitalization. Some commercial pharmacogenetic tests already include *SLC6A4* polymorphisms in their panels (38). However, currently there are no recommendations for drug dosing and selection considering polymorphisms in *SLC6A4*. It also seems noteworthy at this point, that besides effectiveness, *SLC6A4* variants have been associated with antidepressant tolerability (39, 40) and even depression susceptibility with *SLC6A4* variation as a potential disease modifying factor (41, 42). Further prospective studies are warranted before genotyping of the SERT1 can be recommended as an additional basis for antidepressant selection. Besides *SLC6A4*, other pharmacodynamically relevant gene variants may gain importance in the near future. Candidate genes under investigation that have been associated with antidepressant efficacy, include genes encoding for the tryptophan hydroxylase (*TPH*), serotonin receptors (*5-HT1A*, *5-HT2A*, *5-HT6*), dopamine receptors (*DRD2*, *DRD4*) and others (18). It is conceivable that pharmacokinetic as well as pharmacodynamic gene variants have a combined effect on the efficacy and tolerability of antidepressants. Therefore, a broader polygenetic approach with panel PGx tests is expected to further gain relevance for a personalized medicine approach in selection and dosing of antidepressants.

Data availability statement

The original contributions presented in this study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethikkommission Nordwest- und Zentralschweiz (EKNZ), 4056 Basel, Switzerland. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

CS, ML, KH, SA, and HM: conceptualization and study design. CS, RM, ML, and HM: investigation and interpretation of genotyping data. TM and MH: psychiatric clinical assessments. CS: writing—original draft preparation and visualization. RM, TM, MH, ML, SA, KH, and HM: writing—additional content, critical review, and editing. HM: supervision. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.942268/full#supplementary-material

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Pharmacogenetic-Guided Antidepressant Selection as an Opportunity for Interprofessional Collaboration: A Case Report [A-4]

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Case Report

Pharmacogenetic-Guided Antidepressant Selection as an Opportunity for Interprofessional Collaboration: A Case Report

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Abstract: In the herein reported case of a 42-year-old woman diagnosed with anxiety and depression, a long history of antidepressant ineffectiveness and adverse drug reactions was decisive for an in-depth medication review including pharmacogenetic panel testing. In detail, treatment attempts with paroxetine and escitalopram were ineffective and discontinued due to subjective gastrointestinal intolerance. Due to the worsening of the depression after the failed treatment attempts, admission to our clinic became necessary. Herein, owing to the collaboration of psychiatrists with clinical pharmacists, individualized incorporation of pharmacogenetic data into the process of antidepressant selection was enabled. We identified vortioxetine as a suitable therapeutic, namely for being most likely pharmacokinetically unaffected as predicted by pharmacogenetic panel testing and taking into account the current comedication, as well as for its favorable action profile. Herein, our collaborative effort proved to be successful and resulted in the patient's depression remission and clinic discharge with the interprofessionally selected pharmacotherapy. This exemplary case not only highlights the potential benefits and challenges of pre-emptive pharmacogenetic testing in antidepressant prescription, but also proposes an approach on how to put pharmacogenetics into practice.

Keywords: antidepressant drugs; depression; pharmacogenetics; psychiatry; pharmaceutical care; interprofessional relations; vortioxetine; CYP2D6; CYP2C19; ABCB1

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1. Background

Pharmacotherapy, in addition to behavioral therapy and others, is an important pillar in the treatment of major depressive disorder (MDD). Today, prescribing clinicians can choose from a wide range of marketed antidepressants. However, successful treatment of depression remains challenging and inter-individual differences in response to antidepressants are common. Indeed, around half of unipolar depressed patients do not respond to the first treatment attempt [1,2]. Moreover, the experience of serious adverse events under antidepressant pharmacotherapy and discontinuation due to intolerance of the same has been associated with therapy failure [2]. In particular, divergent levels of systemic drug exposure can cause inter-individual drug responses, leading to either toxicity in the case of supratherapeutic drug levels or ineffectiveness due to subtherapeutic drug levels. Apart from avoidable factors such as drug–drug or food–drug interactions and insufficient adherence, deviations in drug levels can also be caused by given predispositions, such as impaired renal or liver function, and, notably, genetics. In fact, many antidepressants are metabolized by highly polymorphic cytochromes P450 (CYP) including CYP2D6 and CYP2C19. For these enzymes, individuals can exhibit phenotypes with altered activity

ranging from poor to ultrarapid metabolizers. Especially for CYP2D6 and CYP2C19, these phenotypes find their origin in the genetic make-up and can therefore be predicted by genotyping of associated single-nucleotide polymorphisms or copy number variations [3]. We have recently reported a case in which CYP genotypes might have substantially impaired antidepressant drug response over the years [4]. Moreover, the known influence of polymorphisms on antidepressant pharmacokinetics, toxicity and treatment response is already highlighted on numerous drug labels of marketed products [5]. Additionally, multiple guidelines with genotype-based recommendations for drug dosing and selection have been published and are currently available for tricyclic antidepressants and selective serotonin reuptake inhibitors [6,7]. Furthermore, the Swiss Society for Anxiety and Depression (SGAD) recommends genotyping of the P-glycoprotein (encoded by *ABCB1*) after experiencing antidepressant treatment failure [8]. The efflux transporter P-glycoprotein has an important gatekeeping role at the blood–brain barrier, where it extrudes xenobiotics and drug molecules including certain antidepressants. It is hypothesized that homozygous carriers of the wildtype allele may experience increased efflux of substrate antidepressants, leading to decreased drug levels within the central nervous system, which is their site of action. This theory is based on a limited number of clinical studies that linked certain *ABCB1* polymorphisms to antidepressant treatment response [9–11].

However, despite the already compiling evidence, especially for SSRIs and tricyclic antidepressants [6,7], pharmacogenetic (PGx) analysis is not yet routinely applied when prescribing these antidepressants. Underlying reasons are diverse and barriers to the implementation of PGx services include fragmentary evidence from prospective clinical trials, limited reimbursement from basic health insurance (which, in Switzerland, is currently only possible if clinical pharmacologists prescribe the specific testing), missing established procedures and, in general, a lack of education and experience among mental health care providers [12,13]. An approach to overcome some of these barriers, to efficiently enable individualized PGx information processing for antidepressant selection and dosing, might involve the interprofessional collaboration of psychiatrists and clinical pharmacists. The added value of an interdisciplinary approach concerning medication review in the psychiatry setting has been investigated before and was found to have a significant impact on the detection and solution of drug-related problems [14]. As described beforehand, pharmacogenetic predisposition might be a cause of drug-related problems such as adverse drug reactions and ineffectiveness. To illustrate the challenges and benefits of such an interdisciplinary PGx service, we herein report an exemplary case where individually interpreted PGx data were used in the course of collaborative decision-making on readjusting antidepressant pharmacotherapy.

2. Case Presentation

2.1. Clinical Case and Medication History

A 42-year-old female patient diagnosed with a generalized anxiety disorder (ICD-10 F41.1) and a recurrent depressive disorder (ICD-10 F33), without any other comorbidity diagnosed, entered our clinic for inpatient treatment due to acute mental decompensation manifested by reduced appetite, weight loss, abdominal pain without underlying somatic cause, sleeping disorder and lethargy. The recent deterioration in the patient's condition was found to be multifactorial, inter alia caused by increasing familiar burden, recent therapy with childhood trauma processing and stress triggered by the COVID-19 pandemic. At admission, the current depressive episode without psychotic symptoms was rated as severe (ICD-10 F33.2), i.e., the rater-assessed 21-item Hamilton Rating Scale of Depression (HAM-D21) [15] yielded a score of 33 and the self-rating scale Beck Depression Inventory (BDI) [16] showed a score of 40. Prior outpatient treatment attempts included pharmacotherapy with paroxetine and escitalopram, both of which were discontinued due to subjective gastrointestinal intolerance and with insufficient therapeutic effect. As a result, the patient developed a strong fear of medication and potential adverse drug reactions, so that she refused a further therapeutic approach in the outpatient setting. At the clinic, an

initial treatment attempt with pregabalin 25 mg daily was discontinued after only two days, upon the patient's complaining of muscle cramps. Additionally, treatment with quetiapine was limited to a low-dose intake at night, due to the occurrence of daytime fatigue at higher dosage. Eventually, a therapy with agomelatine 50 mg at night was implemented and well tolerated. However, due to the limited effect of agomelatine monotherapy in the treatment of the underlying anxiety and the current severe depressive episode, a combination with escitalopram was introduced. With the help of a liquid formulation, a gradual dosage increase over the course of two weeks was attempted, due to the aforementioned subjective intolerance experienced in the past, under escitalopram dosages of up to 15 mg daily. At the present time, the patient tolerated a daily dosage of up to 10 mg escitalopram well. Meanwhile, laboratory parameters for liver and kidney function were assessed, revealing values in a normal range (e.g., serum creatinine, total bilirubin, ALAT and ASAT). However, the patient showed persisting unresponsiveness after almost 4 weeks of inpatient treatment. Therefore, and due to the known involvement of the polymorph CYP2C19 in escitalopram metabolism, the treating physician requested a pharmacogenetic consultation by clinical pharmacists of the hospital. This clinical pharmacy service includes a comprehensive medication review of the current medication as well as a semi-structured interview to gain information on the patient's medication history and prior experiences with therapy failure and adverse drug reactions. At present, this pharmacogenetic consultation is part of an observational case study approved by the local ethics committee (ClinicalTrials.gov identifier: NCT04154553). Written informed consent for genetic testing and health data retrieval was collected from the patient prior to the intervention. Eventually, pharmacists classified the present case as potentially relevant in the context of pharmacogenetics and panel pharmacogenotyping was conducted from a buccal swab, applying the commercial service Stratipharm® offered by humatrix AG (Pfungstadt, Germany). In their laboratory, the polymorphisms are determined by applying real-time PCR using the automated Life Technologies QuantStudio 12 k flex (Thermo Fisher, MA, USA) with the respective optimized and commercially available chemistry. Interpretation of the genotyping results identified the patient as CYP2C19 rapid metabolizer (RM, *17 heterozygous), CYP2D6 and CYP2B6 normal metabolizer (NM, *1 homozygous). Furthermore, the patient exhibited genetic variants associated with increased inducibility of CYP1A2 (*1F homozygous), and no variation in the *ABCB1* polymorphism rs2032583. Additionally, the analyzed *HTR2A* gene locus exhibited a homozygous variation for the rs7997012 polymorphism (Table 1).

Table 1. Selected results of the panel pharmacogenotyping and phenotype interpretation thereof.

Gene	Variant *(Also Tested Variants in Gene Locus)*	Genotype	Predicted Phenotype
CYP1A2	rs762551 g.75041917C>A (in *1F) *(rs2069514)*	A/A	Increased inducibility
CYP2B6	*(rs8192709, rs28399499, rs3745274)*	WT [3], *1	Normal function (NM [1])
CYP2C19	rs12248560 g.4195C>T (in *17) *(rs4986893, rs4244285, rs28399504)*	C/T	Increased function (RM [2])
CYP2D6	*(CNV, rs35742686, rs3892097, rs5030655, rs5030867, rs5030865, rs5030656, rs1065852, rs201377835, rs28371706, rs59421388, rs28371725)*	WT [3], *1	Normal function (NM [1])
ABCB1	rs2032583 c.2685+49T>C *(rs1045642, rs1128503, rs2032582)*	T/T (WT [3])	Substance specific function
HTR2A	rs7997012 c.614-2211T>C *(rs6311, rs6313, rs9316233, rs6314)*	C/C	Substance specific function

[1] NM: normal metabolizer; [2] RM: rapid metabolizer; [3] WT: wild type.

2.2. Pharmacogenetic Data Interpretation

The gastrointestinal adverse drug reactions experienced in the past after the intake of escitalopram and paroxetine are frequently observed (1–10%) [17]. In the herein presented case, the underlying genetic profile, however, was not associated with an increased risk of adverse drug reactions due to the supratherapeutic drug levels of these substances. In the case of paroxetine, which is mainly metabolized by CYP2D6 with herein predicted normal activity (NM, *1 homozygous), treatment can be initiated with the usual recommended starting dose [6]. Escitalopram is extensively metabolized by CYP2C19, which, in the present case, was predicted with increased activity (RM, *17 heterozygous) and associated with an elevated risk of therapy failure [6]. Indeed, the patient did not respond to escitalopram after reintroducing it at our clinic. Nevertheless, side effects cannot be excluded per se. However, considering the fact that the reintroduction of escitalopram in the inpatient setting was well tolerated, a potential psychosomatic cause of the experienced gastrointestinal disorders might be discussed. Polychroniou et al. (2018) stated in their evaluation of treatment-naïve adults (n = 105) that escitalopram-associated side effects are dose-dependent [18]. Whether this also applies to the escitalopram re-exposure remains unclear. It seems noteworthy that there are data linking gastrointestinal distress during paroxetine and escitalopram intake to altered gut microbiota composition [19,20], but whether this also contributes to the disease symptoms remains to be further investigated.

Besides the gastrointestinal side effects, the antidepressants used previously, namely paroxetine, escitalopram and agomelatine, had not been effective. In the case of escitalopram, this most likely can be attributed to the increased activity of CYP2C19, through which escitalopram is extensively metabolized. Accordingly, current guidelines recommend consideration of an alternative antidepressant that is not predominantly metabolized by CYP2C19, due to the risk of inefficacy as a consequence of subtherapeutic drug levels (e.g., [6]). Furthermore, it seems noteworthy that the patient was a homozygous carrier of the CYP1A2 (*1F) variant, which is known to be linked to the enhanced inducibility of this particular CYP enzyme [21,22]. Together with the patient's smoking status, this genetic profile could be linked to an increased degradation of agomelatine, which, when given as a monotherapy, indeed did not improve the patient's depression. However, no guidelines for PGx-guided agomelatine selection and dosing are currently available.

Additionally, other mechanisms than the CYP-related metabolism may have played a role in this individual's medication history. One of these mechanisms may be the activity of the efflux transporter P-glycoprotein (encoded by *ABCB1*). The *ABCB1* rs232583 major allele variant has been associated with reduced therapy response in the treatment with substrates of this efflux transporter. Here, the transporter, which is known to be expressed in the blood–brain barrier, is assumed to limit brain entry, resulting in lower efficacy of centrally active molecules; these also include the molecules used in the herein reported patient, paroxetine and escitalopram [9–11]. It remains to be determined whether genetic variants such as the rs232583, which has been associated with the reduced efficacy of ABCB1 substrates, also influences the effect of P-glycoprotein as a determinant of oral bioavailability due to its apical expression in enterocytes. However, data supporting this notion are rather limited. In the context of antidepressants, which are known to modulate the serotonin homeostasis [23], the genetic profile of the serotonin receptor (*HTR2A*) was also evaluated within the herein applied commercial system by humatrix AG. Here, the patient exhibited the homozygous variant allele rs7997012, which has been linked with a decreased response to therapeutic interventions with es-/citalopram [24]. In this context, it seems to be noteworthy that the frequencies of the previously discussed genetic polymorphisms may vary across different ethnic populations and that, in this case, we are reporting a single patient of European descent.

Based on the analysis of the genetic profile and the patient's medication history, taking into account the known contribution of the altered CYP2C19 and CYP1A2 to the metabolism as well as potentially ABCB1 to the transport of various antidepressants, the clinical pharmacist recommended the following substances for therapy optimization:

vortioxetine, bupropion or venlafaxine. All of these are primarily metabolized by CYP enzymes with normal activity as predicted by panel pharmacogenotyping, i.e., vortioxetine and venlafaxine via CYP2D6 [25,26] and bupropion via CYP2B6 [27]. Moreover, these compounds exhibit slight differences in their pharmacodynamic profile, which, independent of the genotype, impacts the individual's response. The physician decided together with the patient to change the antidepressant therapy to vortioxetine at week five of the hospitalization. This shared decision-making was supported on the one hand by the pharmacist's reasoning that vortioxetine is primarily metabolized via the normally active CYP2D6, is not a relevant P-glycoprotein substrate [25] and would have no expected interaction with the patient's current co-medication. On the other hand, vortioxetine had a suitable pharmacodynamic profile for the present case, i.e., mood-lifting and anxiety-relieving, favored by the psychiatrist. Thus, escitalopram and quetiapine were discontinued and vortioxetine 10 mg daily augmented with low-dose aripiprazole 2.5 mg daily was started instead, as an add-on to the already established agomelatine 50 mg daily. Notably, pharmacologic augmentation is a common strategy in the treatment of therapy-resistant MDD [28]. After five more weeks under treatment with the above-described regimen, the patient was discharged with remitted symptoms as evidenced by a HAM-D21 score of 4 (at admission: 33) and a BDI score of 7 (at admission: 40).

3. Conclusions and Outlook

The interprofessional collaboration between psychiatrists and clinical pharmacists facilitated an individualized therapy approach with interpretation and incorporation of PGx data into the antidepressant selection process. Changing to an antidepressant with most likely unaffected pharmacokinetics as predicted by the genetic panel test and taking into account the current comedication and medication history, in combination with a favorable profile of action, was successful, as shown by good tolerability and remission of depression with the interprofessionally selected pharmacotherapy. It may be speculated that an early approach with PGx testing might have significantly reduced the patient's burden as well as the duration of hospitalization. However, we are aware that we are reporting a single case, which does not allow for generalized conclusions, and the aforementioned hypotheses will have to be further tested in prospective studies [29]. Cases such as this support the notion that pre-emptive genotyping, or perhaps phenotyping, if available in a clinical setting [30], would be of great value for the patient and potentially cost-effective for the health care sector by enhancing the prescription of an effective pharmacotherapy at an early stage and thereby potentially reducing the duration and number of hospitalizations. However, at least in Switzerland, there is no clear or formal structure to support these advances. It is the aim of an ongoing research program to evaluate and establish these structures for an interprofessional collaboration of pharmacists (community and hospital) and the treating physicians [31].

