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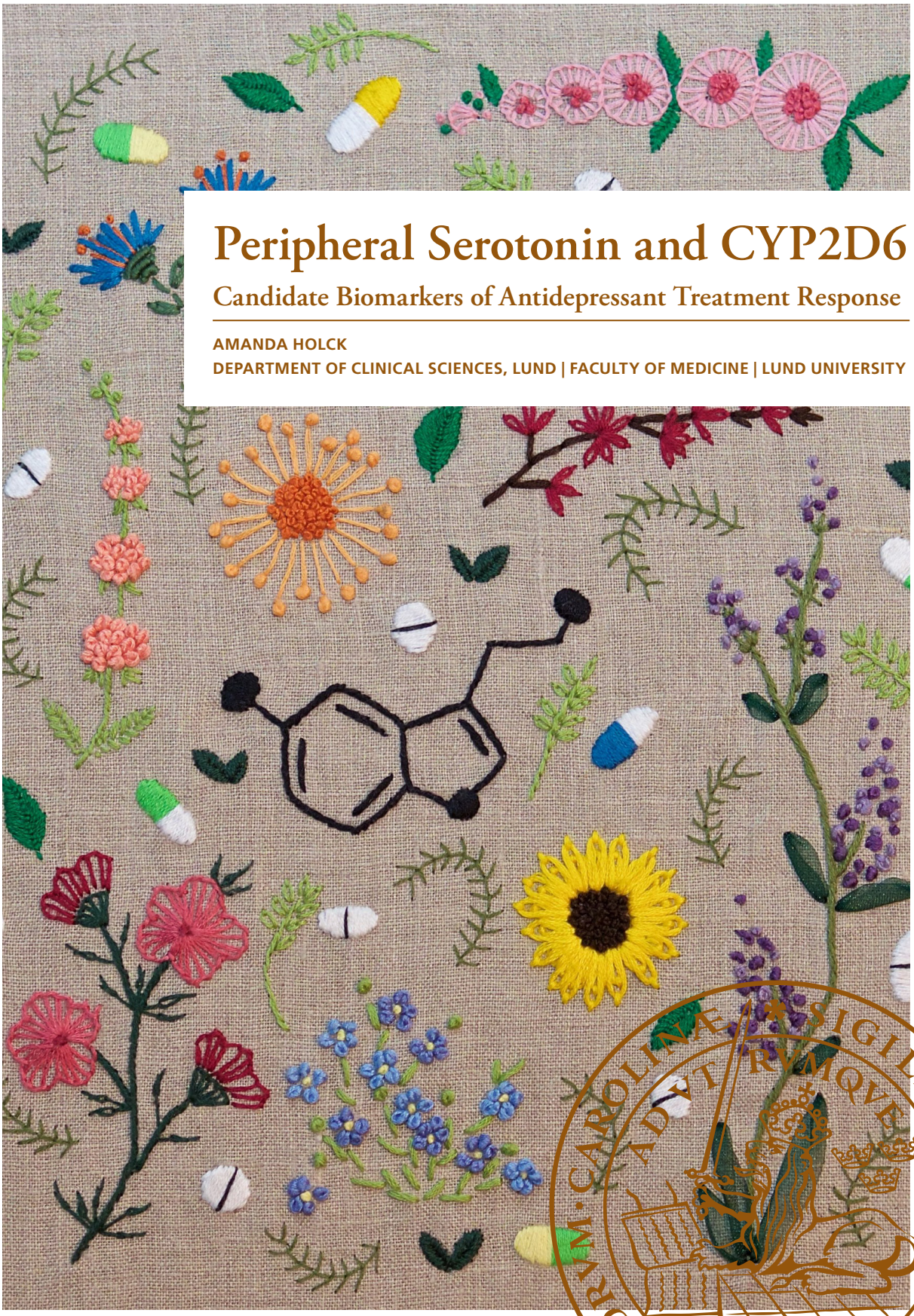

Peripheral Serotonin and CYP2D6

Candidate Biomarkers of Antidepressant Treatment Response

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Amanda Holck

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract:
Background and aim
Many patients with depressive disorders do not improve with first-line treatment with selective serotonin inhibitors (SSRIs). Adverse drug reactions (ADRs) are common. However, there are no reliable methods to predict treatment response.The general aim of this thesis was to investigate the role of peripheral serotonin and the polymorphic CYP2D6 gene as potential biomarkers of treatment response, including ADRs and suicidality, in depressive disorders.
Methods
The thesis includes material from two clinical cohorts and one systematic review. In the first cohort (paper I) plasma serotonin was analysed at baseline and at week 8 in 26 participants with major depressive disorder that were treated with SSRIs. Paper II is a systematic review that examines the association between peripheral serotonin levels and treatment response to antidepressants. Papers III and IV are based on the Genes, Depression and Suicidality (GEN-DS) cohort, a cross-sectional observational study where 415 patients with depressive disorders were subjected to rigorous diagnostic and anamnestic interviewing and genotyped for CYP2D6.
Results
Paper I: Higher baseline serotonin was associated with SSRI treatment response. Treatment was associated with a decrease of serotonin.
Paper II: Several studies reported that higher baseline serotonin was associated with better response to SSRIs, but no studies showed an association between serotonin levels and response to non-SSRIs.
Paper III: Patients with CYP2D6 phenotypes with lower metabolizing effect did not report significantly more ADRs. This result remained unchanged when phenoconversion due to CYP2D6-inhibiting comedication was considered.
Paper IV: A history of suicide attempts was not overrepresented among CYP2D6 ultrarapid metabolizers.
Conclusion
Peripheral serotonin levels may be associated with treatment response specifically to SSRI treatment in depressive disorders, but more studies are needed. The CYP2D6 polymorphism did not predict the occurrence of ADRs in a naturalistic setting. CYP2D6 UM phenotype was not associated with suicide attempts, and further studies of single-gene associations with suicidal behaviours are likely not warranted.

Key words: Depression, biomarkers, treatment response, serotonin, 5-HT, CYP2D6, pharmacogenetics, adverse drug reactions, suicide attempt

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“Hope is the thing with feathers
That perches in the soul,
And sings the tune without the words,
And never stops at all.”

Emily Dickinson

Table of Contents

Abstract

Background and aim

Many patients with depressive disorders do not improve with first-line treatment with selective serotonin inhibitors (SSRIs). Adverse drug reactions (ADRs) are common. However, there are no reliable methods to predict treatment response.

The general aim of this thesis was to investigate the role of peripheral serotonin and the polymorphic *CYP2D6* gene as potential biomarkers of treatment response, including ADRs and suicidality, in depressive disorders.

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The thesis includes material from two clinical cohorts and one systematic review. In the first cohort (paper I) plasma serotonin was analysed at baseline and at week 8 in 26 participants with major depressive disorder that were treated with SSRIs. Paper II is a systematic review that examines the association between peripheral serotonin levels and treatment response to antidepressants. Papers III and IV are based on the Genes, Depression and Suicidality (GEN-DS) cohort, a cross-sectional observational study where 415 patients with depressive disorders were subjected to rigorous diagnostic and anamnestic interviewing and genotyped for *CYP2D6*.

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Populärvetenskaplig sammanfattning

Depressiva tillstånd är mycket vanliga. Över 300 miljoner människor världen över bedöms lida av depression, och depression är en av de vanligaste orsakerna till nedsatt livskvalitet och hälsa. En mycket allvarlig potentiell följd av depression är suicid.

Läkemedel som används för att behandla depression tillhör egentligen flera olika läkemedelsgrupper, men tillsammans kallas de ofta för ”antidepressiva” trots att många av preparaten även används för att behandla andra sjukdomar såsom ångesttillstånd. Även om antidepressiva läkemedel hjälper många människor, så finns det också en stor andel personer som inte blir bra trots ett eller flera försök med läkemedelsbehandling. Det finns i dagsläget inget sätt att veta ifall en person kommer att få bra effekt av ett visst läkemedel eller ifall det till exempel finns en stor risk för att utveckla biverkningar. För att hitta ett läkemedel som hjälper är det inte ovanligt att man som patient med hjälp av sin läkare måste testa sig fram mellan flera olika preparat innan man träffar rätt.

En stor förhoppning inom psykiatrin under de senaste årtiondena har varit att hitta biologiska markörer för att kunna förutsäga vem som kommer att få god effekt av ett visst läkemedel genom att analysera till exempel ett enkelt blodprov. Detta skulle kunna spara både tid och pengar, men framför allt minska lidandet för personer som mår mycket dåligt.

Än så länge finns det inte några så kallade biomarkörer som kan användas på detta sätt inom den kliniska psykiatrin. Mycket forskning pågår, och det sker undersökningar av många olika möjliga biomarkörer. Bland annat har man diskuterat att undersöka koncentrationen av serotonin i blodet och att titta på genetiska variationer av ämnen som är aktiva vid nedbrytning av läkemedel i kroppen.

Serotonin är en signalsubstans som bidrar till att överföra information mellan nervceller i hjärnan. Det är vanligt att höra att depression beror på en serotoninbrist i hjärnan, men man har aldrig kunnat visa att det faktiskt är så. Däremot så vet vi att de vanligaste typerna av antidepressiva läkemedel, så kallade selektiva serotoninåterupptagshämmare (SSRI), påverkar signaleringen med serotonin och att det på ett eller annat vis bidrar till att behandlingen i många fall leder till en förbättring. Ingen vet egentligen hur antidepressiva läkemedel fungerar på detaljnivå.