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study. Written informed consent has been obtained from the patients to publish this paper.

Data Availability Statement: The genetic data presented in this study are available on request from the corresponding author. The data are not publicly available for ethical and privacy reasons.

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5.2 Project B

PGx in pharmaceutical care: Pharmacist training

Pharmacogenetics in pharmaceutical care – piloting an application oriented blended learning concept [B]

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Pharmacogenetics in Pharmaceutical Care—Piloting an Application-Oriented Blended Learning Concept

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Abstract: To enable application-oriented training of Swiss pharmacists on pharmacogenetic (PGx) testing, an advanced, digital training program was conceptualized based on the Miller's Pyramid framework, using a blended learning approach. The PGx advanced training program included an asynchronous self-study online module, synchronous virtual classroom sessions with lectures and workshops, and a follow-up case study for in-depth applied learning including the analysis of the participants' PGx profile. The evaluation of the training program consisted of (a) an assessment of the participants' development of knowledge, competencies and attitudes towards PGx testing in the pharmacy setting; (b) a satisfaction survey including; (c) questions about their future plans for implementing a PGx service. Twenty-one pharmacists participated in this pilot program. The evaluation showed: (a) a significant improvement of their PGx knowledge (mean score in the knowledge test 75.3% before to 90.3% after training completion) and a significant increase of their self-perceived competencies in applying PGx counselling; (b) a high level of satisfaction with the training program content and the format (at least 79% expressed high/very high agreement with the statements in the questionnaire); (c) a mixed view on whether participants will implement PGx testing as a pharmacy service (indecisive 8; agreed/completely agreed to implement 7/1; disagreed 3 (n = 19)). We consider ongoing education as an important driver for the implementation of PGx in pharmacy practice.

Keywords: pharmacogenetics; pharmacogenetic testing; pharmaceutical care; pharmacy service; blended learning; e-learning; digital training; advanced training

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1. Introduction

Interindividual differences in response to pharmacotherapies are common and may be caused by various avoidable factors such as drug–drug and food–drug interactions, as well as insufficient adherence, but also by given predispositions such as renal and hepatic deficiency and notably genetics. Hence, today various drug labels point out the impact of genetic predispositions on drug response and in certain cases even strongly recommend genetic testing prior to treatment start [1]. Genetic testing, so called pharmacogenotyping, is used to identify individuals, who may particularly benefit from a certain pharmacotherapy, or who carry an increased risk of therapy failure, adverse drug reactions and even severe toxicities due to their genetic makeup [2]. Currently, multiple guidelines with recommendations for genotype-based drug selection and dosing are available and comprise a variety of actionable drug–gene interactions such as clopidogrel and *CYP2C19* encoding for the enzyme cytochrome P450 2C19, as well as 5-fluorouracil and *DPYD* encoding for the enzyme dihydropyrimidin dehydrogenase [3,4].

However, despite the already accumulating evidence, the adoption of pharmacogenotyping in clinical practice remains modest, which is attributed to multiple barriers such as the restricted reimbursement of pharmacogenetic tests, the limited evidence from prospective clinical trials, as well as a lack of education of health care professionals [5]. In Switzerland, pharmacogenetic (PGx) testing can currently only be initiated by physicians and in the majority of cases even requires a prescription from a specialized pharmacologist to ensure health insurance coverage [6]. It may be discussed that these rather restrictive requirements for the initiation of PGx testing may additionally hamper its implementation in clinical practice. However, currently, the Swiss law on genetic investigations in humans is being revised and a notion to enable pharmacists to initiate PGx testing is under consideration [7]. It may be speculated that the extension of the pharmacist's competencies in PGx testing may also support further adoption of pharmacogenetics in health care practice.

Nonetheless, as aforementioned limited education of health care professionals remains a relevant barrier in the implementation of PGx testing. In 2018, we conducted a survey among Swiss community (n = 238) and hospital (n = 134) pharmacists, which showed that about 75% of the participating pharmacists perceived their knowledge of PGx as insufficient to adequately advise their patients. However, the same number of pharmacists considered it their duty to counsel patients in the matter of PGx and additionally expressed willingness to participate in an advanced training course on the said topic [8]. A recent survey among Canadian pharmacists showed comparable results and additionally identified digital training as a highly accepted and favoured way of learning [9].

Digital training is a widely used and studied method in under- and postgraduate pharmacy education, where it was shown to improve the immediate gain of knowledge and enjoys a high acceptance rate amongst participants [10]. Notably, there are various approaches in reported educational programs using digital media, including online modules, online reading materials and online, synchronous, or asynchronous lectures [10]. These also included training programs using a blended learning approach, where synchronous face-to-face classroom teaching and asynchronous online learning were combined [11,12]. The mix of learning environments and the use of digital technologies in blended learning was found to improve the students' performance in exams [12] and additionally may promote participant engagement. Digital training approaches have also been used in pharmacogenomics education of pharmacists and pharmacy students, where in a recent international survey especially online open access PGx databases were reported to be frequently used [13]. Overall, the adoption of pharmacogenetics into pharmacy and medical university curricula has increased globally in recent years, but seems to be mainly taught on a genetic level without integration into other scientific fields and may therefore lack application-oriented aspects [13]. The Miller's Pyramid offers a framework for the conceptualization and assessment of application-oriented training for professional services. It defines four levels of performance: (1) knows; (2) knows how; (3) shows how; (4) does [14].

Meanwhile, numerous post-graduate training programs for pharmacists on pharmacogenetics are available, and amongst others are widely offered in the USA and Canada [15,16]. However, to our knowledge, no such in-depth, post-graduate training program is yet available in Switzerland. To fill this educational gap and to properly prepare pharmacists for their anticipated, challenging responsibility in PGx testing, an advanced, digital training program for community and hospital pharmacists was developed. We, a PGx task force group of health care professionals from academia and pharmacy practice, piloted the training program between October 2020 and January 2021, using an application-oriented blended learning concept.

2. Materials and Methods

2.1. Learning Outcomes

Overall, we aimed to enable the participating pharmacists to identify and address PGx issues arising in their daily work and to support patients and other health care providers accordingly. In particular, the following learning outcomes were defined:

- The pharmacists have knowledge of:
  1. The basics of pharmacogenetics;
  2. The current legal situation in Switzerland concerning genetic testing;
  3. The currently available PGx tests and their reimbursement in Switzerland.
- The pharmacists are able to:
  1. Evaluate the evidence and the implications of PGx testing for clinical practice;
  2. Rate information concerning PGx in the Swiss summary of product characteristics;
  3. Interpret results from PGx tests and incorporate them into a medication review;
  4. Support patients and health care providers likewise with their inquiries about PGx.

2.2. Recruitment

For the pilot training program, we aimed to recruit 20 pharmacists interested in clinical pharmacy and interdisciplinary topics, working in hospitals, community pharmacies and other institutions (e.g., authorities). The program was officially announced and open for registration on the website for continuous education of the Department of Pharmaceutical Sciences at the University of Basel. Additionally, we recruited participants via email invitation, who were enrolled at the University of Basel for the advanced certificate studies in clinical pharmacy, or who had subscribed for the email distribution list of the Swiss Association of Public Health Administration and Hospital Pharmacists (GSASA).

2.3. Program Design

To allow best possible location- and time-independent learning, we chose a blended learning approach, combining asynchronous, self-study online modules and synchronous, virtual classroom sessions as well as a follow-up with individual case studies for in-depth applied learning. The program content was selected and structured according to the framework of Miller's Pyramid, which defines four levels for the realization and assessment of a professional service [14] (Table 1). The piloting of the training program was conducted over a period of three months, starting off with an asynchronous, self-study online module using the learning management system ADAM, https://adam.unibas.ch/ (accessed on 18 November 2020) (University of Basel, 2020). This asynchronous, self-study online module was designed to cover the basics of PGx including genetic variation, pharmacologically relevant genotypes and phenotypes, as well as open access sources for PGx information retrieval (Pharmacogenomics Knowledge Base, Dutch Pharmacogenetics Working Group) [17,18]. The participants were able to study independently and self-paced using online content including texts, quizzes, and links to further literature. During this phase, it was also possible to exchange information with participants and instructors via an online discussion board. We anticipated a minimum effort of half a day to complete the asynchronous online module, but the participants were granted time-independent access to the according platform throughout the training program. In preparation for the following synchronous, virtual classroom session at the beginning of the second month, the participants were asked to complete a basic case study covering the contents of the previous asynchronous, online module. The subsequent synchronous, virtual classroom session was held via the video conferencing system Zoom 5.0.4 (Zoom Video Communications, San José, CA, USA). The session contained lectures covering the legal framework for genetic testing in Switzerland, reasonable indications for PGx testing as well as application and interpretation of PGx test results in pharmacy practice. Furthermore, the participants worked in groups of four, in break out rooms of the Zoom session, on three different patient cases using real-life, anonymized pharmacogenetic and health data to solve the cases and come up with recommendations. Following this first virtual classroom training, the participants were given the opportunity to have their personal PGx profile assessed and interpreted using the commercial service Stratipharm® (humatrix AG, Pfungstadt, Germany). Based on their personal and an exemplary PGx profile, the participants were asked to solve a fictional polypharmacy case as a transfer task, writing a report with recommendations for a fictional treating physician. After three months a second synchronous, virtual classroom

session was conducted. Herein, the participants were given individual feedback on their transfer task. Furthermore, the program organisers and experts discussed together with the participants the opportunities and challenges for pharmacogenetics in today's and future pharmacy practice.

Table 1. PGx training program components and assessments charted to Miller's Pyramid framework.

Miller's Pyramid Level	Description	Training Component	Assessment
Level 1: Knows	Knowledge of facts	Asynchronous self-study online module	Knowledge test
Level 2: Knows How	Competences in application	Synchronous virtual classroom (part 1)	Self-assessment questionnaire
Level 3: Shows How	Performance and demonstration of learning	Asynchronous case study with individual PGx profile and synchronous virtual classroom (part 2)	Self-assessment questionnaire and individual feedback
Level 4: Does	Action and integration into practice	Peer group for experience sharing	not available

2.4. Assessments

To assess the participants' progress in learning as well as their development of competencies and attitudes toward PGx testing in pharmacy practice, we used online self-assessment questionnaires, knowledge tests, and satisfaction surveys (Table 1). In order to start the asynchronous, self-study online module, the participants had to answer a 16-item multiple choice knowledge test on the basics of PGx. This test was developed and reviewed by experienced university educators of our task force group. After completion of the asynchronous self-study online module participants had to answer the knowledge test again, to assess their status and progress concerning PGx basic knowledge. Furthermore, we aimed to evaluate the participants' development of competencies and attitudes towards PGx testing in pharmacy practice based on their self-perception. Therefore, we applied a self-assessment questionnaire adapted from a recent project on the topic published by Crown and colleagues [15]. In the adapted questionnaire, participants were asked to score their agreement with 13 statements describing their knowledge, competence and attitude towards PGx testing on a five-point Likert scale, before and after attending the complete digital training program. In addition we surveyed the participants' satisfaction with the training program, using a 67-item questionnaire, which was based on an already available evaluation questionnaire from certified continuous pharmaceutical education courses. The respective questionnaire allowed open-ended and Likert-type responses to assess opinions regarding the content and the realization of the program as well as to collect suggestions on its potential further improvement.

2.5. Data Analysis

Participant characteristics and outcomes of the participant satisfaction survey were summarized and described as either means and standard deviations (SD) for scale variables or total counts and percentage for group variables. Due to the small sample size we chose non parametric testing to compare outcomes of the pre- and post-training knowledge test and self-perception questionnaires. For the knowledge test we used the Wilcoxon matched pairs test. However, as the method of data collection in the self-perception questionnaires did not allow data pairing, pre- and post-training outcomes were compared using the Mann–Whitney test. GraphPad Prism Version 5.01 was applied for all statistical analyses.

3. Results

3.1. Participant Characteristics

The group of 21 participants (women n = 14, 66.7%; mean age = 38 years, SD 8.9), consisted of pharmacists with an average of 12.5 (SD 8.2) years of practical experience and

a majority holding a postgraduate degree in community or hospital pharmacy (n = 15; 71.4%). Participants predominantly had a hospital pharmacy background (n = 12; 57.1%) and were almost exclusively from the German speaking part of Switzerland (n = 20; 95.2%) (Table 2).

Table 2. Participant characteristics.

Characteristic	Category	Mean (SD) or Number (%)
Age	-	38.1 (8.9) range: 26–57
Gender	Women Men	14 (66.7) 7 (33.3)
Years in practice	-	12.5 (8.2) range: 1–32
Practice setting	Community pharmacy Hospital pharmacy Other [1]	7 (33.3) 12 (57.1) 2 (9.5)
Postgraduate degree	-	15 (71.4)
Career level	Senior pharmacist Employed pharmacist Not specified	6 (28.6) 12 (57.1) 3 (14.3)
Percentage employment	-	82.9 (18.7) range: 40–100
Place of work in Switzerland (CH)	German speaking CH Italian speaking CH French speaking CH	20 (95.2) 1 (4.8) 0 (0)

[1] Health authorities, Academia.

3.2. Participant Development of Knowledge, Attitude, and Competence

Prior to the asynchronous, self-study online module all participants (n = 21) answered the multiple choice PGx knowledge test and scored on average 75.3% (SD 8.9). The mean score of the PGx knowledge test participants took after the asynchronous, self-study online module resulted in 90.3% (SD 6.0), which is a mean difference of 15% ($p < 0.001$).

The survey of self-perception of knowledge, attitude and competence was collected from all 21 participants prior to the start of the training program, whereas after completion of the full training program only 20 participants answered the follow-up survey. After finishing the training program, participants on average rather agreed with statements expressing knowledge in the field of pharmacogenetics, which is a significantly increased agreement compared to their rating of the same statements prior to the course (Table 3). When asked to rate statements about their attitude towards pharmacogenetics in pharmacy practice, participants rather agreed with them before and after the training program. Statements on competencies to apply pharmacogenetics in practice were perceived as neutral to rather agreeing before the training. After completion of the program participants showed significantly enhanced agreement with the same statements about competencies (Table 4).

3.3. Participant Satisfaction

The participants did not consistently answer all questions of the satisfaction questionnaire, which is why the number of answers may differ from the total number of participants. Overall participants expressed their satisfaction with the training program, by mainly strongly agreeing to recommend the course to a colleague (79% (15), n = 19) and mainly rating the complete course as excellent (79% (15), n = 19). The asynchronous, self-study online module was generally rated as good or excellent (29/67% (6/14), n = 21) and participants largely agreed or strongly agreed with the user-friendliness of the learning management system used (38/43% (8/9), n = 21) as well as with the desire to attend further trainings with similar online modules (48/38% (10/8), n = 21). When asked about the

usefulness of the assessment of their individual PGx profile, all participants agreed or completely agreed with it having an additional educative effect (15/85% (3/17), n = 20). We further asked about their intentions to implement PGx testing as a service in their pharmacy. A majority expressed to be still indecisive about implementing PGx testing (42% (8), n = 19) and others agreed or completely agreed to implement it (36/5% (7/1), n = 19). The remainder disagreed with implementing a PGx service in their pharmacy (16% (3), n = 19). To explain their reluctance towards introducing a PGx service, participants mentioned lack of time or support from superiors, the current legal requirements regarding the initiation of genetic testing in Switzerland, and the lack of coverage of the service by health insurers. For the overall program, a potential for improvement was especially noted for the extent and time required in the asynchronous learning sequences (self-study online module and transfer task) as well as for the limited opportunities for individual exchange between the participants.

Table 3. Self-perception of knowledge.

Item	Pre-Training Mean (SD) n = 21	Post-Training Mean (SD) n = 20	p-Value (Mann–Whitney Test)
I am sufficiently informed about the availability of genetic testing.	1.8 (0.9)	3.9 (0.8)	<0.001
I am adequately informed about the use of pharmacogenetics in the context of drug selection.	2.1 (0.9)	4.4 (0.6)	<0.001
I am adequately informed about the use of pharmacogenetics in the context of drug dosing.	2.0 (0.7)	4.2 (0.5)	<0.001
I feel comfortable using my current knowledge of pharmacogenetics to recommend medications.	2.0 (1.0)	3.9 (0.4)	<0.001
I feel comfortable using my current knowledge of pharmacogenetics to recommend drug dosages.	1.9 (0.9)	3.8 (0.6)	<0.001

Response Scale: 1 = Do not agree at all; 2 = Rather do not agree; 3 = Neutral; 4 = Rather agree; 5 = Fully agree.

Table 4. Attitude and self-perception of competence.

Item	Pre-Training Mean (SD) n = 21	Post-Training Mean (SD) n = 20	p-Value (Mann–Whitney Test)
Pharmacogenetics will be an important component of pharmacy practice in the future.	4.1 (0.7)	4.2 (0.7)	0.989
As a pharmacist, I am well positioned to interpret information from pharmacogenetics testing for my patients.	4.0 (0.7)	4.2 (0.6)	0.204
Pharmacogenetics is relevant to my clinical practice.	3.9 (0.9)	3.8 (0.9)	0.847
I can identify drugs for which pharmacogenetic testing is an option.	3.1 (1.0)	4.4 (0.5)	<0.001

Table 4. *Cont.*

I am able to accurately apply pharmacogenetic concepts to select medications for my patients.	2.2 (0.9)	3.9 (0.4)	<0.001
I am able to accurately apply pharmacogenetic concepts to determine dosages for my patients.	2.0 (0.8)	3.8 (0.5)	<0.001
I can share information with my patients about how pharmacogenetics can affect the efficacy of their medications.	3.4 (1.0)	4.5 (0.5)	<0.001
I can share information with my patients about how pharmacogenetics can affect the safety of their medications.	3.3 (1.0)	4.5 (0.5)	<0.001

Response Scale: 1 = Do not agree at all; 2 = Rather do not agree; 3 = Neutral; 4 = Rather agree; 5 = Fully agree.

4. Discussion

We conceptualized and piloted an extensive multi-day and application-oriented advanced, digital training program on PGx testing as a pharmacy service, which to our knowledge is the first of its kind reported for Switzerland.

The 21 participants showed good knowledge on the fundamentals of pharmacogenetics already prior to the course, measured by the average of correctly answered questions in the initial knowledge test of over 75%. However, after completion of the asynchronous self-study online module, participants further increased their average scores to 90%, which is a significant improvement ($p < 0.001$), indicating a gain of short time knowledge. Additionally, the according standard deviation was reduced by almost one third ($\pm$8.9% vs. $\pm$6.0%), when comparing the before and after test results, which indicates a reduction in scattering of test results and therefore a potentially more balanced level of basic PGx knowledge among participants after completing the self-study online module. This asynchronous self-study online module was indeed designed to improve the participants' knowledge on PGx fundamentals but should also allow for an individual and self-paced familiarization with the topic to bring all participants to a similar level of knowledge. In terms of personal attitude, participating pharmacists were from the beginning convinced about the importance of PGx in pharmacy practice and further about their significant role as pharmacists in PGx testing. The already apparent good PGx basic knowledge and favorable attitude towards PGx testing in pharmacy practice prior to the program, does not necessarily reflect the general Swiss pharmacist community, but is probably due to a selection bias. Paid program participation was open to all pharmacists and registration was on a voluntary basis, which is why we expected to attract and recruit individuals with an already positive attitude towards pharmacogenetics in pharmacy practice and a general interest in the topic. It may be discussed that the already initially present motivation and positive attitude has additionally positively influenced the participants' learning outcomes.

The average participant could be described as a rather experienced pharmacist, with an average age of over 38 years, experience of over 12 years in pharmacy practice and predominantly holding a postgraduate pharmacy degree. As the course was held in German, we anticipated to mainly attract pharmacists from the German-speaking part of Switzerland, which may indeed limit our findings to this region. However, German is the main language of over 60% of the Swiss population [19].

Participants not only showed an improvement of basic PGx knowledge, but also demonstrated a significant development in self-perceived knowledge and competencies. After completion of the program, the participating pharmacists on average agreed or fully agreed with statements regarding their PGx knowledge and their competencies in applying it in pharmacy practice. Notably, participants perceived their ability to counsel and share information on PGx with a patient as significantly improved. All our reported findings are in line and comparable with other published PGx continuing education programs

for pharmacists conducted in Canada and the United States [15,20,21]. In comparison with these previous programs, it is worth mentioning, that in our program we offered the participants an analysis of their personal PGx profile, which may have allowed them to experience the handling and use of PGx data in a more intuitive and therefore sustainable way. Indeed, participants perceived this opportunity as an additional educational benefit. In a recent study it was shown, that after providing medical doctors with their individual PGx profile, their attitude towards PGx testing and awareness of its potential impact on drug therapy significantly changed. The involved medical doctors were more positive about PGx testing and its utility [22]. Another method to enhance sustainable learning, might include the use of simulated patient interactions. Simulation-based clinical pharmacy training has been found to beneficially impact learning in students and postgraduate pharmacists [23]. However, in the herein presented training program we have used non-simulated, theoretically discussed patient cases. To further address patient interaction in PGx counselling simulation-based training might offer a suitable learning method.

Furthermore, our program was conducted exclusively digitally. Initially, only the introductory module on PGx basics was planned as a digital training. However, due to the ongoing COVID19 pandemic at that time, the complete course was later converted into a digital training with alternating sequences of asynchronous and synchronous learning, so called blended learning. The participants' overall satisfaction with the execution of the program is reflected in their exceedingly positive rating in the final satisfaction survey. Nonetheless, the participants also mentioned a specific drawback of the digital program. They perceived the opportunities for individual exchange between the participating peers as limited. In the case of a further completely digitally conducted program, we would like to specifically ensure that the participants have sufficient opportunities for personal exchange. This may, for example, be enhanced with a kick-off, face-to-face video conference, where participants have the chance to introduce themselves and learn about other participants' and the overall program goals, to create more of a team spirit.

Connecting and learning from each other's experiences may play an important role in the further process of implementing a new pharmacy service. When we asked the participating pharmacists after completing the program about their intentions to implement PGx testing as a service in their pharmacy practice, feedback was mixed. Potential barriers to an implementation of PGx testing were defined as a lack of time or support from superiors, the current legal requirements regarding the initiation of genetic testing in Switzerland, and the lack of coverage of the service by health insurers. To help participants gain confidence and offer support with implementation, we initiated a peer group for pharmacists interested in offering PGx services to share their experiences with peers and adressing further challenges met in daily practice (e.g., patient couselling and education, insurance, data protection).

5. Conclusions

The pilot study on our advanced, digital training program showed measurable improvement of both knowledge and competencies in applying pharmacgenetic testing in pharmaceutical care. Nonetheless, we consider ongoing education, for example within our peer group, as an important driver for the implementation of PGx testing in community and hospital pharmacies. For the future, we hope to also include interested medical doctors in our educational program and in our peer group, to further enhance the undoubtedly necessary interprofessional collaboration in PGx testing to improve patient outcomes [24].

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Institutional Review Board Statement: Ethical review and approval were waived for this study. According to the definition of human research in the Swiss legislation, the project is not subject to approval by the ethics committees, as the anonymously collected/anonymized data are not health-related and were collected for evaluation and quality assurance of the training program.

Informed Consent Statement: Written informed consent has been obtained from the participants to publish this paper.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. Data were collected in German and are not publicly available for privacy reasons.

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5.3 Project C

PGx in pharmaceutical care: Pharmacist-led PGx service

A guide to a pharmacist-led pharmacogenetic testing and counselling service in an interprofessional healthcare setting [C]

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A Guide to a Pharmacist-Led Pharmacogenetic Testing and Counselling Service in an Interprofessional Healthcare Setting

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Abstract: Genetic predisposition is one factor influencing interindividual drug response. Pharmacogenetic information can be used to guide the selection and dosing of certain drugs. However, the implementation of pharmacogenetics (PGx) in clinical practice remains challenging. Defining a formal structure, as well as concrete procedures and clearly defined responsibilities, may facilitate and increase the use of PGx in clinical practice. Over 140 patient cases from an observational study in Switzerland formed the basis for the design and refinement of a pharmacist-led pharmacogenetics testing and counselling service (PGx service) in an interprofessional setting. Herein, we defined a six-step approach, including: (1) patient referral; (2) pre-test-counselling; (3) PGx testing; (4) medication review; (5) counselling; (6) follow-up. The six-step approach supports the importance of an interprofessional collaboration and the role of pharmacists in PGx testing and counselling across healthcare settings.

Keywords: pharmaceutical care; clinical pharmacy; medication review; pharmacy service; pharmacogenomics; personalized medicine; hospital pharmacy; community pharmacy; primary care; secondary care

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1. Introduction

In clinical practice, patients show individual responses to pharmacotherapy. While some experience an adequate effect, others do not respond at all, and some suffer from unwanted adverse reactions or even severe toxicities when taking the same drug at the same dose. Amongst many others, one reason for this may be the patients' individual genetic predisposition. On the one hand, genetic variation can impact drug response by altering the expression and/or activity of genes encoding the enzymes and transporters that are involved in drug absorption, distribution, metabolism, or excretion (ADME), potentially affecting pharmacokinetics. On the other hand, genes encoding drug targets can also show variations, which may alter their structure, expression or activity, potentially affecting pharmacodynamics [1]. As an illustration, the enzyme cytochrome P450 2D6 (CYP2D6), which is involved in the metabolism of over 25% of marketed drugs, exhibits a wide range of metabolic capacities across a population. This is in part due to several known genetic variants translating into normal, increased, reduced, or even lacking enzyme

activity. These enzymatic activities are grouped into four major phenotypes: (i) normal metabolizers (normal activity, NM); (ii) ultra-rapid or rapid metabolizers (increased activity, UM/RM); (iii) intermediate metabolizers (reduced activity, IM); (iv) poor metabolizers (no activity, PM). CYP2D6 genetic variants were found to affect the pharmacokinetics of several substrate drugs and thereby the risk of experiencing adverse drug reactions or therapy failure [2]. Compelling evidence in this context led to the incorporation of pharmacogenetic (PGx) information on drug labels and even to the publication of international PGx dosing guidelines for multiple CYP2D6 substrates, including analgesics, antidepressants, neuroleptics, antiarrhythmics and antiemetics [3–5].

Hitherto, PGx testing has become increasingly applicable in clinical practice as it becomes more and more affordable, and as advances in digital technology enable the integration of PGx information into clinical decision support tools [6]. However, PGx is not the only factor influencing drug response. In particular, other non-genetic factors may affect individual drug response as well, including physiological factors (e.g., age, sex, organ function); environmental factors (e.g., drug–drug interactions (DDI), food–drug interactions, smoking); and behavioral factors (e.g., medication adherence) [7,8]. Notably, a patient that is found to be a CYP2D6 normal metabolizer, based on PGx testing, may thus become an intermediate or even poor metabolizer through the co-administration of a CYP2D6-inhibitor. This deviation from an individual's genotype-predicted phenotype by non-genetic factors is considered a phenoconversion [9]. However, PGx assessments in clinical practice are often focused on drug–gene interactions (DGI) only, without considering the other factors that are potentially needed for a patient-individual evaluation and integration of PGx information. In these cases, if the prediction of enzyme function for pharmacokinetic estimations is challenging, therapeutic drug monitoring (TDM) can be a relevant addition to PGx testing [10]. Clinical pharmacists are trained to consider a wide range of factors influencing drug response when performing a medication review to address drug-related problems [11]. Thereby, pharmacogenetic information may offer an additional piece to complete the medication review puzzle, enhancing more comprehensive and individualized analysis and therapy recommendations.

A glance at clinical practice shows that the integration of PGx in clinical routine is still modest or often lacking. Barriers to the application of PGx are diverse, including restricted reimbursement of PGx tests, partially limited evidence from prospective clinical trials, as well as a lack of education of healthcare professionals [12]. There are numerous notions addressing prospective PGx evidence e.g., [13–15], and the education of healthcare professionals, e.g., [16,17]. However, only a limited number of publications address how a PGx service aiming for patient-individual therapy recommendations can be designed, refined and applied in a real-world multi-professional healthcare setting e.g., [18]. A recent survey of Dutch pharmacists, physicians and patients, participating in a pilot study for an outpatient PGx service, found that the unclear allocation of responsibilities between healthcare professionals was a major barrier to the implementation of the PGx service [19]. Defining a formal structure, as well as concrete procedures and clearly defined responsibilities, may facilitate and increase the implementation of PGx testing in clinical practice. Herein, we describe the design and the refinement of a pharmacist-led PGx service in an interprofessional setting.

2. Materials and Methods

2.1. Service Design

The planning of the service started with the selection of a commercial provider of pharmacogenetic analyses applicable to an intervention in pharmacy practice. After a comparison of several commercial providers, we selected a system that was originally developed for pharmacogenetic testing in pharmacy practice (Stratipharm®, humatrix AG, Pfungstadt, Germany). The system offers sampling by buccal swabs, analyzing a panel of clinically relevant genetic variants (Table S1) that not only reports the geno- or haplotypes, but also provides a sophisticated phenotypic interpretation that is relevant to

most of the drugs that are currently available on the European market. Within this system, accredited healthcare professionals can be granted access to the genetic information by a patient-owned personal code. Interpretation of the genetic data for the impact on selected drugs is continuously updated based on currently available evidence and recommendations extracted from Pubmed (www.pubmed.ncbi.nlm.nih.gov) (accessed on 1 April 2022); PharmGKB (www.pharmgkb.org) (accessed on 1 April 2022); and CPIC (www.cpicpgx.org) (accessed on 1 April 2022), respectively. Moreover, we adapted the proposed procedure of a service that was published by the U-PGx (Ubiquitous Pharmacogenomics) project strategy [14] to the Swiss healthcare system. The adaptation was carried out based on the information that was obtained from stakeholders from different fields who were involved in PGx, including clinical pharmacists, clinical pharmacologists, epidemiologists, the Swiss federal commission for genetic testing and professional associations. The final service description in the Results section follows the recommendations of the TIDieR (template for intervention description and replication) checklist for better reporting of interventions [20].

2.2. Service Refinement

The service, consisting of a comprehensive medication review [11] and supplemented with the individuals' pharmacogenetic information to optimize drug selection and dosing, was originally started with single cases. After further standardization of the intervention, an observational case series study (ClinicalTrials.gov ID: NCT04154553) was launched. The primary objective of the case series was the compilation of case reports, where pharmacogenetic testing was applied to determine the hereditable component of the patient's susceptibility to experience therapy failure (TF) and/or adverse drug reactions (ADR). Patients were recruited in the primary care setting, during hospitalization, or in ambulant hospital care. Eligible were adult patients either experiencing ADR or TF, or patients with a positive family history (of either); or patients with a planned/ new prescription for drugs that were known to be affected by genetic variants that influence their drug metabolism (pharmacokinetics) and/or the activity of the drug target (pharmacodynamics). Following the referral by the treating physician, the recruited patients underwent the process, as depicted in Figure 1. Individual cases from the series were published as case reports [21–26]. The work experience that was gathered within the observational case series study was used to further refine the PGx service over the duration of 3 years between 2019 and 2021, and was based on feedback from patients and treating physicians that was further elaborated in a mixed methods study [27].

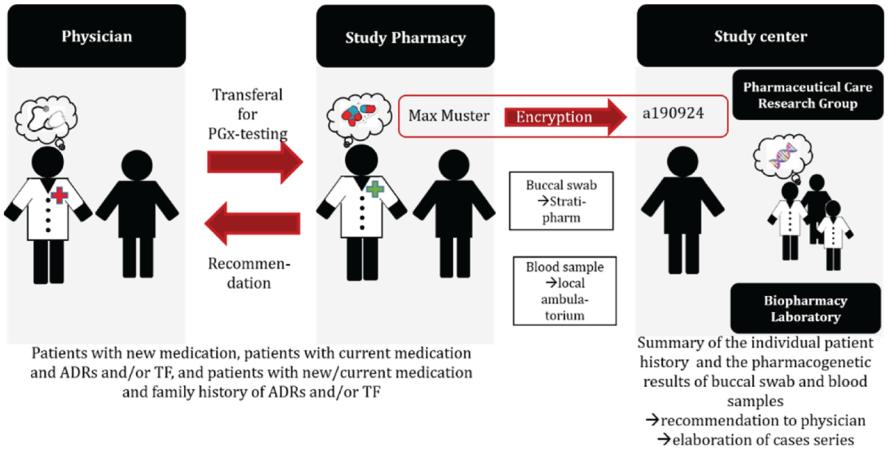

Figure 1. Study procedure of the observational case series study. ADR: adverse drug reaction; TF: treatment failure; PGx: pharmacogenetic.

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3. Results

3.1. Service Description

The herein described service leads, within six steps, to the integration of pharmacogenetic information into a medication review by a pharmacist to serve as a rational basis for shared decision making, together with the treating physicians and the patient, in order to enable individualized pharmacotherapy optimization (Figure 2).

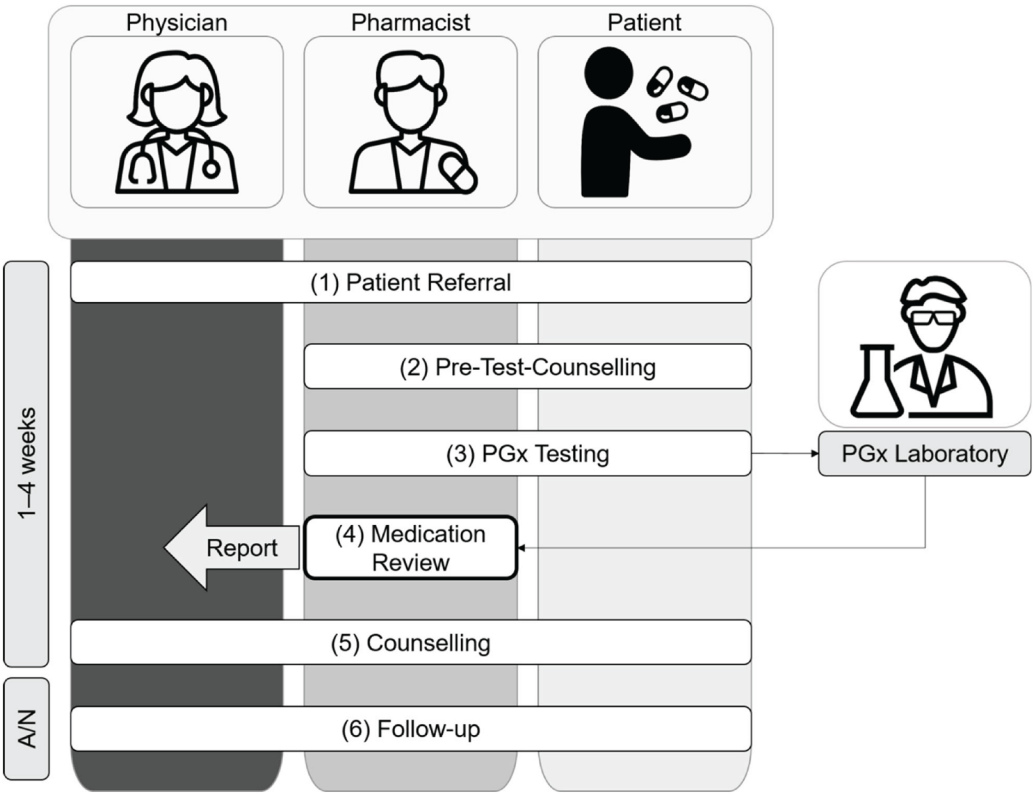

Figure 2. Overview of the pharmacist-led PGx service in an interprofessional setting.