Förutom i hjärnan så finns serotonin bland annat också i mag-tarmkanalen där det bildas samt i blodet, framför allt i blodplättar. Tidigare studier har undersökt ifall

serotoninnivåer kan kopplas till behandlingssvar för antidepressiva läkemedel, och det finns vissa studier som till exempel har beskrivit att högre nivåer av serotonin i blodet skulle kunna vara kopplat till bättre effekt vid behandling med SSRI-preparat.

Två av studierna i avhandlingen har undersökt ifall serotoninnivåer i blodet kan förutsäga vem som får effekt av antidepressiv läkemedelsbehandling. I studie I undersöktes koncentrationen av serotonin i plasma innan och efter åtta veckors behandling med SSRI-preparatet sertralin hos personer med depression. Resultatet visade att de som fick god effekt av behandlingen hade högre nivåer av serotonin i plasma innan behandlingsstart än de som inte svarade på behandlingen. En större sänkning av serotoninnivå under behandlingstiden var också kopplat till bättre behandlingssvar. Studie II genomfördes som en systematisk översiktsartikel, där tidigare publicerade artiklar som undersökte sambandet mellan serotoninnivåer i blod och behandlingssvar med antidepressiva läkemedel vid depression sammanställdes. Resultatet tydde på att det möjligen kan finnas ett samband mellan högre serotoninnivåer innan behandlingsstart och bättre behandlingsutfall vid behandling med SSRI-preparat, men det skulle behövas fler stora och väldesignade studier för att säkert kunna säga ifall det finns ett sådant samband. Resultatet tyder också på att det inte finns något sådant samband för antidepressiva läkemedel med andra typer av verkningsmekanism.

Enzymet CYP2D6 finns i levern. Ett enzym är ett protein som påskyndar kemiska reaktioner, och som i det här fallet bidrar till att bryta ner många vanliga läkemedel. Hur effektivt CYP2D6-enzym man har är ärftligt. Man ärver en variant av CYP2D6-genen som har nedsatt, normal eller ökad aktivitet från båda föräldrar. Den sammantagna funktionen hos enzymet kan då variera från helt nedsatt till något nedsatt, normal eller ultrasnabb. Personer med helt nedsatt enzymvariant kan inte bryta ner läkemedel alls via den här vägen och kommer sannolikt få mycket högre koncentration av just den typen av läkemedel i blodet. Viss tidigare forskning tyder på att det kan leda till mer biverkningar. En tidigare svensk studie har också visat att den ultrasnabba varianten av CYP2D6 var tio gånger vanligare bland personer som hade avlidit genom suicid. En möjlig anledning till det skulle kunna vara att personer med en ultrasnabb variant av CYP2D6 bryter ner vissa läkemedel så snabbt att koncentrationen i blodet blir för låg för att de skulle få någon effekt, och därför oftare än andra lida av en otillräckligt behandlad depression.

Studie III och IV baserades på en patientgrupp med svårbehandlad depression som intervjuades noggrant och genomgick en diagnostisk genomgång i den så kallade GEN-DS-studien. Den genetiska variationen av CYP2D6-enzymet kontrollerades också hos patienterna. Studie III undersökte ifall patienter med olika varianter av CYP2D6-enzymet rapporterade olika mycket biverkningar. Vissa läkemedel kan också blockera CYP2D6-enzymet så att personen i fråga inte kan bryta ner läkemedel genom CYP2D6 oavsett vilken variant av enzymet man har ärvt. Resultatet från studien kunde inte visa att det var någon skillnad i hur mycket biverkningar personerna rapporterade beroende på vilken enzymvariant de hade ärvt

eller ifall de också medicinerade med blockerande läkemedel. Studie IV undersökte ifall den ultrasnabba varianten av CYP2D6 var överrepresenterad bland personer med svårbehandlad depression och suicidförsök någon gång i livet jämfört med de som inte hade genomfört något suicidförsök. Vi kunde inte hitta något sådant samband.

Sammanfattningsvis så visar resultaten från avhandlingen att det kan vara aktuellt att forska mer på serotoninnivåer i blodet som en möjlig biomarkör för att förutsäga effekt vid depressionsbehandling med SSRI-preparat, även om det än så länge inte finns grund för att säga att det finns ett säkert samband. Vad gäller CYP2D6 så verkar det inte finnas någon anledning att undersöka den genetiska variationen hos personer med svårbehandlad depression generellt då vi inte fann någon tydlig koppling till hur vanliga biverkningar var. Däremot är det sannolikt relevant att undersöka CYP2D6-variant hos personer som till exempel har allvarliga biverkningar av läkemedel som bryts ner av CYP2D6. Det verkar inte heller finnas någon grund för att den ultrasnabba varianten av CYP2D6 skulle kunna förutsäga risk för suicidförsök.

List of Papers

Paper I

Holck A, Wolkowitz OM, Mellon SH, Reus VI, Nelson JC, Westrin Å, Lindqvist D. Plasma serotonin levels are associated with antidepressant response to SSRIs. J Affect Disord. 2019 May 1;250:65-70.

Paper II

Holck A, Movahed P, Westrin Å, Wolkowitz OM, Lindqvist D, & Asp M. Peripheral serotonin levels as a predictor of antidepressant treatment response: A systematic review. Prog Neuropsychopharmacol Biol Psychiatry. 2024 Jul 13:133:111031.

Paper III

Holck A, Asp M, Green H, Westrin Å, Reis M. CYP2D6 genotyping and inhibition as predictors of adverse drug reactions in depressive disorders. J Clin Psychiatry. 2024;85(1):23m14937.

Paper IV

Asp M, Holck A, Green H, Westrin Å, Reis M. CYP2D6 UM phenotype is not related to suicide attempts in depressive patients in secondary psychiatric care. Nord J Psychiatry. 2025;79(7):556–63.

Author's contribution to the papers

Paper I

Writing of original draft, data curation, formal analysis, visualization

Paper II

Conceptualization, methodology, writing of original draft, project administration, investigation, contributed to acquisition of data, formal analysis, visualization

Paper III

Substantial contributions to the conceptualization and the design of the study, writing of original draft, contributed to acquisition of data, data curation, formal analysis, visualization

Paper IV

Substantial contributions to the conceptualization and the design of the study, substantial contributions to the writing of original draft, contributed to acquisition of data, data curation

Abbreviations

5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine (serotonin)
5-HTT	5-hydroxytryptamine transporter, serotonin transporter (synonym to SERT)
5-HTTLPR	Serotonin-transporter-linked promoter region
ADR	Adverse drug reaction
BDNF	Brain-derived neurotrophic factor
BMI	Body mass index
CSF	Cerebrospinal fluid
CNS	Central nervous system
CYP	Cytochrome P450
DTD	Difficult-to-treat depression
DSM	Diagnostic and Statistical Manual of Mental Disorders
DST	Dexamethasone suppression test
ECT	Electroconvulsive therapy
EM	Extensive metabolizer (more often referred to as normal metabolizer)
GEN-DS	Genes, Depression and Suicidality
HDRS	Hamilton Depression Rating Scale
HPA	Hypothalamic-pituitary-adrenal
ICD	International Classification of Diseases
IDO	Indoleamine 2,3-dioxygenase
IM	Intermediate metabolizer
MADRS	Montgomery-Åsberg Depression Rating Scale
MAO	Monoamine oxidase

MDD	Major depressive disorder
MINI	Mini International Neuropsychiatric Interview
NM	Normal metabolizer
PGx	Pharmacogenomics
PM	Poor metabolizer
PRS	Polygenetic risk score
PTSD	Post-traumatic stress syndrome
RCT	Randomized controlled trial
RDC	Research Domain Criteria
rTMS	Repetitive transcranial magnetic stimulation
SCID-I	Structured Clinical Interview for DSM-IV Axis I Disorders
SCID-II	Structured Clinical Interview for DSM-IV Axis II Disorders
SERT	Serotonin transporter (synonym to 5-HTT)
SNP	Single nucleotide polymorphism
SNRI	Serotonin and norepinephrine reuptake inhibitor
SSRI	Selective serotonin reuptake inhibitor
TCA	Tricyclic antidepressant
TDM	Therapeutic drug monitoring
TDO	2,3-dioxygenase
TPH	Tryptophan hydroxylase
TRD	Treatment-resistant depression
UHPLC/ MS/MS2	Ultrahigh performance liquid chromatography/tandem mass spectrometry
UKU	Udvalgd før Kliniske Undersøgelser Side Effect Rating Scale
UM	Ultrarapid metabolizer
WHO	World Health Organization

Introduction

Depression

A brief history

The concepts of depression and melancholy have been acknowledged for thousands of years, but the description and the understanding of the depressive states have changed over the centuries. Hippocrates concluded that "If fear and sadness last a long time, such a state is melancholy". According to Hippocrates, moods were the results of the balance of the four humors (blood, phlegm, yellow bile and black bile) [1]. The word melancholy is even derived from the Greek words for "black bile".

For several hundred years, the concept of "melancholia" was roughly a synonym for "madness" and many other symptoms than sadness and emotional disturbance were described in this way [2, 3]. The most well-known text covering the subject was Robert Burton's seminal work *The Anatomy of Melancholy* (1621). Other frequently used concepts were those of "Weltschmerz" and "spleen", terms for being downcast and out of sorts [3].

In the 19th century a distinction was usually made between the two affective illnesses of neurasthenia and melancholia. Neurasthenia, with the meaning of "weak (or tired) nerves", was seen as a chronic, less serious condition of depression and anxiety that would often include pain, fatigue and hypochondria. Melancholia was described as episodical and was frequently associated with psychotic symptoms in addition to the affective symptoms. [3]

The term depression was initially more or less a synonym to neurasthenia [3]. Toward the end of the 19th century, both the term melancholia and the term neurasthenia started to be replaced by the term "mental depression" [2]. The term mental depression seemed to be preferred among many physicians, possibly because the term evoked a "physiological" explanation of the symptoms [2].