1. Patient Referral

Target patients have (a) experienced ADR and/or TF (reactive); (b) a planned new prescription or pharmacotherapy changes (preemptive); or (c) a family history of ADR and/or TF (preemptive). Patients are referred to the pharmacist-led PGx service (i) by their physician (general practitioner or specialist); (ii) based on own initiative (i.e., word-of-mouth); or (iii) by a pharmacist. In any case, treating physicians are informed and asked for their support for the planned pharmacist-led PGx service.

2. Pre-Test Counselling
   After referral to the pharmacist-led PGx service, the pharmacist and the patient meet face-to-face at the community/hospital pharmacy or at the hospital ward for a pre-test counselling visit to decide whether to proceed with PGx testing, following these steps:
   - 2.1 The pharmacist informs the patient about the goals, potential significance, and limits of PGx testing. In addition, the pharmacist answers any questions that the patient may have about PGx testing;
   - 2.2 The pharmacist performs a medication reconciliation and preliminary medication review of type 2a [11], using the Swiss polymedication check form [28] as an interview guide to (i) assess the patient's current medication regimen;

(ii) clarify the patient's medication history, including experienced ADR and TF; (iii) identify any non-genetic drug related problems (e.g., drug–drug interaction, smoking, nutrition, renal and liver function, medication adherence, allergies). The pharmacist then clarifies any remaining ambiguities with family members or institutions providing care (e.g., home care, dispensing pharmacy, prescribing physician), provided that the patient agrees to do so. If urgent action is required due to identified drug-related problems (e.g., contraindications, need for therapeutic drug monitoring), the pharmacist immediately consults with the treating physician;

2.3 The pharmacist decides whether to proceed with PGx testing based on the information that is available from the patient interview (2.2.). More precisely, there must either be pharmacogenetic recommendations available (e.g., CPIC guidelines) or a rationale from the drug's metabolism for potential DGIs, for at least one substance or drug class that is indicated as suspicious. Substances are classified as conspicuous, e.g., either due to ADR and/or TF (reactive approach), or when considered for planned treatments (preemptive approach);

2.4 The pharmacist collects the patient's written informed consent for PGx testing. A copy of the signed informed consent is given to the patient. The pharmacist ensures that any questions the patient may have are answered. If the patient needs more time to decide, the further procedure may be postponed.

3. PGx Testing

The pharmacist collects a swab of the patient's oral mucosa and ships it to the designated and approved PGx laboratory together with the signed informed consent (2.4). The PGx laboratory provides the pharmacist with the analyzed results from PGx testing (e.g., information about genetic variants and corresponding phenotype interpretation, processing time for Stratipharm®—max. 7–10 working days) and an online clinical decision support tool to check for DGIs.

4. Medication Review

The goal of the medication review process is (a) to detect drug-related problems and (b) to recommend specific medication changes or interventions, in order to optimize the patient's pharmacotherapy to better meet his needs, and by this to ultimately improve health outcomes. Therefore, the pharmacist performs a structured evaluation of the patient's past, current and planned medication, considering the available genetic and non-genetic information (2.2 and 2.3). To support this evaluation, the pharmacist consults (i) the PGx laboratory's clinical decision support tool and the pharmGKB database (www.pharmgkb.org) (accessed on 1 April 2022) to assess DGIs; (ii) the summary of product characteristics and a drug interaction database (mediQ, www.mediq.ch) (accessed on 1 April 2022) to assess drug–drug interactions (DDI) and other drug related problems; (iii) a quantitative prediction tool to assess drug–drug–gene interactions (DDGI) (www.ddi-predictor.org) (accessed on 1 April 2022), combining the assessment results from (i) and (ii) (e.g., phenoconversion). Finally, the pharmacist prepares a written report with patient-specific recommendations and sends it to the treating physician.

5. Counselling

The pharmacist and/or treating physician communicate the PGx test results (3) and the medication review conclusions (4) to the patient in a face-to-face visit, phone call or video conference. The setting of the counselling is chosen based on the preferences of the patient and/or physician. In a process of shared decision making, the pharmacotherapy is adapted or additional laboratory analyses are initiated (e.g., therapeutic drug monitoring).

6. Follow-up

The pharmacist actively follows up with the patient one and six months after the counselling (5) to answer any further questions the patient may have and to assess the need for further counselling. The pharmacist offers the physician (i) follow-up counselling for

further questions regarding the PGx test results, and (ii) an update of the medication review; for instance, in the case of major medication changes or shifts of variable non-genetic factors (e.g., renal function).

3.2. Service Refinement

The population of the case series observational study, which formed the basis for the PGx service refinement, consisted of 142 mainly female (66%) patients with a median age of 52 (IQR = 40–63) years. Around 60% of the patients were referred to the PGx service by a medical specialist doctor and about the same proportion was enrolled in the primary care setting (community pharmacy). A majority of the included patients had a main diagnosis of a mental or behavioral disorder (ICD-10 = F, 61%). The number of prescribed medicines reached a median of 6 (IQR = 4–9) per person, resulting in a majority of patients with polypharmacy (≥5 prescribed medicines, 62%), (Table 1).

Table 1. Demographics of the observational case series study.

Characteristic	Category	Number (%) or Median (IQR)
Subjects, n	-	142
Age (years), median (IQR)	-	52 (40–63) (min. 18, max. 88)
Gender, n (%)	Female Male	93 (65.5) 49 (34.5)
Referring party, n (%)	Medical specialist General practitioner Pharmacist	92 (64.8) 25 (17.6) 25 (17.6)
Enrollment setting, n (%)	Community pharmacy Hospital pharmacy	85 (59.9) 57 (40.1)
Main diagnosis, n (%)	Mental and behavioral disorders (ICD-10: F) Diseases of the musculoskeletal system and connective tissue (ICD-10: M) Diseases of the circulatory system (ICD-10: I) Other *	86 (60.6) 30 (21.1) 15 (10.6) 11 (7.8)
Number of prescribed medicines, median (IQR)	-	6 (4–9)
Polypharmacy (≥5 prescribed medicines), n (%)	-	92 (62.2)

* ICD-10: C (neoplasms); -E (endocrine, nutritional and metabolic diseases); -G (diseases of the nervous system); -R (symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified); -U (codes for special purposes); or -Z (factors influencing health status and contact with health services).

The patients were included in the case series study to apply the PGx service based on a total of 549 suspected substances, which corresponded to a median of three suspected substances per patient (IQR 2–5). These were suspicious for DGIs due to clinically observed ineffectiveness (39%), ADR (40%) or both (5%). A smaller proportion gave cause to apply the PGx service preemptively due to planned new prescriptions (15%) or a family history of ADR and/or TF (0.6%). Slightly less than two-thirds of these suspected substances were eventually associated with any of the tested pharmacogenetic variations (n, 318; median, 2; IQR 1–3). The frequencies of genotype-predicted CYP2D6- and CYP2C19-phenotypes in our population correspond to the expected frequencies in the overall European population [29]. The patient-specific recommendations derived from the medication review by the pharmacists were implemented in about two-thirds of the cases that were followed up in both community (64%) and hospital pharmacy (66%) settings. The documented workload to perform the pre-test counselling, the medication review and the final counselling was on average 3 h per patient.

With the experiences from the case series, we refined the processes of patient referral (1); medication review (4); counselling (5); and follow-up (6) (Figure 2).

Initially, the patients were referred (Figure 2, step 1) to the intervention by their treating physicians who were informed about this opportunity in general practitioner quality circles or during hospital briefings. However, in pharmacy practice, drug-related problems are also directly addressed by pharmacists and/or patients during consultations and drug dispensing in the community pharmacy setting, or during interprofessional ward rounds and medication reconciliation in the hospital setting. Therefore, the pharmacists started to directly approach eligible patients. In a few cases, the patients approached the pharmacists through their own initiative due to word of mouth. In any case, treating physicians were informed and asked for their support for the planned pharmacist-led PGx service.

The case series increased the involved pharmacists' and physicians' knowledge and experience with pharmacogenetics, which influenced the medication review structure and content (Figure 2, step 4). Based on the experience, pharmacists started to supplement the medication review report with a concise overview of the patients' pharmacogenetic profile and thereof predicted phenotypes, in order to facilitate the understanding about PGx information and to enable the application of these results to future drug-related problems and questions. Furthermore, the pharmacists provided interpretations for the impact of predicted phenotypes on pharmacokinetics for substances without explicit pharmacogenetic guidelines whenever reasonable.

The counselling visit (Figure 2, step 5) was originally intended to take place only between the pharmacist and the patient. Some physicians however preferred to take part in the counselling or even conduct the counselling themselves to facilitate shared decision making. Therefore, we started to organize the counselling visits individually based on the patients' and physicians' preferences, so that the pharmacist and/or the physician were able to conduct the counselling visit with the patient based on the medication review that was provided by the pharmacist.

The follow-up (Figure 2, step 6) was primarily intended to evaluate the implementation of the pharmacists' recommendations within the case series study. However, the follow-up was additionally appreciated by the involved pharmacists, physicians, as well as patients, and was thus adapted accordingly. Patients and physicians received the opportunity to clarify open questions and place further queries. Pharmacists were able to collect continuous feedback on their recommendations and to remind the patients about the lifelong impact of their pharmacogenetic makeup.

4. Discussion

We propose a pharmacist-led PGx service for interprofessional settings in both primary and secondary care. This service was designed for and refined within the heterogeneous Swiss healthcare system, consisting of 26 different cantonal systems. Therefore, we believe that this service may also be applied in the healthcare systems of other countries. For the adaptation of this service, we have had good experience in consulting a wide range of experts, including clinical pharmacologists and epidemiologists, who are experienced in the field of PGx.

Projects with pharmacists who are involved in pharmacogenetic testing have been described for distinct healthcare settings, from primary care to individual clinics e.g., [18,30,31]. Our goal was to develop step-by-step guidance to encourage the practical implementation of a pharmacist-led pharmacogenetic service across healthcare settings and with the inclusion of other healthcare professionals and patients. Our experiences with the case series study showed that this approach was feasible in different settings and across a diverse sample of patients. However, we would like to highlight several remaining challenges when implementing such a service in clinical practice.

First, the documented workload of patient counselling and conducting the medication review was on average 3 h per patient. This cumulation does not include administrative work to arrange the appointments or sample shipping, nor the time that is invested for

follow-up visits etc. This raises the question of resource management and reimbursement of the provided services. For instance, in Switzerland, PGx testing requires in most cases a prescription from a specialized pharmacologist to ensure basic healthcare coverage. Moreover, the initiation of PGx testing is by Swiss law currently limited to physicians. The Swiss law on genetic testing in humans is currently under revision, considering a notion to enable pharmacists to initiate PGx testing [32]. From our experience in the case series study, pharmacists became aware of drug-related problems that were potentially associated with PGx in their daily practice. Pharmacists, as important points of contact for patients when it comes to drug-related problems, are ideally placed to include pharmacogenetic information in their assessment of medication therapies. Enabling pharmacists to initiate PGx testing might enhance its implementation in clinical practice as an interprofessional service to improve patient outcomes.

Second, we consider equal and strong interprofessional collaboration to be a key factor for the implementation of the proposed service. The service involves at least four parties, namely, a pharmacist, a physician, a PGx laboratory and the patient. However, depending on the individual setting and notably for multimorbid elderly patients, there might also be more parties involved, for instance, additional physicians (general practitioners and medical specialists), therapists, nurses or other caregivers such as family members. Having existing and trusting relationships with all the involved parties proved essential for the success of implementing the service. While in secondary care, already established collaborations between healthcare professionals may facilitate the implementation of the PGx service, our experience shows that this service is also feasible in primary care settings. This is reflected in the fact that pharmacists' recommendations were implemented with equal frequency in both primary and secondary care settings (ca. 65%). Still, implementing such a PGx service in primary care can be associated with an increased effort to establish essential interprofessional relationships. One prerequisite for a beneficial interprofessional collaboration in PGx is the continuous education of the involved healthcare professionals. Lacking knowledge of PGx amongst healthcare professionals has been described as a barrier to the implementation of PGx in clinical practice [12]. To address this, we have developed a blended-learning continuous educational program for pharmacists based on our work experience from the case series study. In the future, we plan to also include physicians, which may enhance interprofessional collaboration from the very start [16].

Third, the lack of digital data exchange between healthcare providers hinders communication and data sharing. So far, Switzerland lacks a consistent e-health strategy to overcome this barrier. Improved digital networks, considering data security, could enhance the continuous use of PGx information across healthcare settings and professions. Notably, germline genetic information has a lifelong validity. To overcome the large heterogeneity of data management systems, the U-PGx Consortium has adopted a so-called Safety-Code card system for their Europewide clinical trial (PREPARE). This personal card includes a basic overview of the individual's PGx profile and a QR-code to access a web-based decision support tool with individual PGx dosing recommendations. The Safety-Code card allows for easy sharing of the genetic information between healthcare providers and empowers the patient to decide who can access their data [14]. Apart from the accessibility of PGx information, digital interfaces are also important to facilitate access for healthcare providers to other relevant non-genetic information. As mentioned before, PGx information should be analyzed in context with non-genetic information, including co-medication and medication history. Therefore, a nationwide electronic health record (EHR) system would be of great benefit to ensure access for all healthcare providers to both genetic, as well as non-genetic health data. One of the early adopters of such an EHR system is Estonia, where a central digital repository provides access to an individual's lifelong medical history, including PGx information (https://e-estonia.com/) (accessed on 1 April 2022).

5. Conclusions and Outlook

Our proposed PGx service was feasible in an interprofessional and heterogeneous healthcare setting. Over 60% of our recommendations were implemented and we recorded a continuous referral of over 140 patients for 3 years. Our experience shows that a PGx service within an interprofessional setting needs a clear structure and assignment of tasks. Access to (electronic) patient data and remuneration for the service remain important barriers to the implementation in clinical practice. Moreover, follow-up studies are warranted to assess the impact (e.g., clinical outcome, cost-effectiveness) of such a PGx medication review intervention in selective patient cohorts. Based on our experiences, we selected psychiatric patients with major depression for a first ongoing outcome study [13]. Finally, pharmacists as specialists in pharmacotherapy and important points of contact for drug-related problems are ideally placed to initiate PGx testing and support other healthcare professionals with patient-specific medication reviews.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pharmacy10040086/s1, Table S1: Stratipharm® (humatrix AG, Pfungstadt, Germany) SNPs and annotations.

Author Contributions: Conceptualization and methodology, C.K.S., C.J., M.L.L., K.E.H., H.E.M.z.S. and S.S.A.; investigation, formal analysis and data interpretation, C.K.S., C.J., A.B., F.M.W., M.L.L., K.E.H., T.I., M.H., T.M. and H.E.M.z.S.; writing—original draft preparation, C.K.S.; writing—review and editing, C.J., A.B., F.M.W., M.L.L., K.E.H., T.I., M.H., T.M., H.E.M.z.S. and S.S.A.; visualization, C.K.S. and C.J.; supervision, S.S.A. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The underlying observational case study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local ethics committee of "Ethikkommission Nordwest- und Zentralschweiz" (2019-01452, 3 October 2019).

Informed Consent Statement: Informed consent was obtained from all the subjects involved in the study. Written informed consent was obtained from the patients to publish this paper.

Data Availability Statement: The data (incl. genetic data) presented in this study are available on request from the corresponding author. The data are not publicly available for ethical and privacy reasons.

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5.4 Project D

Pre-emptive pharmacist-led PGx service in MDD patients (PrePGx)

Pharmacist-guided pre-emptive pharmacogenetic testing in antidepressant therapy (PrePGx) study protocol for an open label, randomized controlled trial [D]

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Trials

STUDY PROTOCOL Open Access

Pharmacist-guided pre-emptive pharmacogenetic testing in antidepressant therapy (PrePGx): study protocol for an open-label, randomized controlled trial

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Abstract

Background: It is known that only 50% of patients diagnosed with major depressive disorders (MDD) respond to the first-line antidepressant treatment. Accordingly, there is a need to improve response rates to reduce healthcare costs and patient suffering. One approach to increase rates of treatment response might be the integration of pharmacogenetic (PGx) testing to stratify antidepressant drug selection. The goal of PGx assessments is to identify patients who have an increased risk to experience adverse drug reactions or non-response to specific drugs. Especially for antidepressants, there is compiling evidence on PGx influencing drug exposure as well as response.

Methods: This study is an open-label, randomized controlled trial conducted in two study centers in Switzerland: (1) the Psychiatric Clinic of Solothurn and (2) the Private Clinic Wyss in Münchenbuchsee. Adult inpatients diagnosed with a unipolar moderate or severe depressive episode are recruited at clinic admission and are included in the study. If the adjustment to a new antidepressant pharmacotherapy is necessary, the participants are randomized to either Arm A (intervention group) or Arm B (control group). If no new antidepressant pharmacotherapy is introduced the participants will be followed up in an observational arm. The intervention is the service of pharmacist-guided pre-emptive PGx testing to support clinical decision making on antidepressant selection and dosing. As a comparison, in the control group, the antidepressant pharmacotherapy is selected by the treating physician according to current treatment guidelines (standard of care) without the knowledge of PGx test results and support of clinical pharmacists. The primary outcome of this study compares the response rates under antidepressant treatment after 4 weeks between intervention and control arm.