From several depressive disorders to major depressive disorder

Emil Kraepelin (1856-1926) separated manic-depressive illness from the progressive deterioration of dementia praecox [1, 4]. Manic-depressive illness included all types of melancholia, psychotic depression and all forms of serious

depressive and manic illness [3]. Kraepelin's term "psychogenic" depression was separated from manic-depressive illness as it was responsive to changes in the patient's social situation, and was similar to Sigmund Freud's (1856-1939) "neurotic" or "reactive" depression that was seen as the result of a psychic conflict. [1, 3]. The term "endogenous depression" was sometimes used for melancholia [3].

Throughout history distinctions have almost always been made between melancholic depression and non-melancholic depression. These two groups have generally been separated clinically, with patients with melancholic depression usually responding well to electroconvulsive therapy (ECT) and pharmacotherapy and presenting clinically as very slowed and with a non-reactive mood [5]. The non-melancholic depression has been less well demarcated, with symptoms such as a mixture of unhappiness, anxiety, phobia and character disorder, often with poorer response to treatment with ECT and antidepressant pharmacotherapy [5].

The concept of different types of depression ended abruptly in the public mind with the introduction of the DSM-III (Diagnostic and Statistical Manual of Mental Disorder) in 1980. The various depressive disorders that were diagnosed at the time were reduced to one: major depressive disorder (MDD) [5]. Melancholia was no longer considered a separate illness but could be coded as a subclass of MDD [6].

The lumping together of the previously separated types of depression into MDD has influenced the treatment offered to patients suffering from depression, who generally all tend to be offered "antidepressants". It has been suggested that this may be one reason why antidepressant treatment is often unsuccessful, as some evidence shows that anxiety, dysthymia, milder depression and other more chronic states may be less responsive to antidepressant drug treatment [5].

The heterogeneity of depression

The goal of medical diagnoses has always been to "carve nature at its joints". In psychiatry, however, this has not been as simple as was once hoped. Major depressive disorder is not a clearly defined illness. Like all other psychiatric diagnoses it is a syndrome, meaning that symptoms have been grouped together as they are believed to belong to the same disorder (for diagnostic criteria of MDD see Table 1). While not all patients with the same syndrome will exhibit the same symptoms, there will generally be enough of an overlap or similarity to suggest a distinctive disorder [7]. However, in MDD there is so much heterogeneity in what symptoms the patients may present with that there are 227 possible symptom combinations that all fulfil the diagnostic criteria. If all specific symptoms of compound criteria are considered separately (for example psychomotor agitation and psychomotor retardation), there are 14,528 possible combinations of criteria [8]. Some of the symptoms such as fatigue, loss of appetite or weight loss and insomnia are also common in other medical illnesses [9]. This raises the question if two patients who present with quite different – sometimes entirely different – symptoms really suffer from one common disorder [7].

The classification system International Classification of Diseases 10th edition (ICD-10) published by the WHO (World Health Organization) is used for coding diagnoses and for statistical analyses in Sweden [10]. The diagnostic criteria for MDD are more or less interchangeable with those of the DSM.

Table 1. DSM-5 diagnostic criteria for major depressive disorder.

DIAGNOSTIC CRITERIA FOR MAJOR DEPRESSIVE DISORDER	
A. Five or more of the following symptoms have been present during the same 2-week period and represent a change from previous functioning. At least one of the symptoms depressed mood (1) or loss of interest or pleasure (2) is present. All symptoms except for thoughts of death or suicide must be present for most of the day/nearly every day.	1. Depressed mood 2. Diminished interest or pleasure 3. Weight loss or weight gain, or decrease or increase in appetite 4. Insomnia or hypersomnia 5. Psychomotor agitation or retardation 6. Fatigue or loss of energy 7. Feelings of worthlessness or guilt 8. Diminished ability to think or concentrate, or indecisiveness 9. Recurrent thoughts of death, suicidal ideation, suicide attempt or suicide plan.
B. The symptoms cause clinically significant distress or impairment in important areas of functioning.	
C. The episode is not attributable to effects of a substance or to another medical condition.	
D. The episode is not better explained by another psychiatric disorder.	
E. There has never been a manic or hypomanic episode.	

Adapted from the Diagnostic and Statistical Manual of Mental Disorders Fifth Edition [11].

Subtypes of depression

The most recent DSM version, DSM-5, describes several subtypes of depression. These are grouped by for example pattern (single or recurrent episodes, rapid cycling and seasonal); clinical features (anxious distress, mixed features, atypical, melancholic, catatonic, psychotic); and onset (early, late, post partum) [9, 11]. The MDD subtypes have never been shown to be absolutely distinct from one another; for example, melancholic and psychotic depression have some overlap but also independent categorical features [12].

In DSM-5, patients with depressed mood for two years or more can be diagnosed with persistent depressive disorder (a combination of the DSM-IV syndromes of dysthymia and chronic depression) [11]. Bipolar depression is classified as a clinical presentation of bipolar disorder, and other depressive disorders include premenstrual dysphoric disorder, substance/medication-induced depressive disorder and depressive disorder due to another medical condition [11].

Epidemiology

Depressive disorders are common afflictions. More than 300 million people globally are estimated to suffer from depressive disorders. Prevalence rates worldwide have been estimated at 5.1 % in females and 3.6 % in males. [13] Twelve-month prevalence and lifetime prevalence in adults were found to be 10.4 % and 20.6 %, respectively, in a large American study [14]. Equally high numbers of cumulative probabilities for developing first incidence depression were found in the classic Lundby study that was conducted in Sweden, with numbers of 22.5 % in men and 30.7 % in women based on data from 1972-1997 [15]. Most studies report higher incidence of depression in women than in men [16]. Depressive episodes commonly debut in relative youth, with peak years of onset between 15 and 29 years of age [17].

The natural course of depression

Depressive symptoms frequently improve over time regardless of treatment. A meta-analysis has reported that in primary care settings, 32 % of untreated depression will remit within 6 months and 53 % within 12 months [18]. The spontaneous remission rate in the short term is likely to be somewhat lower, and different numbers have been reported from 8-23 % in three months [18, 19]. However, the phenomenon of spontaneous remission contributes to a high percentage of placebo responders in antidepressant trials [20], with as many as 35-40 % showing improvement in the placebo arm in randomized trials for antidepressant treatments [21].

A previous study showed that in persons with MDD who sought treatment, 50 % of those with a first-onset episode recover and have no further episodes [22]. Another study has reported a possibly larger risk of recurrent episodes, reporting that 50-85 % of those who have suffered one episode of depression will have a subsequent episode, and that the corresponding number is as high as 80-90 % for those who have suffered two episodes [23]. If individuals have experienced several previous episodes of depression, the time to recurrence seems to shorten [17].

Suicidality

Suicide attempts are common in depression. A meta-analysis showed that the pooled lifetime prevalence of suicide attempts in people with MDD was 31 % (95 % CI 27-34 %) [24], as compared to about 3 % in the general population [25]. More than 700 000 people die by suicide each year [26]. Bipolar disorder and MDD has been reported to be two of the psychiatric disorders with the highest suicide risk [27], and

the most common psychiatric disorder reported in people who die by suicide is depression [28].

Suicide is more frequent among men than among women; in high-income countries men account for more than three times as many suicides as women [26]. Even though suicide is more common among older people, it is the third leading cause of death among people aged 15-29 years [26].

The age-standardized global suicide rate has dropped by 35 % between the years 2000 and 2021 [26]. In Sweden, suicide rates have decreased since the 1980s; since the beginning of the 2000s, a decrease by about 0.5 % per year has been reported. The decrease in suicide rates in Sweden is especially pronounced among older men, while the decrease has stagnated in the younger population in recent years. [29]

Epidemiologic and family studies have estimated that the genetic component of suicide behaviour is somewhere between 17 % and 55 % [30], and the heritability in non-mortal attempts has been estimated at 17 % to 45 % [31]. Several studies, including family, twin, and adoption studies have shown that the hereditary transmission of suicidality remains after controlling for the transmission of psychiatric disorders [32]. This indicates the importance of genetic susceptibility that is separated from psychiatric disorders.

Suicidal behaviour

Suicidal behaviour can be separated into suicidal ideation, suicide attempt and suicide death. These have been shown to be three distinct groups, with differing but overlapping risk factors [33].

Suicidal ideation is defined as thinking about, considering or planning suicide [33].

Suicide attempts include self-inflicted, potentially harmful behaviour exhibiting explicit or implicit evidence of the individual's intent to die [34]. While suicide death is more common among men than among women, suicide attempts are more common among women [35]. Time to full remission from a major depressive episode has been reported to be the most robust predictor of a subsequent suicide attempt in depression [36]. Other risk factors that have been reported include a history of suicide attempts, psychotic features, severity of depression, severity of hopelessness and substance misuse [36, 37].

Suicidal ideation along with a history of suicide attempt has been reported to be associated with future suicide death at both the short and long term (6 weeks to 10 years) [36]. Male sex, anxiety disorders, severe depression and alcohol or substance abuse has also been reported to predict suicide death [37]. Previous studies have further reported that compared to suicide attempters, suicide completers were more likely to be older, have alcohol use disorder, have comorbid health problems and frequently use more lethal methods [38-40].

Suicide prevention

Suicide prevention has been the focus of much research and debate during the last decades. Suicide prevention has long been dominated by suicide risk assessments. However, this method has been shown to be largely ineffective [41]. Recent studies have shown that almost 74 % of people who died by suicide in Sweden in 2015 were in contact with a health care provider during the three months prior to the suicide, and 60 % within four weeks [42]. Dishearteningly, studies show that when mental health clinicians estimated the immediate risk of suicide at the last service contact before a suicide, the majority judged the risk to be low or absent, and more than two thirds of patients had denied having thoughts of suicide in mental health conversations in the last 30 days before death by suicide [41]. Studies have shown that suicidal ideation often varies greatly over the course of days or even hours, and thoughts of suicide may return unexpectedly after having been accurately reported as low during an assessment [43, 44]. Suicide risk assessment tools have also been used, but have low positive predictive values and low sensitivity and are only marginally superior to chance; the majority of suicides occur among patients who are classified as low or medium risk [45, 46].

Current research into suicide prevention often favours therapeutic risk assessment and formulation. This includes building a therapeutic alliance with the patients during conversations, and to utilize the alliance to identify modifiable risk factors and make a collaborative treatment plan (so called “safety planning”) [41].

Options for suicide prevention that have been reported to be effective and scalable according to a meta-analysis include educating primary care physicians, educating high school students about mental health, restricting means and following up and reaching out to patients who are discharged after a suicide crisis [47].

Serotonin

Serotonin – production and metabolism

The serotonin molecule (also referred to as 5-hydroxytryptamine or 5-HT) consists of an indole ring with two functional groups, an acidic OH group at the five position and a flexible ethylamine side chain at the three position [48]. Serotonin is mainly synthesized in the enterochromaffin cells of the intestinal mucosa from the precursor tryptophan, an essential amino acid [48]. The first step of the reaction is catalyzed by the enzyme tryptophan hydroxylase (TPH). TPH1 is expressed in the gastrointestinal (GI) tract and TPH2 is expressed in the central nervous system (CNS) [49]. Tryptophan can be converted through two main pathways, where the serotonin pathway is one. The other is the kynurenine pathway, that is responsible for the degradation of almost 95 % of dietary tryptophan [50]. Factors such as stress,

infections and changes in the gut microbiome can shunt tryptophan metabolism from serotonin production towards the kynurenine pathway [51].

Serotonin has functions in many different organ systems including the regulation of energy balance and food intake, GI and endocrine function, and cardiovascular and pulmonary physiology (see Figure 1) [52]. Serotonin is also involved in various behavioural and neuropsychological processes such as mood, perception, reward, anger, aggression, appetite, memory, sexuality and attention [52].

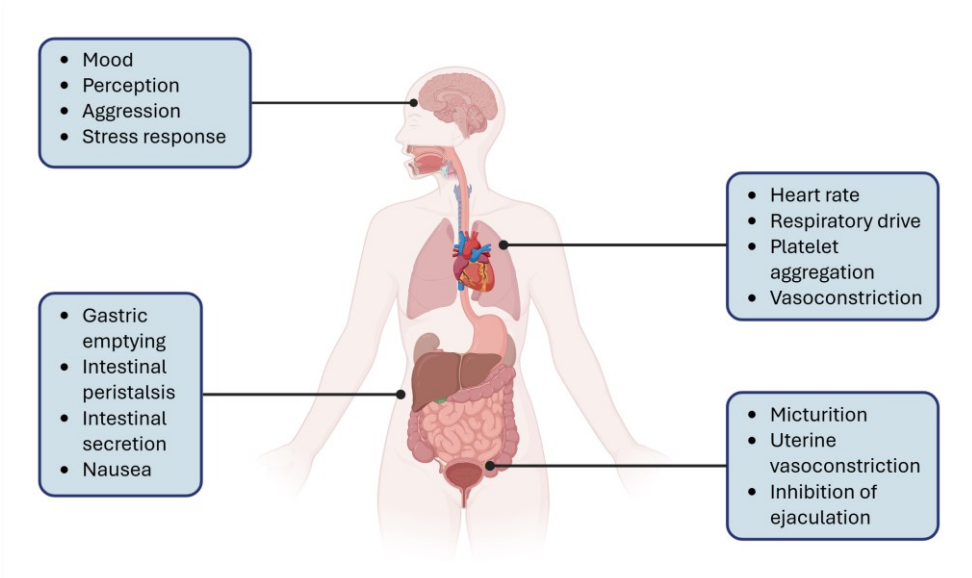

Figure 1. An illustration of some of the many effects of serotonin in the body.
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The majority of serotonin is present in the enterochromaffin cells but approximately 5-8 % is present in platelets, and a very low concentration is present in plasma [48]. Serotonin is released into the bloodstream by the enterochromaffin cells and then taken up into the platelets. In platelets, the serotonin is stored in dense granules and promotes aggregation and thrombus formation as it is re-released into the circulation in response to signals produced by for example damaged endothelium and ischemia. [48]

Less than 1 % of serotonin is present in the CNS [48]. Tryptophan is converted to serotonin in the neurons, as serotonin cannot pass the blood-brain barrier [48]. Serotonergic neurons project from the raphe nuclei located in the middle of the brainstem throughout the brain and the spinal cord (see Figure 2) [52]. Serotonin is stored in synaptic vesicles in serotonergic neurons, and the vesicles fuse with the

neuronal membrane to release serotonin into the synapse as a response to stimulation of the neuron [48]. The action of serotonin in the synaptic cleft is terminated by serotonin reuptake transporters (SERTs) or by its diffusion away from the synaptic cleft; while the SERTs are highly selective for their substrate, there is also some affinity for the other monoamines (norepinephrine and dopamine) [53]. The SERT is also expressed on platelets where it is responsible for serotonin uptake from plasma [54].

The primary metabolic pathway for serotonin is through monoamine oxidase (MAO), mainly by MAO-A as MAO-B only represents a small portion of serotonin metabolism and is the predominant form in platelets [53]. Metabolization of serotonin mainly occur in the brain, gastrointestinal tract, lungs, liver and platelets [53].

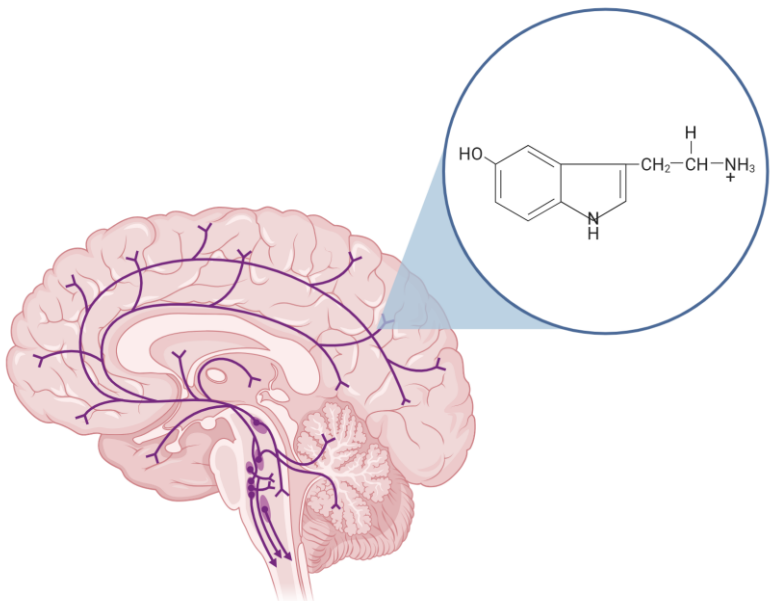

Figure 2. The serotonin molecule and the serotonin pathways of the brain.
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Serotonin receptors

Serotonin has diverse effects throughout the body which are mediated via serotonin receptors. Seven subtypes of serotonin receptors, mainly G-protein-coupled receptors, have been recognized [53]. Table 2 summarizes the different known 5-HT receptors, their most frequent locations and their proposed roles in depressive disorders. The fact that each individual serotonin receptor is involved in multiple physiologic processes presents a challenge for serotonergic drugs, since even a drug targeting a single serotonin receptor subtype is likely to have effects on multiple body systems [52].

Table 2. Description of the different serotonin receptors and their proposed roles in depressive disorders.

	LOCATION	PROPOSED ROLE IN DEPRESSIVE DISORDERS
5-HT1A	Raphe nuclei; also hippocampus, septum, amygdala and corticolimbic areas	Presynaptic autoreceptor, post-synaptic receptor. Reduced 5-HT cell firing with activation (e.g. with SSRI treatment); with desensitization increased 5-HT cell firing. Neuronal inhibition, behavioural effects (sleep, feeding, thermoregulation, and anxiety)
5-HT1B	Substantia nigra, globus pallidus and basal ganglia; blood vessels	Presynaptic autoreceptor, post-synaptic receptor. Reduced 5-HT cell firing with activation (e.g. with SSRI treatment); with desensitization increased 5-HT cell firing. Presynaptic inhibition, behavioural effects. Pulmonary vasoconstriction
5-HT1D	Brain, cranial blood vessels	Not well-known. Believed to be responsible for regulating firing rate of dopamine containing cells and release of dopamine
5-HT1E	Frontal cortex, striatum	Unknown
5-HT1F	Cortex, hippocampus	Unknown
5-HT1P	Enteric nervous system	Likely unimportant in depression; regulates chloride secretion and intestinal peristalsis
5-HT2A	Brain, mainly neocortex; also platelets and GI tract	GABAergic interneurons, glutamatergic projection neurons. Neuronal excitation, behavioural effects, and learning. Drugs with high affinity to this receptor subtype may augment SSRI effect in resistant patients through blocking 5-HT2A mediated effects. Antagonism increases noradrenaline release under SSRI treatment. Also involved in obesity and diabetes. Increases micturition reflex. Platelet aggregation
5-HT2B	Dorsal raphe; mainly in stomach fundus	Increases serotonin release. Stimulates smooth muscles, involved in heart development