Discussion: The findings from this clinical trial are expected to have a direct impact on inter-professional collaborations for the handling and use of PGx data in psychiatric practice.

Trial registration: ClinicalTrials.gov NCT04507555. Registered on August 11, 2020. Swiss National Clinical Trials Portal SNCTP000004015. Registered August 18, 2020.

Keywords: Pharmacogenomics, Depression, Antidepressant, Pharmaceutical care, Psychiatry

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Administrative information

Note: the numbers in curly brackets in this protocol refer to SPIRIT checklist item numbers. The order of the items has been modified to group similar items (see http://www.equator-network.org/reporting-guidelines/spirit-2013-statement-defining-standard-protocol-items-for-clinical-trials/).

Title {1}	**Pharmacist-guided pre-emptive pharmacogenetic testing in antidepressant therapy (PrePGx): study protocol for an open-label, randomized controlled trial.**
Trial registration {2a and 2b}.	ClinicalTrials.gov, ID: NCT04507555 Swiss National Clinical Trials Portal, ID: SNCTP000004015
Protocol version {3}	Version 3.1, dated 14.09.2021
Funding {4}	Pharmaceutical Care and Biopharmacy Research Groups, University of Basel, 4056 Basel, Switzerland Solothurner Spitäler AG, 4500 Solothurn, Switzerland Privatklinik Wyss AG, 3053 Münchenbuchsee, Switzerland Stiftung zur Förderung des pharmazeutischen Nachwuchses in Basel, 4054 Basel, Switzerland
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Name and contact information for the trial sponsor {5b}	Psychiatrische Dienste Solothurn PD Dr. med. Thorsten Mikoteit Weissensteinstrasse 102 4503 Solothurn Switzerland Phone: +41 32 627 11 11 URL: https://www.solothurnerspitaeler.ch/unsere-spitaeler/psychiatrische-dienste/
Role of sponsor {5c}	This study is an investigator-initiated trial with a sponsor-investigator. The third party funding source had no influence on the study design and will not be involved in its conduct, analysis, and publication of the results.

Introduction

Background and rationale {6a}

Successful treatment of depression remains challenging, considering the fact, that only 50% of patients suffering from major depressive disorders respond to the first-line antidepressant treatment [1, 2]. Furthermore, a drug exposure of at least 4 weeks is necessary to assess clinical treatment response [3], possibly making the trial and error approach time-consuming and exhausting for the patient.

It is well known that patients are exhibiting diverse reactions following drug intake. In many cases, interindividual variability in drug response can be attributed to changes in systemic drug exposure (area under the curve). Meaning the risk of low-drug serum levels resulting in treatment failure and high-drug serum levels leading to toxicity. The organism influences systemic drug exposure by multiple mechanisms namely absorption, distribution, metabolism, and excretion (ADME) of a taken drug molecule. This concept is summarized in the term of pharmacokinetics and comprises a variety of proteins acting on drug molecules in terms of transport (absorption, distribution, and excretion) as well as enzymatic reactions (metabolism). Changes in the activity of the aforementioned proteins will therefore affect systemic exposure and hence drug response. Moreover, the activity of drug transporters and enzymes is influenced by avoidable factors such as drug-drug interactions or drug-food interactions, but also by given predispositions including disease factors and genetics. In fact, a wide range of genes encoding drug transporters and enzymes are polymorphs, occasionally translating into proteins with altered activity [4].

A relevant drug-gene interaction is for example *CYP2D6* with tricyclic antidepressants. The gene encoding for the cytochrome P450 enzyme 2D6 is known to be highly polymorphic including variants that are translated into metabolizing enzymes with increased or reduced activity. Associated phenotypes are termed as ultra-rapid metabolizer or poor metabolizer, respectively [5]. Tricyclic antidepressants are often metabolized via CYP2D6 and have repeatedly been associated with altered pharmacokinetics due to the individual's genetic predisposition. Based on the rich data on pharmacogenetics, a CPIC (Clinical Pharmacogenetics Implementation Consortium) guideline was published with recommendations for tricyclic antidepressant dosing and for compound selection based on the respective CYP2D6 metabolizer status [6]. Nevertheless, sparse evidence from prospective trials in terms of therapy outcome and cost-effectiveness has so far been an obstacle for implementing *CYP2D6*-guided tricyclic antidepressant prescribing in clinical practice [7].

Remarkably, a wide range of studies is currently being conducted with the aim to identify biomarkers for early and reliable prediction of treatment outcomes of marketed antidepressants, e.g., [8, 9]. However, there is already compiling evidence on pharmacogenetics influencing both, antidepressant exposure and treatment response. This data is gathered and rated according to its level of evidence in the Pharmacogenomics Knowledge Base (PharmGKB) [10]. The aforementioned Clinical Pharmacogenetics Implementation Consortium (CPIC, https://cpicpgx.org/) and the Dutch Pharmacogenetics Working Group (DPWG, https://www.knmp.nl/patientenzorg/medicatiebewaking/farmacogenetica) are publishing guidelines on genotype-guided drug dosing and/or drug selection, which currently includes recommendations for tricyclic antidepressants and selective serotonin reuptake inhibitors. Besides, the Swiss Society for Anxiety and Depression (SGAD) recommends genotyping of *ABCB1* upon antidepressant treatment failure [3]. The latter gene encodes for p-glycoprotein an efflux transporter known for its function in the extrusion of drug molecules and xenobiotics at the blood-brain barrier. Even if not fully validated, it has been hypothesized that patients carrying the wildtype allele of the transporter exhibit increased efflux of substrate antidepressant drugs at the blood-brain barrier, which would translate into decreased drug levels within the central nervous system, and therefore at the place of action. This assumption is based on a limited number of studies, where the *ABCB1* genotype was linked to antidepressant treatment response [11–13].

Even though pharmacogenotyping is not part of routine patient care, pharmaceutical companies cite the known influence of certain polymorphisms on serum levels, adverse drug reactions, and treatment failure in their drug labels [14]. This also applies to several antidepressants authorized in Switzerland namely the tricyclic antidepressants clomipramine, amitriptyline, nortriptyline, and opipramol; the selective serotonin reuptake inhibitors escitalopram, citalopram, fluoxetine, paroxetine, and fluvoxamine; the monoamine oxidase A inhibitor, moclobemide, and the serotonin; and noradrenaline reuptake inhibitors venlafaxine and duloxetine, to name some of them.

Today, pharmacogenetic panel tests are commercially offered. These panel tests consider multiple polymorphic genes involved in pharmacokinetics as well as in the pharmacodynamics of antidepressant drugs. Stratipharm® (humatrix AG, Pfungstadt Germany, https://www.stratipharm.de) is one of the commercial products offering pharmacogenetic panel testing from buccal swabs combined with an evidence-based genotype interpretation. Hitherto, there is only a limited number of prospective clinical studies and to our knowledge non conducted in Switzerland, testing the influence of pre-emptive pharmacogenotyping on patient outcome, whereby limiting the evidence for being advantageous for depression remission or cost-effectiveness over the standard of care, e.g., [15–17]. In fact, pre-emptive panel testing is not yet state of the art in psychiatric practice.

Objectives {7}

We hypothesize that it is beneficial to incorporate PGx information to guide drug selection and dosing in the treatment of depression, involving clinical pharmacists in processing and evaluating the PGx test results in the context of the individual patient history and current co-medication.

The primary objective of this clinical study is to compare the service of pharmacist-guided PGx testing with the current standard of care for antidepressant selection and dosing with regard to treatment outcome. Accordingly, the following null hypothesis results for the primary endpoint: Therapy response rates after antidepressant treatment for 4 weeks do not differ whether the service of pharmacist-guided pre-emptive pharmacogenetic testing was applied or not.

The secondary objectives are to compare tolerability of the antidepressant pharmacotherapy and overall duration of hospitalization between the intervention and standard care study arms.

Trial design {8}

This is an open-label, randomized controlled trial, investigating the effectiveness and tolerability of registered antidepressants in adult inpatients with diagnosed major depressive episode.

To prevent selection bias, eligible patients in need of a new antidepressant pharmacotherapy are randomized at the same ratio into either the control or the intervention arm (parallel study arms).

Methods: Participants, interventions, and outcomes

Study setting {9}

This is a multicenter clinical trial conducted in Switzerland, at the Psychiatric Clinic of the Solothurner Spitäler AG in Solothurn and the Private Clinic Wyss in Münchenbuchsee.

Eligibility criteria {10}

Patients are considered eligible for trial inclusion if all of the following criteria are met: (1) ≥ 18 years old, (2) diagnosis of unipolar moderate or severe depressive episode (ICD10: F32.1/32.2/33.1/33.2), and (3) Hamilton

Depression Rating Score, version 17 items (HAM-D17) ≥ 17.

If a patient meets any of the following criteria, he or she cannot be included in the trial: (1) acute suicide risk, (2) psychotic symptomatology, (3) other acute serious psychiatric disorder other than depression, (4) excessive consumption of alcohol and/or drugs, (5) severe acute or severe chronic somatic diseases, (6) pregnant or lactating women, and (7) under current treatment with fluoxetine.

Who will take informed consent? {26a}

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits, and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he or she may withdraw from the study at any time and that withdrawal of consent will not affect his or her subsequent medical assistance and treatment. All participants of the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participants to make an informed decision about their participation in the study. Participants will be granted enough time to decide whether to participate or not. The formal consent of a participant, using the approved consent form, will be obtained before the participant is submitted to any study procedure. The consent form will be signed and dated by the investigator or his designee at the same time as the participant[1].

Additional consent provisions for collection and use of participant data and biological specimens {26b}

With an additional consent form, the patient is asked for permission for further use of the collected biological samples and genetic data, in encrypted form, for not yet further defined future research projects. If the patient consents to the further use of the biological samples and genetic data, the remaining biological material is stored in the Biobank Biopharmazie at the University of Basel, Switzerland.

Interventions

Explanation for the choice of comparators {6b}

The intervention described in the following section {11a} is compared with the current standard of care, where the treating investigator alone selects and doses the antidepressant pharmacotherapy, considering clinical factors only, without taking genetics into account.

[1]Binding wording by the Swiss Association of Research Ethics Committees - Clinical Protocol Template for ClinO, Chapter 4 »Other Clinical Trials«, Version 1.0, 30.08.2018

Intervention description {11a}

The study intervention is the service of pharmacist-guided pre-emptive PGx testing to support clinical decision making for antidepressant selection and dosing.

This service involves genotyping and thereof evidence-based genotype interpretation commercially offered as Stratipharm® (humatrix AG, Pfungstadt Germany, https://www.stratipharm.de). Stratipharm® provides substance-specific recommendations based on current evidence of international guidelines (Clinical Pharmacogenetics Implementation Consortium, CPIC, and Dutch Pharmacogenetics Working Group, DPWG) as well as evidence from clinical studies annotated in the Pharmacogenomics Knowledge Base (PharmGKB, www.pharmgkb.org). Furthermore, clinical pharmacists will process and evaluate the results from PGx testing (Stratipharm®) in the context of the individual patient medication history, medical, and laboratory data (including drug serum levels if available) as well as current comedication (drug-drug interactions) and forward an individualized recommendation for antidepressant selection and dosing to the treating physician. This intervention is applied pre-emptively, meaning before initiation of a new antidepressant pharmacotherapy during the first week after inclusion into the study.

Criteria for discontinuing or modifying allocated interventions {11b}

The intervention under investigation is the service of pharmacist-guided pharmacogenetic testing for the selection and dosing of a new antidepressant pharmacotherapy. Discontinuation or modification of the investigator's chosen drug and dosage due to any reason is not impaired by the allocated intervention.

Strategies to improve adherence to interventions {11c}

The study is conducted on inpatient depression wards of the psychiatric clinics, where monitoring of medication intake and basic laboratory tests (hematology, clinical chemistry, and the like) are part of the routine clinical practice. Furthermore, a clinical study coordinator is supporting the investigators in the conduct of all study-specific assessments, which are internally monitored.

Relevant concomitant care permitted or prohibited during the trial {11d}

The introduction of a new antidepressant pharmacotherapy or augmentation strategy (e.g., lithium and the like) are not possible until after randomization when genotyping results are available (7±2 days after inclusion). However, the following measures can be taken in the interim: (1) if an antidepressant therapy has already been taken before entering the clinic, it can be continued during this period, and (2) additional

supportive measures are possible, including sleep-promoting pharmacotherapy (e.g., benzodiazepines or low-dose trazodone, mirtazapine, trimipramine).

Provisions for post-trial care {30}

After the intervention phase of the study, patients in the control and observation groups as well as their treating physicians will gain access to the PGx data collected.

Study participants do not receive any compensation. However, there are no additional costs for the study participants or the respective health insurance company due to study participation.

In the event of study-related damage or injuries, the liability of the respective institution, Psychiatric Services Solothurn or Private Clinic Wyss, provides compensation, except for claims that arise from misconduct or gross negligence.

Outcomes {12}

The primary endpoint is defined as the rate of response to the antidepressant therapy at the end of week 4 of the treatment phase. This time point was chosen based on the recommendations of the SGAD, which advise to assess clinical effectiveness after four weeks of antidepressant pharmacotherapy [3]. Moreover, the response is determined as a reduction in the Hamilton Depression (HAM-D) Scale score of at least 50% from the baseline score [18]. In this study, the 17-item HAM-D questionnaire (HAM-D17) is used.

Secondary endpoints will be assessed to further evaluate the clinical effectiveness of pharmacist-guided PGx testing as an intervention in antidepressant pharmacotherapy. Included are the following endpoints: (1) time to response—time span from the start of antidepressant pharmacotherapy until first assessed response (= HAM-D17 reduction of at least 50% compared to baseline), until end of week 4; (2) remission rate—HAM-D17 score ≤ 8, at week 4; (3) overall change in HAM-D17 score from baseline to end of week 4; (4) time till discharge—time span from admission to discharge from inpatient treatment, assessed up to 3 months; (5) patient depression self-rating—two weekly assessment with Beck-Depression-Inventory (BDI-II) questionnaire [19], until end of week 4; (6) side effect measure—weekly assessment of self-rated frequency, intensity, and burden of side effects (FIBSER score) [20], until end of week 4; and (7) Number of AEs related to antidepressant pharmacotherapy—severity grading ≥ 2 (using CTCAE version 5.0) [21] and causality to antidepressant pharmacotherapy assessed as possible, probable, or definite, until end of week 4.

Participant timeline {13}

Run-in phase (days -7–0) When patients have signed the informed consent and are included in the study, a smear of their oral mucosa is taken and sent to humatrix AG (Pfungstadt Germany, https://www.stratipharm.de) for pharmacogenotyping and phenotype prediction (day -7, see Fig. 1). During the run-in phase, liver and kidney functions will be assessed, taking the following laboratory values: creatinine, eGFR (calculated using CKD-EPI formula), ASAT, ALAT, gamma GT, and total bilirubin. Additional laboratory values will be determined: TSH, C-reactive protein, serum levels of vitamin B12 (total concentration), vitamin D (25-hydroxy-cholecalciferol), and basic hematology (hemoglobin, total erythrocytes, total thrombocytes, total leukocytes). These values will be reassessed at discharge. Moreover, the patient's antidepressant medication history including the reasons for discontinuation is documented (see Table 1).

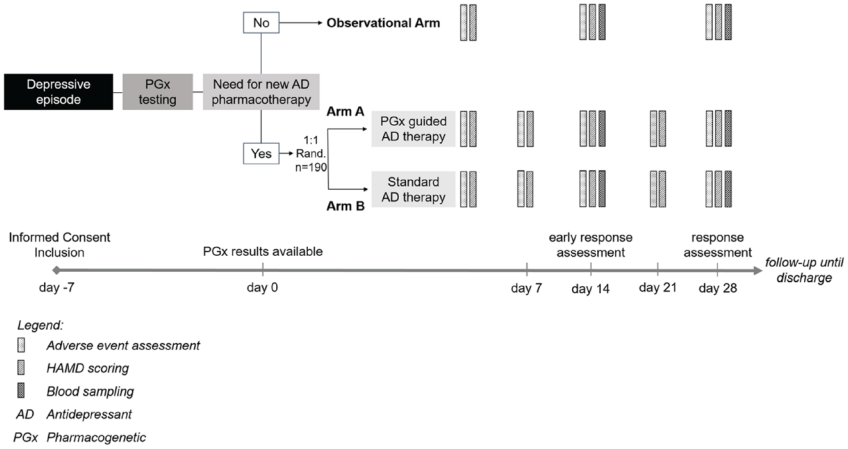

Fig. 1 Study procedures

Table 1 Schedule of assessments

Day	Pre-study	Run-in phase		Treatment phase					Follow-up	Clinic discharge
	−8	−7	−7–0	0	7	14	21	28	35, 42..	
Informed consent for trial participation	x									
Eligibility (pregnancy test, drug screening, inclusion, & exclusion criteria)	x									
Buccal swab (Stratipharm®)		x								
Medical history (previous antidepressant therapies)			x							
Lab values (basic hematology, creatinine (eGFR), ASAT, ALAT, total bilirubin, gamma-GT, CRP, TSH, vit. B12, and vit. D)			x							x
Concomitant medication documentation			x	x	x	x	x	x	x	x
RANDOMIZATION (only if new antidepressant indicated)				x						
PGx pharmaceutical recommendation (only arm A)				x						
Start NEW antidepressant				x						
HAM-D17	x			x[a]	x[c]	x	x[c]	x	x	x
AE assessment (antidepressant therapy only)					x[c]	x	x[c]	x	x	x
FIBSER patient self-assessment				x[a]	x[c]	x	x[c]	x	x	x
BDI-II patient self-assessment				x[a]		x		x	x[b]	x
Blood sample collection (EDTA and serum)						x		x		

[a]Baseline scoring before the first intake of new antidepressant
[b]BDI-II assessed in two weekly intervals
[c]Only for study arms A and B

Observational arm If after the run-in phase, at day 0 (±3 days), the adjustment to a new antidepressant pharmacotherapy is evaluated by the treating investigator as not necessary, the patient will be followed up in the observational arm, and the following scores will be assessed on days 0, 14, and 28 ±3 days: (1) HAM-D17, (2) FIBSER, and (3) BDI-II. Additionally, also blood samples will be collected on days 14 and 28 (±3 days) for further genotyping and retrospective assessment of serum drug concentrations (see Fig. 1). After day 28 of the study, the already collected genotyping data will be interpreted by a clinical pharmacist and made accessible to the treating physician.