	LOCATION	PROPOSED ROLE IN DEPRESSIVE DISORDERS
5-HT2C	Choroid plexus, hippocampus, substantia nigra	GABAergic interneurons. 5-HT2C antagonists may augment SSRI efficacy. Chronic treatment with SSRIs causes desensitization. CSF secretion. Reduces food intake and triggers weight loss
5-HT3 family	Parasympathetic terminals in the gastrointestinal tract, in solitary tract nucleus and area postrema	Controls dopamine, acetylcholine release and GABAergic system, excites cortical GABAergic neurons. Stimulates norepinephrine release in the locus coeruleus which decreases firing rates and leads to decrease of noradrenaline release in terminal areas. Involved in control of the vomiting reflex
5-HT4 family	Various peripheral and central nervous system structures and GI tract, on neurons of the myenteric plexus and on smooth muscle and secretory cells	5-HT4 knockout mice show an exaggerated inhibitory response of 5-HT neurons to the SSRI citalopram
5-HT5A	Hippocampus	Unknown
5-HT6 family	Striatum; also nucleus accumbens, olfactory tubercle and cortex	Antagonists potentiate effects of antidepressants and agonists may initiate some outcomes of antidepressants like fluoxetine. Effect on the sleep cycle and memory disorders. May be efficacious in treatment of obesity and drug abuse
5-HT7 family	Hypothalamus and intestine; also thalamus, hippocampus and cortex, blood vessels	Involved in circadian rhythms. Downregulated binding with chronic antidepressant treatment. Modulates memory. Responsive to inflammatory stimuli

Based on Taciak et al 2017 [55] and Mohammad-Zadeh et al 2008 [53].

Serotonin levels in different body compartments

As described above, serotonin is present in platelets, plasma and serum as well as in the CNS [48]. Since central serotonin levels are difficult to measure, the different blood components have been proposed as alternatives. It has been shown that platelet levels, and to a lesser degree plasma levels, of serotonin correlate to cerebrospinal fluid (CSF) serotonin levels [56, 57]. There is a lack of similar comparisons with serum and whole blood serotonin levels. Platelets have also been suggested to be comparable to neurons in serotonergic actions as they both express the SERT that is involved in reuptake and metabolism [54]. Plasma has by some been suggested to be less appropriate for the measurement of serotonin levels, as the levels may be very low and review articles have reported discrepant concentrations in studies [58, 59]. However, as of today, there is no clear consensus

as to whether blood components other than platelets can reliably be used for serotonin analyses or not.

A systematic review by Anderson and Bruno-Pacella investigated using platelet serotonin content to assess the bioeffect of SSRIs at the serotonin transporter [60]. The reduction of platelet serotonin content in percent was comparable to the extent of transporter blockade or inhibition that were reported by studies that used neuroimaging to evaluate the effect of the same type of antidepressants. This suggests that platelet serotonin content provides a reliable peripheral estimation of SERT inhibition both centrally and in the periphery [60].

The serotonin transporter gene

The human serotonin transporter SERT or 5-HTT, that is present on neurons and on platelets, is encoded by the *SLC6A4* gene on chromosome 17 [61]. A polymorphism in the transcriptional control region leads to short (S) and long (L) variants of this serotonin-transporter-linked promoter region (5-HTTLPR), where the S allele (and a later discovered L_G variant) has been associated with lower transcription efficiency, leading to lower expression, binding and uptake [61, 62].

A study from 2003 by Caspi et al found that the 5-HTT polymorphism seemed to moderate the influence of stressful life events on depression, showing evidence of a gene-by-environment interaction [63]. Many studies on the serotonin transporter gene followed. However, a large meta-analysis was not able to find any evidence of a strong interaction between stress and 5-HTTLPR genotype in the development of depression, and reported no impact on risk for depression [64]. The alleles with lower transcription efficiency have also been suggested to be related to less favourable outcomes with antidepressant medications and the *SLC6A4* has been investigated as a candidate gene of suicidal behaviour; results have been inconclusive [65-67].

Explanatory models of depression

The monoamine hypothesis

The monoamine systems in general, and the serotonin system in particular, started drawing attention in the 1950s. It was discovered that the antihypertensive agent reserpine precipitated depression in certain patients while also depleting brain serotonin and norepinephrine stores and increasing concentrations of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in urine. [68].

Soon after, it was discovered that the antimycobacterial agent iproniazid improved mood in tuberculosis patients with depression [69]. Iproniazid was found

to inhibit monoamine oxidase (MAO), a mitochondrial enzyme that prevents the degradation of serotonin and norepinephrine in the presynaptic nerve terminal [70]. An additional substance, imipramine, was investigated as a treatment for schizophrenia but instead reduced depressive symptoms and was found to block the serotonin and norepinephrine transporters [70].

These two discoveries that indicated a reduced monoamine transmission in patients lead to the proposition of the "catecholamine hypothesis" of depression in 1965 [71]. A few years later, serotonin was suggested as the most important monoamine, leading to the proposition of the "serotonin hypothesis" by Coppen in 1967 [72, 73].

There have always been unsolved issues attached to the monoamine hypothesis. One of these is the so-called therapeutic delay; even though SSRIs inhibit the serotonin transporter within hours with an increased availability of serotonin in the synaptic cleft as a result, there is typically a delay of several weeks in the onset of antidepressant response [69]. It has furthermore never been conclusively shown that depression is a result of, or leads to, lowered serotonin levels, or that SSRI treatment increases serotonin levels [74-76]. Still, the connection between depression and low serotonin levels managed to take root in the public mind, leading to the popular notion of a "chemical imbalance" as the etiology of depression [77].

While depression does not seem to be associated with low serotonin levels [74], there is still evidence that serotonin plays a role in the treatment effect of certain antidepressant medications. Low serotonin function has been suggested to affect mechanisms involved in reaching and maintaining recovery rather than lowering mood generally [78]. Possibly the best example of this is the results from tryptophan depletion studies, where a transient lowering of brain serotonin activity is achieved through diminishing the availability of its precursor tryptophan. Tryptophan depletion does generally not have an effect on mood in healthy controls without a history of depression, but can lead to the temporary return of depressive symptoms in subjects with depression in remission, especially in subjects treated with antidepressant medication [79]. However, it would seem that impairing serotonin function is neither necessary nor sufficient to cause clinical depression [78].

Other biological models

The hypothalamic-pituitary-adrenal (HPA) axis is activated when the body is subjected to stress. This leads to effects such as the mobilization of substrates for energy metabolism and the dampening of immune and inflammatory reactions by secretion of cortisol from the adrenal cortex [80]. While this activation is effective for handling acute stress, chronic stress leads to the hypersecretion of corticosteroids, which has been shown to increase the risk for depression and other diseases [81]. The feedback mechanism where the activation of the HPA axis normally leads to an automatic downregulation of cortisol release seems to be

dysfunctional in subgroups of patients with depressive disorders [81]. Treatments that modify the HPA axis function have not yielded results in clinical trials [9].

Inflammation has also been suggested to relate to depression. Patients treated with cytokine therapies for immune-mediated illnesses such as chronic hepatitis C are often reported to exhibit symptoms of major depression [82]. Depression often presents with symptoms of anhedonia, social avoidance, hypersomnia, lethargy and loss of libido [83]. These symptoms overlap with symptoms of inflammation, and have been suggested to be part of a "sickness behaviour" which promotes energy preservation and shunting of energy resources to fighting infection and wound healing [84]. Depressive patients have also been reported to show elevations of peripheral inflammatory markers [85].

Tryptophan, the precursor to serotonin, passes the blood-brain barrier in competition with other amino acids, and is taken up into serotonergic neurons. Serotonin itself does not cross the blood-brain barrier, and the levels of tryptophan available are crucial to the synthesis of serotonin in the brain [86]. Most dietary tryptophan is degraded through the kynurenine pathway [50]. An increased shunting of tryptophan into the kynurenine pathway reduces the availability of serotonin (see Figure 3). Tryptophan is converted to kynurenine by the enzymes 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO-1 and IDO-2) [50] that are stimulated by an increased activity of the HPA axis and pro-inflammatory cytokines, respectively [50].

In summary, the explanatory models of depression that include monoamines, the HPA axis and inflammation may all be connected and contribute to the pathophysiology and treatment response in at least some subgroups of patients.

Other hypotheses

The examples described above are in no way an exhaustive list of the different mechanisms that have been implied in the pathophysiology of depression. It is widely accepted that depressive disorders are associated with multiple pathogenic factors, such as mental factors, genetic factors, social stress and other diseases [87]. Comprehensive reviews are available; see for example Malhi & Mann [9] and Cui et al [87].

The diathesis-stress model stipulates that a person who carries a biological or psychological diathesis (vulnerability), or both, for a certain type of stressor and is then exposed to an acute stressor is likely to develop depression. Models of depression that incorporate, among other things, genetic, developmental and environmental factors are usually variants of the diathesis-stress model. [28]. Severe events especially, such as financial difficulties, job loss, separations, bereavement and being subjected to violence, have been shown to contribute to the development of MDD [9]. The exact mechanisms are not known but may involve stress leading to long-term elevated glucocorticoids and an imbalance in the HPA system as described above [87]. In line with the "kindling hypothesis", the association

between stressful life events and onsets of major depression seems to be progressively weaker with each new depressive episode [88].

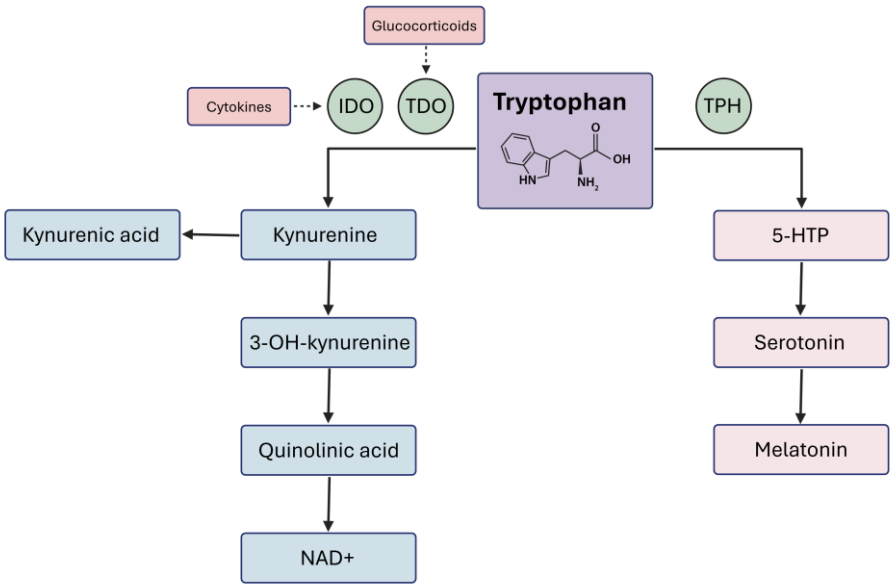

Figure 3. Tryptophan metabolism through the kynurenine and the serotonin pathways. IDO: indoleamine 2,3-dioxygenase. TDO: 2,3-dioxygenase. TPH: tryptophan hydroxylase. NAD: nicotinamide adenine dinucleotide. Created with BioRender.com.

Treatment of depression

Development of antidepressant medications

The first MAO inhibitor iproniazid was initially only used off label as an antidepressive agent. Its antidepressant effect has been attributed to increased monoamine transmitter levels in the presynaptic terminal [68, 89]. While effective, MAO inhibitors have the disadvantage of increasing concentrations of tyramine and norepinephrine in the sympathetic nervous system when combined with foods containing high amounts of tyramine such as cheese, beans and wine, leading to a risk of hypertensive crisis [90].

The tricyclic antidepressants (TCAs), starting with imipramine, was the second group of antidepressants to be discovered and was approved for the treatment of MDD in the United States in 1959 [68, 91]. Its main effect derives from inhibition of norepinephrine and serotonin reuptake transporters; however, the antagonism of adrenergic, muscarinic and histaminergic receptors contribute to side effects such as dizziness, memory impairments and drowsiness [68]. Tricyclic antidepressants are also highly toxic in higher doses, and have historically frequently been used in fatal overdoses [92].

Pharmaceutical companies then began developing compounds that would selectively inhibit the reuptake of serotonin in the synapse and decrease the risk of side effects. The first compound that was made clinically available was the Swedish drug zimelidine; however, this drug was shortly discontinued due to reports on neurotoxicity and immunogenicity [93]. Fluoxetine was the first selective serotonin reuptake inhibitor to be launched in the United States in 1988 under the trade name Prozac. SSRIs are 20-1500-fold more selective for inhibiting serotonin over norepinephrine at their transport proteins and have minimal binding for other postsynaptic receptors [68]. SSRIs are still associated with side effects, mostly of a serotonergic character such as nausea, insomnia and sexual dysfunction, but undesired effects are overall less frequent than with the older antidepressant drugs [91]. As the SSRIs were safer to prescribe than the older TCAs because of lower toxicity, they were widely prescribed by general practitioners which lead to a large increase of pharmacological treatment of depressive disorders [91].

After SSRIs, drugs that selectively target both serotonin and norepinephrine transporters have been marketed, the first one being venlafaxine in 1993. Unlike TCAs, the selective serotonin-norepinephrine reuptake inhibitors (SNRIs) have much less pharmacological action at adrenergic, histamine, muscarinic and dopamine receptors, leading to a less pronounced side effect profile. [68] SNRIs have been reported to be more effective than SSRIs; however, the advantage has been suggested to be modest [94].

Other drugs that have been developed to treat depressive disorders include the noradrenergic and specific serotonergic antidepressant mirtazapine, the serotonergic drug vortioxetine and the dopamine-norepinephrine reuptake inhibitor bupropion [68, 90].

How do antidepressants work?

The initiating effect of SSRIs and several other types of antidepressants has long been established to include inhibition of the neuronal serotonin transporter (SERT) located on presynaptic axon terminals of serotonin neurons (see Figure 4). The function of the SERT is to terminate the actions of serotonin in the synaptic cleft by reuptake and to allow serotonin to be stored for reuse [95]. However, this initial effect is not sufficient to explain the delayed effect of antidepressants that typically takes several weeks. The exact mechanisms behind this delayed effect remains

unknown, although many different hypotheses both regarding the serotonergic system and beyond have been proposed [96].

One known delayed action is the desensitization of presynaptic 5-HT_{1A} receptors. Stimulation of these receptors by serotonin in the synaptic cleft decreases neuronal firing rates, working as a feedback mechanism. However, the 5-HT_{1A} receptors become desensitized with a decreased disinhibiting effect during a longer SSRI treatment leading to an increase in serotonergic neurotransmission. [95] Experiments have been made where SSRIs were combined with drugs that selectively block 5-HT_{1A} autoreceptors to try and accelerate the onset of therapeutic response to SSRIs; however, this has not proved clinically effective [96].

Since medications such as SSRIs are also effective in several other conditions than depressive disorders such as OCD, panic disorder and bulimia, is has been suggested that the drugs are likely to have different actions in different brain regions, possibly due to differences in properties of serotonin receptors in these areas [95]. While some antidepressants are designed to be more "selective" for serotonin or norepinephrine, it has been shown that serotonin and norepinephrine receptors interact anatomically and pharmacologically, and may also have modulatory effects on dopamine [93]. It is currently believed that all licensed oral antidepressants relieve depression by increasing serotonin or norepinephrine availability, at least initially, regardless of the variations in proposed mechanisms [96].

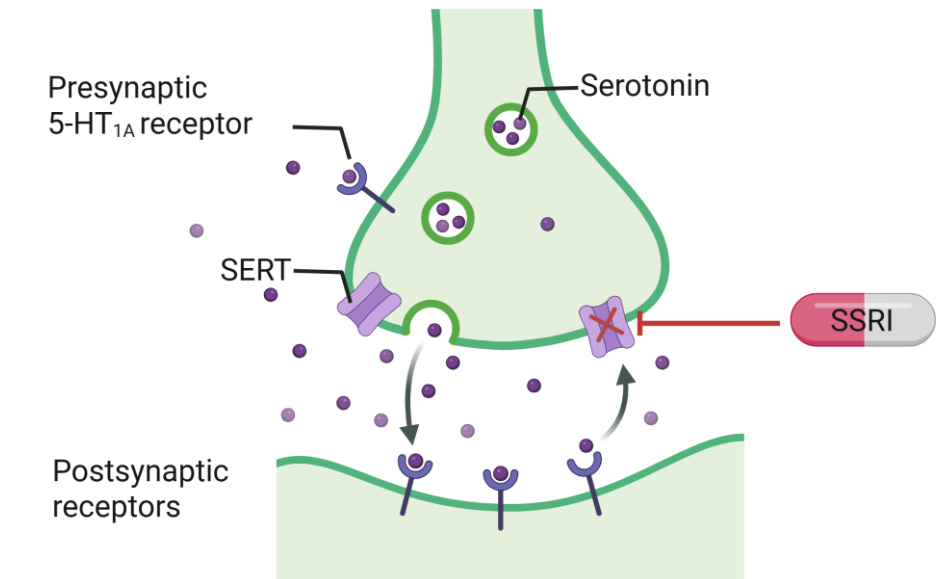

Figure 4. Proposed mechanism of action of a selective serotonin receptor inhibitor (SSRI).
SERT: Serotonin transporter. Created with BioRender.com.

Slow adaptive changes in post-receptor signaling cascades and downstream mechanisms could be mediators of the delayed antidepressant actions, producing secondary changes that involve transcriptional and translational changes that mediate molecular and cellular plasticity over time [97, 98]. For example, BDNF-mediated signaling is believed to be involved in the opposing neuroplastic responses to stress and antidepressant treatment [96, 97]. Treatment with antidepressant drugs may also lead to homeostatic mechanisms by which the brain attempts to regulate monoamine neurotransmission in the presence of a monoamine enhancing drug, and some observed reactions may not actually be related to treatment effect at all [96].

Antidepressant medications have also been shown to affect glutamate neurotransmission by downregulating NMDA-receptor subunits and dampen NMDA function. Ketamine, an NMDA receptor antagonist, produces rapid but transient antidepressant effects in individuals with depression; however, little is known as to the cellular and molecular mechanisms involved. [97-99] Another hypothesis involves antidepressant-induced neurogenesis in certain brain areas, but this hypothesis is based on preclinical studies [98].

In conclusion, much is still unknown about how antidepressant medications exert their effects. Monoaminergic transmission is likely to be involved in some manner in most cases, but the underlying mechanisms largely remain hypothetical.

Effectiveness of antidepressant treatment

The effectiveness of antidepressant drugs has long been discussed. A systematic review and network meta-analysis that collected data from both published and unpublished double-blind randomized controlled trials (RCTs) included more than 116 000 participants and found that all 21 investigated antidepressants were more effective than placebo; however, the effect sizes were mostly modest [20].