Randomization and procedures for arms A and B If after the run-in phase, at day 0 (±3 days), the treating physician assesses the adjustment to a new antidepressant pharmacotherapy as necessary, and the patient is randomized to either arm A (intervention group) or arm B (control group) (see Fig. 1).

In arm B, the treating physician alone, according to the current standard of care considering clinical factors only, will determine the selection and dosing of the new antidepressant pharmacotherapy. The treating physician and patient will be blinded to the results of the previously conducted pharmacogenotyping for patients randomized to arm B until day 28. For patients in arm B, results from prior pharmacogenetic testing will be interpreted by a clinical pharmacist and made accessible to the treating physician after day 28.

In arm A, a clinical pharmacist will process and evaluate the results from PGx testing (Stratipharm®) in context of the individual patient history as well as current co-medication and forward an individualized recommendation for antidepressant selection and dosing to the treating physician at day 0.

In both arms, the newly prescribed antidepressant pharmacotherapy intake is continuously documented and therapy response observed over a period of 28 days with weekly assessments of adverse events related to the antidepressant medication (using CTCAE version 5.0) and scorings of HAM-D17 (days 0, 7, 14, 21, and 28; ±3 days) as well as patient self-assessments of FIBSER score (days 0, 7, 14, 21, and 28; ±3 days) and BDI-II score (days 0, 14, and 28; ±3 days) (see Table 1).

If patients in either study group remain in the clinic after day 28 (±3 days), a weekly follow-up of HAM-D17, FIBSER, and two-weekly BDI-II are continued until discharge (see Table 1).

Sample size {14}

The sample size was calculated to be 95 patients per study arms A and B. This was done taking into account the following criteria: power = 80%, α = 5%, response rate standard care = 0.5 [1, 2] and response rate PGx guided = 0.7 [16]. For the observational study arm, there

is no sample size calculation needed, since this arm does not contribute to the primary endpoint.

Recruitment {15}
Participants are recruited and screened for eligibility by the treating investigator in daily clinical practice, during the regular hospital admission interview, when entering the clinic for an inpatient stay.

Assignment of interventions: allocation
Sequence generation {16a}
Allocation of participants to study arms A or B is based on a computer-generated allocation sequence without any stratifying factors (static unstratified multi-block randomization).

Concealment mechanism {16b}
Randomization for participant allocation into study arms A and B is performed by the appointed clinical pharmacist without knowledge of the allocation sequence, within the web-based electronic data capture program secuTrial® (interactive Systems GmbH, Berlin, Germany). This is an open-label study; however, the service of individual processing and evaluation of the genotyping data is only conducted by the respective clinical pharmacist for participants allocated to arm A (intervention arm) at baseline (day 0). To further guarantee blinding to the genotyping results, assessed during the run-in phase, only the appointed clinical pharmacists do have password-protected access to the genetic data from Stratipharm®.

Implementation {16c}
Separated departments conduct each process of generating the allocation sequence, enrolling the participants, and assigning participants to interventions. The allocation sequence is implemented by an independent data management team of the clinical trials unit at the University of Basel in Basel, Switzerland. The investigators at the Psychiatric Clinic in Solothurn and Private Plinic Wyss in Münchenbuchsee, in Switzerland, conduct the enrolment of participants. Furthermore, the responsible clinical pharmacists of the Solothurner Spitäler AG association perform randomization electronically.

Assignment of interventions: blinding
Who will be blinded {17a}
This is an open-label study, hence no blinding of intervention allocation is possible and needed. However, the genetic data assessed during the run-in phase will only be shared with the treating physician and patients allocated to the control or observational group after day 28 of the treatment phase.

Procedure for unblinding if needed {17b}
Not applicable, as described in section {17a}, this is an open-label trial.

Data collection and management
Plans for assessment and collection of outcomes {18a}
Outcomes described in section {12} are assessed and collected using validated questionnaires and scoring tools (HAM-D17, FIBSER, BDI-II). FIBSER and BDI-II are patient-self-rated scores where the patients fill the according questionnaires independently [19, 20]. The HAM-D17 score is assessed by a trained rater (e.g., treating physician or specifically designated personnel) [18] who is however, not blinded to the patient allocation. The treating physician does the antidepressant adverse event assessment and grading according to the CTCAE version 5.0 [21]. The described data is collected on paper forms approved by the local ethics committee or directly entered into the patient electronic medical record when appropriate.

Plans to promote participant retention and complete follow-up {18b}
Assessments and follow-ups are only conducted during the participants' inpatient stay at the psychiatric clinic. Therefore, the risk of loss to follow-up and deviation to the protocol due to failure to comply with the study visits are considered negligible. However, patients who withdraw their consent (e.g., refuse further data collection) will be informed that all data collected until the time point of their withdrawal will be kept coded and used for analysis.

Data management {19}
The data collected on paper forms or in the patient electronic medical record are regularly transferred to an approved electronic database by a designated clinical research coordinator. The data will be coded with the according patient identifier, once transferred to the electronic database.

Study personnel will be trained on all important study-related aspects. After inclusion and trial participation of the first patient and regularly thereafter, the quality and accuracy of data collection will be checked internally.

All study data are archived for 10 years after study termination or premature termination of the study. The source data and all trial material will be stored in the archive of the study clinic.

Confidentiality {27}
Trial and participant data will be handled with uttermost discretion and is only accessible to authorized personnel who require the data to fulfill their duties

within the scope of the study. On the CRFs and other study-specific documents, participants are only identified by a unique participant number[2]. The participant identification list is kept in a locked place under the supervision of the principal investigator at the study site. Only encrypted data, which cannot be traced back to the individual study participant without knowledge of the identification list, will ever leave the study site. Furthermore, all collected data is stored on password and safety-back-up protected drives of the study clinic, which can only be accessed by authorized personnel. However, the data gathered is always traceable to the source data (e.g., patient medical records or questionnaires) at the study site, based on the accordingly documented patient identifier and the original collection date.

For quality assurance the sponsor, the Ethics Committee or an independent trial monitor may visit the research sites. Direct access to the source data and all study-related files is granted on such occasions. All involved parties keep the participant data strictly confidential[3].

Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in this trial/future use {33}

Collected oral mucosa on day -7, for pharmacogenotyping at humatrix AG in Germany (Stratipharm®), is destroyed 3 weeks after completion of the analysis and only the genetic data remain. These are encrypted with the sample number for evaluation and thus assigned to a database (server without internet access). Only designated employees have access to the data and must comply with strict data protection regulations. The genetic data will be kept until withdrawal. The laboratory in Germany has standards equivalent to those in Switzerland. Results of the pharmacogenetic testing by Stratipharm are made available for the responsible clinical pharmacist at the study site through a web portal, which is password protected and therefore only accessible by authorized personnel. The genetic data assessed by Stratipharm will be coded with the according patient identifier once transferred to the CRF and archived after study termination for at least 10 years.

Collected blood samples, 4 ml EDTA whole blood, and 6 ml serum on days 14 and 28 are not identified by participant name but by a unique participant identifier. For processing and further analysis, the biological material is transferred using dry ice shipment when necessary, to the biobank Biopharmazie at the University of Basel. There, the blood samples and thereof isolated DNA and processed blood serum are appropriately stored between −20 and −80°C in a restricted area only accessible to authorized personnel. Laboratory personnel handling the biological material outside of the study clinic do not have access to the patient identification list and therefore cannot trace the samples back to the individual participants. The planned analysis includes further pharmacogenetic targets and substance blood concentrations using standardized and established methods (e.g., TaqMan® genotyping assays, direct DNA Sanger sequencing, and LC-MS/MS or HPLC). The results of these examinations are not taken into account for the study treatment decisions and will only be assessed after day 28.

The collected blood samples and thereof processed samples (DNA and blood serum) will be stored in the biobank Biopharmazie at the University of Basel until study publication or early study termination. However, if participants consent to the further use of the assessed and encrypted genetic data and biological material, these will be stored over a yet undetermined time period and for a yet undetermined use in the study clinic or in the Biobank Biopharmazie respectively.

Statistical methods

Statistical methods for primary and secondary outcomes {20a}

The primary outcome of this study is the response rate at day 28 in study arms A and B. The response is defined as a reduction of the HAM-D17 score of at least 50% compared to baseline at day 0. Additionally, the following secondary outcomes are assessed: time to response, remission rate, overall change in HAM-D17 score, laytime in the clinic, change in BDI-II score, change in FIBSER score, and number of AEs related to antidepressant pharmacotherapy. For statistical analyses, the software packages of “IBM SPSS Statistics” and “GraphPad Software” are used. A descriptive statistics analysis for all variables is performed. Fisher’s exact test or *t* test is used to compare pairwise differences between groups and between baseline and follow-up visits as per data type. To measure the correlation between variables, the Spearman coefficient will be used. Significance level is two-sided, $\alpha = 0.05$. For further statistical analyses, adjustments for confounding factors will be taken into account. Any deviation from the original statistical plan will be described and justified in the final trial report.

Interim analyses {21b}

Interim analyses are possible, according to time points that are not previously defined. If an interim

[2]Binding wording by the Swiss Association of Research Ethics Committees - Clinical Protocol Template for ClinO, Chapter 4 »Other Clinical Trials«, Version 1.0, 30.08.2018

[3]Binding wording by the Swiss Association of Research Ethics Committees - Clinical Protocol Template for ClinO, Chapter 4 »Other Clinical Trials«, Version 1.0, 30.08.2018

analysis reveals an undue disadvantage of PGx intervention (e.g., increased number of adverse events) compared to the standard care group, the study will be stopped.

Methods for additional analyses (e.g., subgroup analyses) {20b}

Currently, there are no subgroups or adjusted analyses planned. However, the stored samples allow the assessment of additional genetic biomarkers, which may be used for further stratification of the patient cohort and therefore subgroup analyses.

Methods in analysis to handle protocol non-adherence and any statistical methods to handle missing data {20c}

Missing data will be retrospectively retrieved from medical records if possible. If the missing data cannot be retrieved, the last observed value will be used for analysis. Study drop-outs are replaced to achieve the final calculated study size of 95 patients per study arms A and B.

Plans to give access to the full protocol, participant level-data, and statistical code {31c}

To grant public access to the study procedure and status, it is registered and updated whenever necessary in the Swiss National Clinical trial Portal (SNCT P000004015) and the ClinicalTrials.gov register (NCT04507555), of which the latter is listed in the WHO International Clinical Trials Registry Platform (ICTRP; http://www.who.int/ictrp/en/).

Oversight and monitoring

Composition of the coordinating center and trial steering committee {5d}

This trial is an investigator-initiated multicenter clinical study. There is no external coordinating center or trial steering committee involved.

Composition of the data monitoring committee, its role, and reporting structure {21a}

The local ethics committee classified this trial as low risk. Therefore, internal monitoring by designated personnel is applicable. Internal monitoring is performed after the inclusion of the first participant and after study termination. In between internal monitoring will be applied as needed. The accuracy and completeness of the transfer of data from the original source to the CRF as well as completeness and storage of blood samples are checked. Study relevant source data and documents are accessible to internal monitors and in any case to external auditing. Questions are answered during monitoring and auditing. An internal data monitoring plan has been approved by the local ethics committee prior to study initiation.

Adverse event reporting and harms {22}

The intervention under investigation consists of a clinical pharmacist's service resulting in a recommendation of an antidepressant drug approved in Switzerland. Study procedures include taking a swab of the oral mucosa and drawing blood samples for further analysis. These procedures are considered low-risk and routine clinical practice. Only AEs related to the antidepressant pharmacotherapy are reported, since this is a secondary outcome of the study.

Frequency and plans for auditing trial conduct {23}

External auditing by the local ethics committee is possible at any time of the study conduct but not planned and communicated in advance.

Plans for communicating important protocol amendments to relevant parties (e.g., trial participants, ethical committees) {25}

Substantial changes to the study setup and study organization, the protocol, and relevant study documents are submitted to the local ethics committee for approval before implementation. Under emergency circumstances, deviations from the protocol to protect the rights, safety, and well-being of human subjects may proceed without prior approval of the EC. Such deviations shall be documented and reported to the Ethics Committee as soon as possible[4]. Patients still involved in the study conduct are asked to reconsent in case of a substantial amendment concerning the study procedures. Any non-substantial amendments are communicated to the EC in an annual report.

Dissemination plans {31a}

The investigators will publish and will make the study results available to the public in peer-reviewed journals. Besides, our findings will be communicated during national and international congresses relevant to clinicians and academics of associated fields.

Discussion

Despite the growing evidence already incorporated in international pharmacogenomics guidelines, PGx testing for antidepressant selection and dosing is not yet part of routine psychiatric practice. An important reason for this is the sparse number of prospective clinical trials, thereby limiting the evidence for the

[4]Binding wording by the Swiss Association of Research Ethics Committees - Clinical Protocol Template for ClinO, Chapter 4 »Other Clinical Trials«, Version 1.0, 30.08.2018

potential advantage over the current standard of care approach in antidepressant prescribing. Furthermore, in Switzerland, PGx testing requires prescribing by a pharmacologist, to ensure health insurance coverage. Pharmacologists however are not routinely involved in psychiatric clinics and are therefore hard to reach out to in daily practice. Another very likely reason that prevents psychiatrists from incorporating PGx information into their prescribing habits may include missing established procedures and in general a lack of resources in psychiatric clinics to substantially enable individualized PGx information processing to support drug selection and dosing. Therefore, an inter-professional collaboration between psychiatrists and clinical pharmacists may be of benefit for the treatment and provide a supportive framework for an individual interpretation and use of PGx data [22]. Independent of PGx, this interdisciplinary approach has been studied before and was found to have a positive impact on identifying drug-related problems [23]. Moreover, psychiatrists have been reported to mainly seek help from involved pharmacists in terms of drug selection [24]. As described beforehand, PGx information might add substantial value in answering this question. Therefore, the goal is to investigate the service of pharmacist-guided pre-emptive PGx testing in antidepressant therapy.

In the herein described clinical trial, the intervention in question (pharmacist-guided pre-emptive PGx testing) is compared to the current standard of care approach in antidepressant selection and dosing in an open-label, parallel-arm, randomized trial. This trial design allows direct comparison of the two approaches, minimizing selection bias by randomly assigning patients to either intervention or control group. Furthermore, the trial design was intended to fit as naturally as possible into the clinic's daily routine, enabling direct transfer of any study results into practice. However, the pragmatic setup of the trial does not allow blinding of the treating physician nor the patient on group allocation. Another limitation is that the field of pharmacogenetics is constantly evolving, with new findings resulting in further stratification of the recommendations published by CPIC, DPWG, and PharmGKB. Within the study, we will use the drug-genotype interpretation of Stratipharm®, which is based on the aforementioned sources. Therefore, a certain change in recommendations from beginning to end of the clinical trial cannot be excluded. Furthermore, we cannot rule out a potential training effect of the involved physicians, which may lead to favoring the prescription of antidepressants without PGx implications. It should also be emphasized that this trial investigates the effect of an integrated approach to clinical pharmaceutical consulting, of which pharmacogenetic data are a part and cannot be evaluated in isolation. Nevertheless, this trial entails only minimal risks comparable to routine clinical procedures. These relatively minimal risks face a high expected gain of knowledge and evaluation of potential benefits for future patients. In summary, we expect this trial to have a direct impact on routine psychiatry and pharmacy practice.

Trial status

Protocol version number and date: Version 3.1, September 14, 2021.

Date recruitment began: September 15, 2020.

Date recruitment approximately will be completed: September 14, 2023.

Abbreviations

AE: Adverse event; BDI-II: Beck depression-inventory II; CPIC: Clinical Pharmacogenetics Implementation Consortium; CRF: Case report form; CTCAE: Common Terminology Criteria for Adverse Events; CYP: Cytochrome P450; DPWG: Dutch Pharmacogenetics Working Group; eCRF: Electronic case report form; FIBSER: Frequency, Intensity, and Burden of Side Effects Rating; GCP: Good Clinical Practice; HAM-D: Hamilton rating scale for depression; MDD: Major depressive disorder; PGx: Pharmacogenetic; PharmGKB: Pharmacogenomics Knowledge Base; SGAD : Swiss Society for Anxiety and Depression

Acknowledgements

Not applicable

Authors' contributions {31b}

Stäuble CK and Lampert ML contributed equally in the writing of this manuscript. Mikoteit T is the sponsor-investigator (investigator-initiated trial) and led the protocol development. Hersberger KE, Meyer zu Schwabedissen HE, Hatzinger M, Allemann S, and Imboden C were involved in the study design, contributed to the additional content, and were responsible for the critical revision of the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials {29}

Investigators will have full access to the final trial dataset.