The largest clinical trial to date that was not sponsored by the industry and that investigated the effect of pharmacological drug treatment was the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial. 3 671 patients with a diagnosis of non-psychotic MDD received one to four successive acute treatment steps. This study found that only 36.8 % of the patients remitted with the first treatment step (citalopram); the overall cumulative remission rate from all four steps was 67 % [100]. This means that more than 30 % of the included patients did not achieve remission of MDD even after four different treatments.

Maintenance therapy with antidepressant medication has been estimated to reduce the absolute risk of depressive relapse with around 50 % [101, 102].

Treatment-resistant depression/difficult-to-treat depression

Cases where remission has not been reached despite two or more adequate courses of antidepressants are sometimes referred to as “treatment-resistant depression”

(TRD); however, other definitions are sometimes used [103]. The two treatments are not consistently described as needing to belong to different pharmacological classes [104]. The lack of a well-established definition of treatment-resistant depression has resulted in highly variable estimates of its prevalence rate, ranging from 6-55 % [105].

This description of treatment-resistant depression has been criticized for several reasons. Examples include that other treatment alternatives are not taken into account and that two treatment attempts are equated to as many as ten or 20 [104, 105]. The concept of "difficult-to-treat depression" (DTD) has been proposed as an alternative [104]. DTD has been described as "depression that continues to cause significant burden despite usual treatment efforts" [106]. DTD may be suspected after two failed treatment attempts but is not confirmed until other possible contributing factors such as diagnostics, environmental and developmental factors, adherence to treatment and comorbidity have been addressed [104].

Adverse drug reactions

Adverse drug reactions (ADRs) are common problems in the pharmacological treatment of depressive disorders. Some of the most common ADRs related to treatment with SSRIs and SNRIs are GI symptoms such as nausea, diarrhea, and dyspepsia, as serotonergic drugs can affect gastric motility and act on 5-HT_3 receptors that are present in the gut [107]. Other common side effects include weight gain, sexual dysfunction, sweating, sleep disturbance and emotional blunting [107, 108]. The more selective antidepressants are reported to have fewer side effects and be safer in overdose than the older tricyclic antidepressants and are associated with lower drop-out rates; however, SSRIs may have comparatively higher risks of certain ADRs such as hyponatremia, bleeding and sexual dysfunction [108]. While the therapeutic effect is delayed for several weeks, antidepressant side effects are commonly most intense in the initial treatment period. Braund et al found that for the SSRIs escitalopram and sertraline and the SNRI venlafaxine, side effects were greatest at week two and gradually decreased up to week 6 [109]. While decreasing over time, the effect of early ADRs should not be overlooked, as the authors also reported that the burden of side effects was associated with poorer treatment outcome from as early as four days into treatment.

In the STAR*D study, 16.7 % of the patients withdrew completely from first line therapy due to drug intolerance [100]. Other studies have reported that as many as 43 % of patients discontinued or switched antidepressants (SSRIs) due to side effects within the first three months of treatment [110]. Associations have also been made between the occurrence of adverse drug events (ADRs) in general and high overall cost of illness in the Swedish general population [111].

A very serious possible ADR that has been proposed for antidepressant medications, and for SSRIs in particular, is an increased suicide risk. However, large

meta-analyses have not supported an increased frequency of suicides in adults taking SSRIs [112].

CYP2D6

CYP2D6 and drug metabolism

An important contributor to the interindividual variability in drug response and drug intolerance is the inherited differences in drug metabolism mediated by the genes of the cytochrome P450 (CYP) family [113].

About 20-30 % of all marketed drugs are metabolized by the highly polymorphic CYP450 hepatic enzyme CYP2D6, including many antidepressants [114]. The many different variants of the *CYP2D6* gene are divided into alleles causing an absence of, decreased, normal or increased enzymatic activity. Each person's predicted CYP2D6 phenotype is the result of the two alleles, and the different possible phenotypes are labelled as poor metabolizer (PM), intermediate metabolizer (IM), normal metabolizer (NM; previously often referred to as extensive metabolizer or EM) and ultrarapid metabolizer (UM) [115, 116]. An absence of function of the CYP2D6 enzyme is inherited as an autosomal recessive trait [117]. CYP2D6 variants with a decreased enzymatic activity (PM and IM) may lead to increased serum concentrations of CYP2D6 dependent drugs with possible ADRs as a result, whereas CYP2D6 variants with increased enzymatic activity (UM) may lead to unexpectedly low serum concentrations and less effect than anticipated [118] (see Figure 5). The frequency of the different alleles varies greatly between different ethnic groups [119]. In Caucasians, the frequencies have been reported as 6.5 % PMs, 39.0 % IMs, 51.1 % NMs and 3.1 % UMs [120]. The UM frequency may be even lower in Swedish populations with numbers as low as 1 % reported [121].

CYP2D6 and adverse drug reactions

Previous studies have repeatedly shown that the predicted PM phenotype, compared to the other phenotypes, is associated with higher plasma levels of the parent drug or drug metabolites when they are CYP2D6 substrates [122]. This implies that people with a PM phenotype may also experience more adverse drug reactions. However, previous studies into this area have been inconclusive [113, 122]; this may be due to the fact that there is a weak relationship between antidepressant and antipsychotic drug blood levels and clinical response [122, 123]. In a 2019 review, Solomon et al reported that in studies published between 2013 and 2018, four studies found no significant differences in ADRs based on CYP2D6 metabolizer status,

while two studies found positive ADR associations; however, most included studies were reported to be underpowered, and study designs were heterogenous with much variation in which alleles were tested and how phenotypes were classified based on genotypes [113]. Additionally, co-medication was usually not considered, and many studies were deemed to be of poor quality.

Another factor that has been suggested to be associated with CYP2D6 and ADRs is phenoconversion. This phenomenon occurs when patients receive CYP2D6 substrates and concurrent medication with drugs that inhibit the CYP2D6 system. The enzymatic activity is then decreased in a way that mimics the genetic defect and may convert the subjects into a PM phenotype regardless of genotype. It has been suggested that this may occur to a greater extent in IMs than in NMs and UMs as IMs already have a somewhat compromised metabolic capacity. [124]

Based on these uncertainties, it remains unclear if routine CYP2D6 testing may lead to fewer ADRs and a better clinical outcome.

Examples of psychotropic medications that are metabolized by, or inhibits, the CYP2D6 system are given in Table 3.

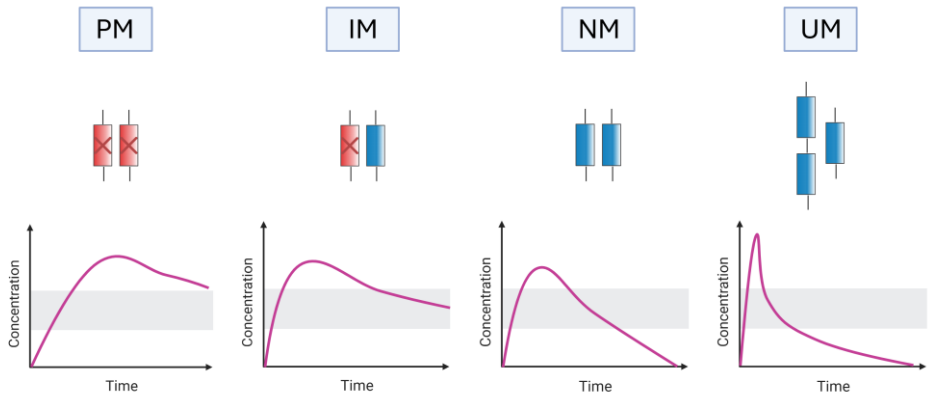

Figure 5. CYP2D6 and drug concentrations

A schematic illustration of the different CYP2D6 genotypes and how they may lead to varying drug concentrations. Concentrations above the grey area may lead to ADRs, and concentrations below to absence of effect. PM: Poor metabolizer. IM: Intermediate metabolizer. NM: Normal metabolizer. UM: Ultrarapid metabolizer. Created with BioRender.com.

CYP2D6 and suicidality

Apart from influences on drug effect and ADRs, the genetic variation of CYP2D6 has also been implicated in suicidality. In a Swedish study from 2010, Zackrisson et al compared the frequency of predicted CYP2D6 phenotypes in autopsies between intoxication cases, suicide cases excepting intoxications and natural deaths

[125]. They found that the frequency of CYP2D6 UMs was almost 10-fold higher in suicide cases (4.7 %) than in natural deaths (0.5 %). It has been hypothesized that a potential overrepresentation of CYP2D6 UMs among suicides or suicide attempters may be the result of ineffective antidepressant treatment due to ultrarapid metabolization of drugs [125].

Several later studies have investigated whether this effect can also be shown in suicide attempts. Most recently, the effect of CYP2D6 and CYP2C19 genotype was investigated in relation to repeated suicide attempts at six or 12 months after an index attempt. Results showed that the risk of repeated suicide attempts increased at six months in CYP2D6 UMs, and at both time-points for patients with high combined metabolic capacities for the two genes [126]. The same research team has previously reported an increased frequency of UMs among suicide attempters in patients with eating disorders [127] and higher scores among UMs on the Suicide Intent Scale after failed suicide attempts [128]. However, other studies have not reported any association between CYP2D6 genotype and suicide attempts [129-131].

Table 3. Examples of psychotropic medications that are substrates and/or inhibitors of CYP2D6 [132-135].