Declarations

Ethics approval and consent to participate {24}

The local ethics committees (Ethikkommission Nordwest- und Zentralschweiz, Basel, Switzerland, and Kantonale Ethikkommission Bern, Bern, Switzerland) approved the described study protocol version 3.1, dated on September 14, 2021, as of September 22, 2021 (reference number 2020-01535). We will obtain consent from all trial participants.

Consent for publication {32}

Not applicable, there is no patient data included in this study protocol.

Competing interests {28}

The authors declare that they have no competing interests.

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Publisher's Note

6 Discussion and Conclusion

Today, healthcare professionals in Switzerland do not consider PGx information as a standard for the pharmacotherapeutic management of MDD patients. The work and findings of this thesis presented here contribute to an understanding of the opportunities and challenges of PGx in the clinical practice of antidepressant therapy, and to the recognition of the role of pharmacists in this context. These findings are based on four overarching projects (A-D). In summary, **Project A** provides an insight into five exemplary depressive disorder patient cases from a prospective observational case study. There, PGx information was used by pharmacists to elucidate histories of TF and ADR, as well as to elaborate recommendations for further therapy optimization. It became apparent that applying PGx information to real patient cases requires individual patient consideration and evaluation, which in turn require advanced knowledge that has not yet been adequately covered in either Swiss undergraduate or in continuing pharmacy education. Therefore, **Project B** was concerned with overcoming this educational gap by developing and conducting a continuing education program to prepare pharmacists for the application of PGx information in clinical practice. In order for the PGx information processed by pharmacists to be taken into account comprehensively in the treatment of patients, an equal and close interprofessional collaboration with other healthcare professionals, especially the treating physician, appeared to be of importance. In this context, **Project C** defined a six-step-approach for the implementation of a pharmacist-led PGx testing and counseling service (PGx service) in an interprofessional healthcare setting. To evaluate the impact of the proposed pharmacist-led PGx service on patient outcomes, an open-label randomized controlled clinical trial in MDD patients was developed in **Project D**.

Based on the findings of **Project A**, I conclude that applying individual PGx information to real-world, depressive-disorder patient cases is not always straightforward and requires individual patient consideration and evaluation. In our studies we came across two particular challenges highlighted by the exemplary case reports of **Project A-1–3**.

First, although there are international initiatives such as the PharmGKB and the CPIC to facilitate the use of PGx information for patient care, evidence for precise PGx-based drug selection and dosing is still fragmentary. Detailed PGx-based dosing guidelines are currently only available for a limited number of antidepressant–gene pairs, including SSRIs, tricyclic antidepressants, *CYP2C19*, *CYP2D6* and *ABCB1* [13,14,27,61]. **Project A-1 and A-2** highlight that, despite the well-known involvement of polymorphic CYPs in the metabolism of bupropion and quetiapine, there is a lack of studies investigating the role of genetics in the interindividual differences in their pharmacokinetic behavior. Moreover, too few clinical studies investigating the relationship between genetics and therapy outcome and tolerability of bupropion and quetiapine have been conducted to form a basis for PGx guidelines. However, genetically predicted CYP metabolizer phenotypes could provide insights into the pharmacokinetic behavior of substrate drugs that are not yet covered by therapeutic recommendations analogous to drug–drug-interaction predictions. Moreover, in addition to pharmacokinetics, pharmacodynamics-related gene variants may also have an impact on antidepressant response. In this context we highlighted a case in **Project A-3** where the patient's *SLC6A4* genotype likely had an effect on the antidepressant treatment course. Genetic variation in antidepressant targets such as the serotonin transporter (SERT1 encoded by *SLC6A4*) have been shown to affect therapeutic response [28,32].

However, current guidelines do not recommend that polymorphisms in *SLC6A4* or in other pharmacodynamics-related genes be considered in antidepressant selection and dosing. In addition to the lack of PGx recommendations, complex genetic variants such as the serotonin transporter linked promotor region polymorphism (5-HTTLPR) may hamper the adoption of *SLC6A4* genotyping in clinical practice. 5-HTTLPR is a variable number tandem repeat polymorphism (VNTR), which cannot be genotyped with real-time polymerase chain reaction (PCR) TaqMan® assays, as used in the commercial PGx panel test applied here (Stratipharm®, Appendix B), that primarily targets single nucleotide polymorphisms (SNP) [e.g. 62].

For the genotyping of complex genetic variants such as VNTR, other more elaborate methods have to be applied. In Project A-3 we used a PCR assay with subsequent gel electrophoresis for visual size analysis of the PCR product to determine 5-HTTLPR variants [63]. A more recent alternative for the genotyping of VNTR offers next-generation sequencing (NGS). Although NGS methods allow for timely whole genome sequencing (WGS), this approach also poses major challenges for the application of genetic testing including PGx in clinical practice [64]. WGS generates a large amount of data that requires analysis by experts using sophisticated bioinformatics tools. Further, the large amount of data increases the likelihood of detecting genetic variants with unknown clinical significance, as well as incidental findings of genetic variants with disease risks or disease modifying risks [64]. In the context of *SLC6A4* it is important to mention that there are genetic variants that not only influence drug response, but also have disease modifying properties. The 5-HTTLPR, which we investigated in Project A-3, has been linked to depression susceptibility [65,66]. A meta-analysis that pooled the results of 54 studies showed an increased risk for carriers of the

variant 5-HTTLPR short allele (S-allele) to develop depression when exposed to stress [67]. In clinical practice, it must therefore be taken into account that genetic information collected to predict drug response may also be associated with disease risks or disease-modifying risks in certain cases. Under these conditions, the evaluation and communication of PGx test results would have to involve more sophisticated approaches. In Switzerland, PGx tests that can also detect disease risks could jeopardize the involvement of pharmacists in PGx, as the assessment of genetic diseases and genetic disease risks is legally reserved for experts such as human geneticists.

Second, PGx-based dosing guidelines are often derived from considerations of single drug–gene interactions (DGI) [7,10]. However, DGI may be affected by additional factors, including physiological, environmental, and behavioral factors [2,3]. In addition, different genetic predispositions may also influence each other.

In **Project A-1** we describe a potential combined effect of *CYP2B6* and *CYP2C19* pharmacogenotypes, both known to be involved in the metabolism of bupropion. It is conceivable that the pharmacokinetic behavior of a drug metabolized via multiple polymorphic enzymes may be affected by the combined effect of the genetic predisposition of these enzymes. Indeed, this effect has been described as drug–gene–gene interaction (DGGI) before [68], and may also need to be taken into account for certain antidepressants which are metabolized by multiple polymorphic CYPs (Table 1). Notably, the CPIC already considers the combined effect of *CYP2D6* and *CYP2C19* genotypes in their dosing recommendations for tricyclic antidepressants. As mentioned before, such effects could also affect other antidepressants, including bupropion, not yet covered by therapeutic PGx recommendations.

Moreover, non-genetic factors such as a patient's comedication can also affect DGI. In **Project A-2** we identified a potential combined effect of the known CYP2D6 inhibitor escitalopram and the patient's genetic predisposition for CYP2D6 intermediate metabolizer on the tolerability of quetiapine. Such effects have been described before as drug–drug–gene interaction (DDGI) [69], which are a possible form of phenoconversion [56]. As previously mentioned, additional, non-genetic predispositions may influence DGI. In Project-A2, the patient's impaired renal function might have influenced quetiapine excretion and therefore tolerability in addition to the CYP2D6 genetic predisposition. In summary, additional factors such as other genetic predispositions, renal function and polypharmacy (DDI) may counterbalance or enhance the expected clinical effects of DGI. To accurately assess the impact of these additional factors on DGI and drug response, stratified outcome studies would need to be conducted, taking into account all types of additional factors and their combinations as previously described.

Still, PGx may provide an opportunity to optimize pharmacotherapy. Clinical pharmacists already consider several interindividual factors when analyzing a patient's medicines in medication reviews to propose interventions for therapy optimization, including clinical data (e.g., age, weight, lab values and comorbidities) [58]. Moreover, pharmacists are an important point of contact for patients and healthcare professionals in the event of drug-related problems [70]. The patient case in **Project A-4** demonstrates, based on the example of psychiatric practice, that pharmacists are well positioned and equipped to include PGx information in medication reviews and give individualized recommendations for therapy optimization in an interprofessional healthcare setting.

Notably, in Project A-1–4 we are reporting the cases of single patients in clinical routine care. Accordingly, this certainly limits the conclusions that can be or should be drawn from the observations. Nevertheless, these exemplary cases provide important insights into the challenges and opportunities of PGx in the clinical practice of depression therapy.

Applying PGx to individual patient cases requires patient-specific consideration and evaluation, which in turn requires advanced knowledge of PGx that is to our understanding so far not sufficiently covered in either Swiss undergraduate or continuing pharmacy education. A recent survey of Swiss pharmacists (n = 372) found that nearly 75% of them rated their knowledge of PGx as inadequate to counsel their patients. Nevertheless, just as many pharmacists felt a responsibility to advise their patients about PGx and therefore showed interest to participate in a continuing education program [71]. Similar findings have been made in surveys conducted in other countries. Pharmacists and healthcare professionals practicing in Singapore, Canada, and the Netherlands, among other countries, considered PGx testing useful but judged their knowledge as insufficient to apply it in practice [72-74]. Indeed, lack of education has been described as a major barrier to the adoption of PGx in clinical practice [52]. In **Project B** we therefore, developed and piloted a continuing education program to prepare Swiss pharmacists for the application of PGx information in clinical practice, which to our knowledge is the first of its kind in Switzerland. Prior to the program, participants already expressed a favorable attitude towards PGx and were convinced about its importance in pharmacy practice. After attending the program, participants showed measurable improvement in their knowledge of PGx and their competence in integrating PGx information into the pharmaceutical care of patients. I conclude that

pharmacists can be enabled to integrate PGx information in clinical practice through a continuing education program. At this point, it should be mentioned that the participants of the education program are a selected cohort subject to selection bias and therefore do not necessarily reflect all Swiss pharmacists. Registration on the course was voluntary and open to all pharmacists for a fee. We, therefore, expected pharmacists with a positive attitude and general interest in PGx to participate. This may have beneficially influenced the participants' learning outcomes. However, at the end of the training the participants expressed mixed intentions to integrated a PGx service into their pharmacy practice. Several barriers to the adoption of a PGx service were indicated, including lack of resources and lack of coverage by health insurers. To further assist participants with implementation, we initiated a peer group for pharmacists who were generally interested in offering PGx services. In our experience with the peer group, even six months after completing the continuing training program, none of the participants had implemented a PGx service in their practice. At the time, this may have been influenced by the situation surrounding the COVID-19 pandemic, including staff shortages and the implementation of other new services such as COVID testing and vaccination. In addition, a major challenge appeared to be the lack of interprofessional networks and physician support for such a PGx service. Broader coverage of PGx during the undergraduate education of healthcare professionals, using interprofessional education concepts, could perhaps promote collaboration between pharmacists and physicians at an early stage, and increase the uptake of PGx in clinical practice. Such programs do already exist, for instance in the United States [75,76]. Still, for PGx to actually be implemented in clinical practice, appropriate interprofessional procedures need to be defined. Indeed, a recent study piloting an outpatient PGx service in the

Netherlands found that unclear allocation of responsibilities between the involved pharmacists and physicians was a major barrier to the adoption of the service [77].

In **Project C** we described and proposed a structured PGx service considering the multi-professional setting in both primary and secondary care. This structured procedure was designed and refined based on our working experience in the observational case series study, where we applied PGx testing and counseling in both settings. Exemplary patient cases recruited within the secondary care setting are described in Project A. As mentioned above, we also recruited cases in the primary care setting, of which two exemplary cases were published by Jeiziner *et al.* [78,79]. In Project C we defined a six-step-approach for PGx testing and counseling, supporting the importance of interprofessional collaboration for the adoption of PGx in clinical practice. In this approach, pharmacists play a key role in enabling an individual, comprehensive evaluation of the patient's PGx profile by integrating this information into a medication review. Thereby, non-genetic factors (e.g. co-medication, renal function), that may enhance or compensate the genetic predisposition (e.g. phenoconversion), are also taken into account. With this structured medication review, pharmacists aim to identify drug-related problems and make individualized recommendations to optimize the patient's medication to improve health outcomes. Finally, pharmacists are responsible for the counseling of patients, physicians and other care givers prior to and after PGx testing. This service was designed and refined for the Swiss healthcare system and may therefore not be directly transferable to other countries. However, since the Swiss healthcare system is heterogeneous, with 26 different cantonal systems, the service could still be adapted to a different healthcare system.

The role of pharmacists in PGx considerations in clinical practice has been previously described as essential by several parties. For instance, a working group of the National Health Service England (NHS) recently concluded in its report on personalized prescribing that PGx services should be multidisciplinary, including pharmacists to guide therapeutic decisions across medical disciplines. They further highlighted that the role of pharmacists in the implementation of PGx in clinical practice is an important area of research [80]. Notably, the Mayo Clinic in the United States has already established a PGx service ("nine-gene pharmacogenomics profile service") in which pharmacists are responsible for interpreting PGx test results to enable their consideration in clinical practice [81].

Until now, specific outcome analyses of such structured PGx services have been limited, inter alia concerning the treatment of depression. In our observational case series study, over 60% of the enrolled patients were diagnosed with a psychiatric disorder, predominantly a depressive disorder. A limited number of industry-sponsored studies has shown that response rates to antidepressants are higher when reports from commercial combinatorial PGx test panels are available [50,51]. In these studies, physicians generally adopted their patients' PGx information from the commercial test reports without consulting other healthcare professionals such as pharmacists [50,51]. However, as already described, the involvement of pharmacists in the individual analysis and transfer of PGx information into recommendations for clinical practice may have an additional benefit. Pharmacists are specialists in pharmacotherapy and key contacts for drug-related problems. They are therefore in an ideal position to assist other healthcare professionals with patient-specific medication reviews that take PGx information into account.

Ideally, such a pharmacist-led PGx service is applied to prevent ADR and TF. So-called pre-emptive PGx testing is performed prior to drug prescription. With this strategy, PGx information that is already available can be considered to guide drug selection and dosing in order to enhance medication safety and efficacy [82]. Pre-emptive PGx testing often entails a panel-testing approach, where multiple genes, relevant for the response to several drugs, are genotyped simultaneously. In the projects described here we applied a commercial PGx panel test (Appendix B). This approach may be particularly useful for MDD patients, as multiple antidepressants can be impacted by the patients' genetic predisposition in several genes (e.g., *CYP2D6*, *CYP2C19*, *ABCB1*) [7,13,14,61].

Furthermore, a recent analysis of Swiss drug claims showed that antidepressants with PGx recommendations, namely escitalopram and trimipramine, are readily used. In a population of almost 890'000 people registered with the Swiss health insurer Helsana, 5.3% were treated with escitalopram and 1.9% with trimipramine between 2016 and 2020 [83]. Moreover, combinatorial PGx panel testing of MDD patients (n = 1149) revealed that current or planned antidepressant medications were prevalently associated with gene–drug interactions. In detail, over 40% of the patients showed moderate gene–drug interactions and around 20% even clinically significant gene–drug interactions [84]. Therefore, a majority of patients under treatment or with planned treatment for MDD could benefit from PGx.

In Project D, we followed-up on our proposed pharmacist-led PGx service from Project C with an outcome analysis of adult MDD inpatients who required a change or an initial prescription of their antidepressant therapy. The PrePGx study (pharmacist-guided pre-emptive pharmacogenetic testing in antidepressant therapy,

ClinicalTrials.gov ID: NCT04507555, Swiss National Clinical Trials Portal ID: SNCTP000004015) is a multi-center, open-label, randomized controlled, parallel three-arm trial. The focus of this study is on interprofessional collaboration for the handling and use of PGx information in the psychiatric practice of depression therapy. We are comparing a PGx intervention (pharmacist-guided pre-emptive PGx testing integrated in a medication review) to the current standard of care for the selection and dosing of antidepressants. In the standard of care (control group), the psychiatrist selects and doses the antidepressant without information on the patient's PGx profile and without a pharmacist consultation and medication review. The primary endpoint is therapy response after four weeks of treatment with the newly introduced antidepressant. This study design facilitates the direct comparison of the two approaches and minimizes selection bias by randomly assigning patients to the intervention or control group. The third study arm is observational and follows up on patients for whom the introduction of a new antidepressant was evaluated as not necessary or possible. Because PGx information is collected for all patients, the observational arm allows for further explorative analyses beyond the primary endpoint. Notably, the trial design was developed in close collaboration with psychiatrists and clinical pharmacists. The pragmatic setup was chosen to allow direct transfer of the findings into clinical practice. However, such a pragmatic approach is also associated with certain limitations, such as the fact that patients and investigators are not blinded to group allocation, which may lead to expectation bias. Furthermore, because the study arms are conducted in parallel, a training effect among the physicians cannot be ruled out, which may lead to a preference for prescribing antidepressants without PGx implications. Regardless of the trial design, PGx is a constantly evolving field in which new evidence may further stratify

the currently available guidelines for antidepressant dosing and selection published by, for example, the CPIC and the DPWG. Therefore, the pharmaceutical recommendations based on those guidelines may be subject to certain changes from the beginning to the end of the study. It should also be emphasized here that this study does not allow for an isolated assessment of the impact of PGx data on treatment outcomes, as we are examining an integrated approach to PGx in the pharmaceutical care of patients with MDD. This sets our work apart from previous and ongoing studies, where the impact of commercial combinatorial PGx test panels is evaluated in randomized clinical trials without an interprofessional approach or pharmacist consultation in antidepressant therapy for MDD patients [50,85]. So far, we have been unable to perform an outcome analysis of the PrePGx study, as patient recruitment is still ongoing in two psychiatric clinics in Switzerland. Based on a sample-size calculation (power = 80%, α = 5%), we intend to enroll 85 patients each in the intervention and control arm (n = 190). As of September 2022 we have enrolled a total of 56 patients. Completion of the study is scheduled for the end of 2024. In conclusion we expect this trial to have a direct impact on the use and handling of PGx information in routine psychiatric and pharmacy practice.

Based on the underlying work from **Project A–D**, I conclude that:

- First, PGx information should not be analyzed in isolation but within the context of other individual patient factors such as physiological factors (e.g., organ function), environmental factors (e.g., drug–drug interactions (DDI), smoking) and behavioral factors (e.g., medication adherence). Therefore, PGx information should be integrated into medication reviews in order to analyze and optimize a patient's medicines.

- Second, pharmacists are well positioned to initiate PGx testing and can be further trained to support other healthcare professionals with medication reviews that include PGx considerations. For this purpose, a pharmacist-led PGx testing and counseling service is feasible in an interprofessional setting of primary and secondary healthcare.

- Third, the impact of a pharmacist-led PGx testing and counseling intervention needs to be evaluated in selective patient cohorts. MDD patients who require a change or an initial prescription of their antidepressant therapy are an appropriate cohort for such an outcome analysis.

7 Outlook

PGx is an active field of research. Further insights will increase our knowledge on the impact of interindividual genetic predisposition on the pharmacokinetic and pharmacodynamic behavior of various drugs. However, there is already compelling evidence for certain drug–gene interactions, which has been processed by international consortia (e.g. CPIC, DPWG) into recommendations and guidelines for application in clinical practice. Still, today the utility of applying PGx in clinical practice is questioned and controversially discussed. On the one hand, this thesis points out the importance of practice-oriented research in PGx. In addition to MDD patients, other patient cohorts should also be studied to gain a broader understanding of the utility of a pharmacist-led PGx service. Patient cohorts that are particularly dependent on adequate medication management could be suitable for this purpose, for example elderly and chronically ill patients with polypharmacy. On the other hand, this thesis emphasizes interprofessional collaboration in PGx. Herein, interprofessional PGx education could further encourage the understanding of the role and competences of other healthcare professionals in PGx, as well as promote the formation of interprofessional networks for the implementation of PGx. Ideally, this form of interprofessional PGx education would already be introduced at an undergraduate level.

Despite these notions, two major challenges to the implementation of PGx in Swiss clinical practice remain.

First, currently PGx testing generally requires a prescription by a specialized pharmacologist to ensure basic healthcare coverage. However, in large parts of clinical practice pharmacologists are usually not readily available for consultation. Expanding reimbursement for PGx testing and related cognitive services to other healthcare

professionals could greatly improve the accessibility and uptake of PGx in clinical practice.

Second, communication and exchange of PGx- and health data between healthcare providers is hampered by the lack of a coherent and interoperable e-health system. Improved digital networks, that consider data security, could enhance the continued use of PGx information across multiprofessional healthcare settings. This is particularly important, as PGx information derived from germline genes has lifelong validity and such data could be used pre-emptively for PGx guidance of patients' future pharmacotherapies. Therefore, the implementation of a national e-health system could promote sustainability and cost-effectiveness through the clear documentation and accessibility of PGx data.

Overall, this thesis contributes to the implementation of PGx in clinical practice by emphasizing the role of pharmacists as experts in pharmacotherapy and PGx in an interprofessional and collaborative healthcare setting. As of December 1, 2022, the revised Swiss law on genetic investigations in humans and associated ordinances came into force, officially allowing pharmacists to initiate PGx testing [53]. Nonetheless, further practice- and implementation-oriented research and notions are warranted to assess the utility of PGx in clinical practice and to improve societal recognition of pharmacists' competencies in this context.

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Appendix

A. Supplementary information Project A-3

CARE case report guidelines

Topic	Item	Checklist item description	Reported on Line
Title	1	The diagnosis or intervention of primary focus followed by the words "case report"	Title
Key Words	2	2 to 5 key words that identify diagnoses or interventions in this case report, including "case report"	13-14 and Title
Abstract (no references)	3a	Introduction: What is unique about this case and what does it add to the scientific literature?	16-18
	3b	Main symptoms and/or important clinical findings	28
	3c	The main diagnoses, therapeutic interventions, and outcomes	16-29
	3d	Conclusion—What is the main "take-away" lesson(s) from this case?	30-31
Introduction	4	One or two paragraphs summarizing why this case is unique (**may include references**)	78-81
Patient Information	5a	De-identified patient specific information.	85
	5b	Primary concerns and symptoms of the patient.	86-90
	5c	Medical, family, and psycho-social history including relevant genetic information	93-117
	5d	Relevant past interventions with outcomes	93-117
Clinical Findings	6	Describe significant physical examination (PE) and important clinical findings.	90-92
Timeline	7	Historical and current information from this episode of care organized as a timeline	Figure 1
Diagnostic Assessment	8a	Diagnostic testing (such as PE, laboratory testing, imaging, surveys).	97-100, 106-110, 152-154
	8b	Diagnostic challenges (such as access to testing, financial, or cultural)	137-150
	8c	Diagnosis (including other diagnoses considered)	85, 90-92
	8d	Prognosis (such as staging in oncology) where applicable	NA
Therapeutic Intervention	9a	Types of therapeutic intervention (such as pharmacologic, surgical, preventive, self-care)	93-95, 101-102, 115, 120
	9b	Administration of therapeutic intervention (such as dosage, strength, duration)	Table 2
	9c	Changes in therapeutic intervention (with rationale)	93-98, 101-104, 113-120
Follow-up and Outcomes	10a	Clinician and patient-assessed outcomes (if available)	122-124
	10b	Important follow-up diagnostic and other test results	123-124
	10c	Intervention adherence and tolerability (How was this assessed?)	101, 122-124
	10d	Adverse and unanticipated events	96, 100-101
Discussion	11a	A scientific discussion of the strengths AND limitations associated with this case report	170-185
	11b	Discussion of the relevant medical literature **with references**.	156-185
	11c	The scientific rationale for any conclusions (including assessment of possible causes)	166-167, 185-186, 148-152
	11d	The primary "take-away" lessons of this case report (without references) in a one paragraph conclusion	166-188
Patient Perspective	12	The patient should share their perspective in one to two paragraphs on the treatment(s) they received	85-86, 105-106, 123-124
Informed Consent	13	Did the patient give informed consent? Please provide if requested	Yes ☑ No ☐

B. Supplementary information Project C

Supplementary Material

Table S1. Stratipharm® (humatrix AG, Pfungstadt, Germany) SNPs and annotations

Gene	Chromosome	Annotation	Position	Amino acid replacement	Base
ABCB1	Chromosom 7q21.12	**rs1045642**	NM_000927.4:c.3435T>C	I1145I	T>C
ABCB1	Chromosom 7q21.12	**rs1128503**	NM_000927.4:c.1236T>C	G412G	T>C
ABCB1	Chromosom 7q21.12	**rs2032582**	NM_000927.4:c.2677G>A	A893T	G>A
ABCB1	Chromosom 7q21.12	**rs2032582**	NM_000927.4:c.2677G>T	A893S	G>T
ABCB1	Chromosom 7q21.12	**rs2032583**	NM_000927.4:c.2685+49T>C	-	T>C
ABCG2	Chromosom 4q22-q23	**rs2231142**	NM_004827.2:c.421C>A	Q141K	C>A
ABCG2	Chromosom 4q22-q23	**rs13120400**	NM_004827.2:c.1194+928A>G	-	A>G
ABCG2	Chromosom 4q22-q23	**rs17731538**	NC_000004.11:g.89055379G>A	-	G>A
ADRB1	Chromosom 10q24-q26	**rs1801252**	NM_000684.2:c.145A>G	S49G	A>G
ADRB1	Chromosom 10q24-q26	**rs1801253**	NM_000684.2:c.1165G>C	G389R	G>C
ADRB2	Chromosom 5q31-q32	**rs1042713**	NT_029289.11:g.9369367G>A	G16R	G>A
ADRB2	Chromosom 5q31-q32	**rs1042714**	NC_000005.9:g.148206473G>C	E27Q	G>C
COMT	Chromosom 22q11.21	**rs4680**	NM_000754.3:c.472G>A	V158M	G>A
COMT	Chromosom 22q11.21	**rs165599**	NM_000754.3:c.*522G>A	-	G>A
COMT	Chromosom 22q11.21	**rs4646316**	NM_000754.3:c.615+310C>T	-	C>T
COMT	Chromosom 22q11.21	**rs9332377**	NM_000754.3:c.616-367C>T	-	C>T
COQ2	Chromosom 4q21.23	**rs4693075**	NC_000004.11:g.84192168G>C	-	G>C
COQ2	Chromosom 4q21.23	**rs6535454**	NM_015697.7:c.894T>C	D298D	T>C
CYP1A2	Chromosom 15q24.1	**rs2069514**	NC_000015.9:g.75038220G>A	-	G>A
CYP1A2	Chromosom 15q24.1	**rs762551**	NC_000015.9:g.75041917C>A	-	C>A
CYP2B6	Chromosom 19q13.2	**rs8192709**	NM_000767.4:c.64C>T	R22C	C>T
CYP2B6	Chromosom 19q13.2	**rs28399499**	NM_000767.4:c.983T>C	I328T	T>C
CYP2B6	Chromosom 19q13.2	**rs3745274**	NM_000767.4:c.516G>T	Q172H	G>T
CYP2C8	Chromosom 10q24.1	**rs10509681**	NM_000770.3:c.1196A>G	K399R	A>G
CYP2C8	Chromosom 10q24.1	**rs11572080**	NM_000770.3:c.416G>A	R139K	G>A
CYP2C8	Chromosom 10q24.1	**rs1934951**	NG_007972.1:g.35707G>A	-	G>A
CYP2C9	Chromosom 10q24.1	**rs1799853**	NM_000771.3:c.430C>T	R144C	C>T
CYP2C9	Chromosom 10q24.1	**rs1057910**	NM_000771.3:c.1075A>C	I359L	A>C
CYP2C9	Chromosom 10q24.1	**rs9332131**	NM_000771.3:c.817delA	K273X	delA
CYP2C9	Chromosom 10q24.1	**rs7900194**	NM_000771.3:c.449G>A	R150H	G>A
CYP2C9	Chromosom 10q24.1	**rs28371685**	NM_000771.3:c.1003C>T	R335W	C>T
CYP2C19	Chromosom 10q24	**rs4244285**	NM_000769.1:c.681G>A	-	G>A
CYP2C19	Chromosom 10q24	**rs4986893**	NM_000769.1:c.636G>A	W212X	G>A
CYP2C19	Chromosom 10q24	**rs12248560**	NG_008384.1:g.4195C>T	-	C>T
CYP2C19	Chromosom 10q24	**rs28399504**	NM_000769.1:c.1A>G	M1V	A>G

CYP2D6	Chromosom 22q13.1	**-**	copy number variation	-	CNV
CYP2D6	Chromosom 22q13.1	**rs35742686**	NM_000106.4:c.775delA	-	delA
CYP2D6	Chromosom 22q13.1	**rs3892097**	NM_000106.4:c.506-1G>A	-	G>A
CYP2D6	Chromosom 22q13.1	**rs5030655**	NM_000106.4:c.454delT	-	delT
CYP2D6	Chromosom 22q13.1	**rs5030867**	NM_000106.4:c.971A>C	H324P	A>C
CYP2D6	Chromosom 22q13.1	**rs5030865**	NM_000106.4:c.505G>T	G169X	G>T
CYP2D6	Chromosom 22q13.1	**rs5030865**	NM_000106.4:c.505G>A	G169R	G>A
CYP2D6	Chromosom 22q13.1	**rs5030656**	NM_000106.5:c.841_843delAAG	K281del	delAAG
CYP2D6	Chromosom 22q13.1	**rs1065852**	NM_000106.4:c.100C>T	P34S	C>T
CYP2D6	Chromosom 22q13.1	**rs201377835**	NM_000106.5:c.181-1G>C	-	G>C
CYP2D6	Chromosom 22q13.1	**rs28371706**	NM_000106.4:c.320C>T	T107I	C>T
CYP2D6	Chromosom 22q13.1	**rs59421388**	NM_000106.4:c.1012G>A	V338M	G>A
CYP2D6	Chromosom 22q13.1	**rs28371725**	NM_000106.4:c.985+39G>A	-	G>A
CYP3A4	Chromosom 7q21.1	**rs2740574**	NG_000004.3:g.135607G>A	-	G>A
CYP3A4	Chromosom 7q21.1	**rs2242480**	NM_017460.5:c.1026+12G>A	-	G>A
CYP3A5	Chromosom 7q21.1	**rs776746**	NM_000777.3:c.219-237G>A	-	G>A
DPYD	Chromosom 1p22	**rs3918290**	NM_000110.3:c.1905+1G>A	-	G>A
DPYD	Chromosom 1p22	**rs72549303**	NM_000110.3:c.1898delC	-	delC
DPYD	Chromosom 1p22	**rs72549309**	NM_000110.3:c.298delTinsTCAT	-	delTinsTCAT
DPYD	Chromosom 1p22	**rs55886062**	NM_000110.3:c.1679T>G	I560S	T>G
DPYD	Chromosom 1p22	**rs67376798**	NM_000110.3:c.2846A>T	D949V	A>T
DPYD	Chromosom 1p22	**rs2297595**	NM_000110.3:c.496A>G	M166V	A>G
GNB3	Chromosom 12p13	**rs5443**	NM_002075.2:c.825C>T	S275S	C>T
GSTP1	Chromosom 11q13.2	**rs1695**	NM_000852.3:c.313A>G	I105V	A>G
HLA-A	Chromosom 6p21.3	**rs1061235**	NM_002116.7:c.*66A>T	-	A>T
HLA-A	Chromosom 6p21.3	**rs1633021**	NC_000006.12:g.29779092T>C	-	T>C
HLA-B	Chromosom 6p21.3	**rs3909184**	NM_005803.2:c.724-507C>G	-	C>G
HLA-B	Chromosom 6p21.3	**rs2395029**	NM_006674.3:c.*568T>G	-	T>G
HLA-B	Chromosom 6p21.3	**rs2844682**	NC_000006.11:g.30946148G>A	-	G>A
HMGCR	Chromosom 5q13.3-q14	**rs17238540**	NM_000859.2:c.2457+117T>G	-	T>G
HMGCR	Chromosom 5q13.3-q14	**rs17244841**	NM_000859.2:c.451-174A>T	-	A>T
HTR2A	Chromosom 13q14-q21	**rs6311**	NC_000013.10:g.47471478C>T	-	C>T
HTR2A	Chromosom 13q14-q21	**rs6313**	NM_000621.3:c.102C>T	S34S	C>T
HTR2A	Chromosom 13q14-q21	**rs7997012**	NM_000621.3:c.614-2211T>C	-	T>C
HTR2A	Chromosom 13q14-q21	**rs9316233**	NC_000013.10:g.47433355C>G	-	C>G
HTR2A	Chromosom 13q14-q21	**rs6314**	NC_000013.10:g.47409034G>A	H368Y	G>A
IFNL3	Chromosom 19q13.13	**rs8099917**	NC_000019.9:g.39743165T>G	-	T>G
IFNL3	Chromosom 19q13.13	**rs12979860**	NC_000019.9:g.39738787C>T	-	C>T
ITPA	Chromosom 20p	**rs1127354**	NM_181493.1:c.43C>A	P32T	C>A

NAT2	Chromosom 8p22	**rs1801280**	NM_000015.2:c.341T>C	I114T	T>C
NAT2	Chromosom 8p22	**rs1799930**	NM_000015.2:c.590G>A	R197Q	G>A
NAT2	Chromosom 8p22	**rs1799931**	NM_000015.2:c.857G>A	G286E	G>A
OPRM1	Chromosom 6q24-q25	**rs1799971**	NM_000914.3:c.118A>G	N40D	A>G
SLC19A1	Chromosom 21q22.3	**rs1051266**	NM_194255.1:c.80A>G	H27R	A>G
SLCO1B1	Chromosom 12p12	**rs4149056**	NM_006446.4:c.521T>C	V174A	T>C
SLCO1B1	Chromosom 12p12	**rs11045819**	NM_006446.4:c.463C>A	P155T	C>A
SLCO1B1	Chromosom 12p12	**rs2306283**	NM_006446.4:c.388A>G	N130D	A>G
SLCO1B1	Chromosom 12p12	**rs4149015**	NG_011745.1:g.4195G>A	-	G>A
TPMT	Chromosom 6p22.3	**rs1800462**	NM_000367.2:c.238G>C	A80P	G>C
TPMT	Chromosom 6p22.3	**rs1800460**	NM_000367.2:c.460G>A	A154T	G>A
TPMT	Chromosom 6p22.3	**rs1142345**	NM_000367.2:c.719A>G	Y240C	A>G
TPMT	Chromosom 6p22.3	**rs1800584**	NM_000367.2:c.626-1G>A	-	G>A
TPMT	Chromosom 6p22.3	**rs12201199**	NM_000367.2:c.419+94T>A	-	T>A
VKORC1	Chromosom 16p11.2	**rs9923231**	NC_000016.9:g.31107689C>T	-	C>T
VKORC1	Chromosom 16p11.2	**rs7294**	NM_024006.4:c.*134G>A	-	G>A
VKORC1	Chromosom 16p11.2	**rs17708472**	NM_024006.4:c.173+525C>T	-	C>T
VKORC1	Chromosom 16p11.2	**rs2359612**	NM_024006.4:c.283+837T>C	-	T>C
VKORC1	Chromosom 16p11.2	**rs8050894**	NM_024006.4:c.283+124G>C	-	G>C
VKORC1	Chromosom 16p11.2	**rs9934438**	NM_024006.4:c.174-136C>T	-	C>T