SUBSTRATES OF CYPD6	INHIBITORS OF CYP2D6
Amitriptyline	Bupropion (strong)
Aripiprazole	Duloxetine (moderate)
Atomoxetine	Fluoxetine (strong)
Clomipramine	Paroxetine (strong)
Haloperidol	
Mirtazapine	
Nortriptyline	
Paroxetine	
Risperidone	
Venlafaxine	

Precision psychiatry

Personalized medicine

Personalized medicine is a term that is used to describe the tailoring of treatment to each patient's individual characteristics and needs [136]. Currently, psychiatric diagnosis and treatment is based on signs and symptoms that are generally presumed to be similar in all patients with a particular diagnosis, with the seeming underlying assumption that one-size-fits-all. In comparison, the goal of personalized medicine

is to be able to offer 'the right drug at the right dose the first time', making treatment both safer and more effective [124]. To some degree, a personalized approach has always been used, in that for example the patient's history and circumstances, symptom profile, gender, weight and smoking status are taken into account [136]. Specific domains that have been suggested to be considered in the clinical characterization of a patient with depression and to function as a base for personalization of management include, among others, symptom profile, clinical subtypes, severity, functioning and quality of life, personality traits, antecedent and concomitant psychiatric conditions, physical comorbidities, family history, environmental exposures, and protective factors and resilience [137].

Precision psychiatry

As compared to personalized medicine, precision medicine, including precision psychiatry, also introduces measurable biomarkers [136]. The definition of a biomarker has been proposed as 'a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention' [138]. In certain non-psychiatric disorders such as Alzheimer's disease classification has been greatly helped by the use of biomarkers, especially when combining multimodal neuroimaging and fluid biomarkers [136].

Three different classes of biomarkers may be of particular relevance in the clinical management of mental disorders: a) diagnostic biomarkers (the probability that a particular condition is present), b) prognostic biomarkers (probability of particular outcomes such as disease relapse) and c) predictive biomarkers (forecasting the response to specific interventions; can include both treatment effect and side effects) [139]. Different types of biomarkers in MDD that have been proposed include genetic markers, peripheral biomarkers and neuroimaging markers (see Figure 6) [140].

The best use of biomarkers in mood disorders would likely not be to differentiate between depressed and non-depressed individuals, as non-depressed individuals rarely present to the clinic for care. One of the greatest clinical utilities in MDD has been proposed to be the matching of individuals to the treatment most likely to be efficacious [141]. Other possible areas of clinical utility would be biomarkers that help distinguish between different diagnoses, such as unipolar and bipolar depression, or that might indicate probable MDD recurrence and in that way inform about the necessity of maintenance treatment [141].

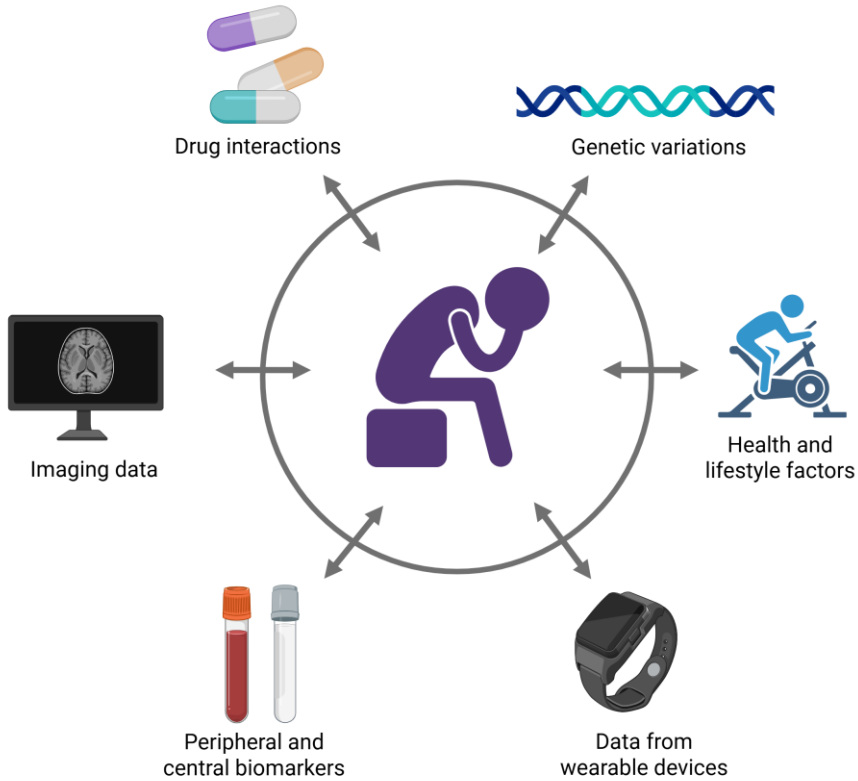

Figure 6. Examples of domains that may be included in precision psychiatry.
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Predicting treatment response

Studies have shown a substantial pharmacokinetic variability of antidepressant medications, with concentrations sometimes varying more than 20-fold between individuals [123, 142]. Therapeutic drug monitoring (TDM), where an individual's drug concentration is measured in serum or plasma, may be used as a tool to provide guidance for individual dosing strategies. However, its role in treatment with antidepressant drugs is less clear as there is limited evidence of an association between concentration and clinical effects such as efficacy and safety, especially for the commonly used SSRIs and SNRIs [123]. Despite this, TDM can be of great value in following intra-individual variations over time and possibly relate changes in drug concentrations to treatment failure or ADRs.

As many antidepressant drugs are believed to perform their effects through the serotonin system, serotonin has been proposed as a biomarker to predict treatment response [143]. While the serotonergic synapses where medications such as SSRIs exert their effect are located in the CNS, biomarkers from the brain and the CSF are difficult to collect. As such, peripheral molecules such as serotonin in peripheral blood may be more appropriate potential biomarkers. An earlier study used plasma metabolomic profiles of MDD patients being treated with SSRIs to identify metabolites that were associated with treatment response, and found that higher baseline serotonin levels as well as larger decrease in serotonin during treatment were associated with a better treatment outcome [144].

Pharmacogenomics

The field of pharmacogenomics (PGx; also referred to as pharmacogenetics) has grown rapidly during the last decades. The purpose of pharmacogenomics is to evaluate the genetic basis for drug response and adverse drug reactions in individual patients [136]. In medicine, pharmacogenomics has mostly been used in the field of oncology; in psychiatry, the first pharmacogenetic testing that has yielded more convincing results is the CYP450 genes [136, 145]. Consensus groups have described current published evidence, prescribing guidelines, and product labels to support the use of PGx testing for CYP2D6 and CYP2C19 for selection and dosing of several commonly-used antidepressant and antipsychotic medications [120].

The PREPARE study, a prospective, real-world implementation study, enrolled 6944 patients that received a first prescription for a drug with clinical recommendations in the guidelines for the Dutch Pharmacogenetics Working Group [146]. The patients were assigned to receive genotype-guided drug treatment or standard care. The study found that genotype-guided prescribing using a 12-gene pharmacogenetic panel significantly reduced the incidence of clinically relevant patient-reported ADRs with around 30 % in various medical conditions, somatic and psychiatric.

A systematic review from 2022 analysed clinical trials for an association between the use of PGx-guided antidepressant treatment and treatment response in 4767 patients with MDD [147]. While unable to determine which gene or combination of genes that were driving the observed effect, the authors reported that individuals that received PGx-guided antidepressant treatment were 41 % more likely to achieve remission compared to those who received treatment as usual. A similar systematic review and meta-analysis was performed shortly thereafter in 2023, with a large overlap in included studies (5347 patients) [148]. This second study drew a different conclusion and reported that the test-guided treatment did not improve long-term outcome as the test-guided treatment contributed to moderately improved response and remission rates at week 8 and 12 but not at week 24. Almost all included studies were funded by the industry and were thus assessed as having a high risk for bias [147, 148].

Despite inconclusive results, pharmacogenomics has been suggested to contribute to reduced health care costs, for example by reducing the cost for treating avoidable ADRs, reducing drug costs by reduced switching and dose adjustments, and reducing the time until disease remission. In addition to direct costs, PGx may reduce disease burden and related costs such as loss of productivity. [149] A Canadian study from 2023 created a microsimulation model of care pathways for MDD and reported that if pharmacogenomic testing of CYP2D6 and CYP2C19 was implemented for adults with moderate to severe depression it could slow or avoid the transition to treatment-resistant depression and thereby substantially reduce health care costs, increase life length and increase quality-adjusted life years [150].

In later years, pharmacogenomics and the study of genetic biomarkers in general have moved from the use of candidate genes toward genome-wide association studies (GWAS). GWAS include millions of variants throughout the genome and have enabled the construction of polygenic risk scores that provide a genetic risk summary of for example a disorder or a trait such as treatment resistance [136, 145]. However, very large sample sizes are necessary to see any statistically meaningful effect, and these studies have yet to produce clinical applications [145].

Guidelines and commercial tests

The research into a few genetic markers, most notably the *CYP2D6* and *CYP2C19* genes, has led to the production of clinical guidelines despite the caveats discussed above. The Clinical Pharmacogenetics Implementation Consortium (CPIC) began producing SSRI and TCA guidelines as early as 2013, despite limited evidence of clinical effects (more evidence exists for its relevance for TCAs than for SSRIs) [113]. The CPIC has curated 29 different guidelines for different classes of drugs, including antidepressants, and how to use PGx results in clinical practice when available; however, they do not discuss when such tests should be ordered [151, 152]. The other wide-spread source of PGx-based dose recommendations is the Dutch Pharmacogenetics Working Group (DPWG) that make gene-drug recommendations that are updated every three months [153].

Several pharmacogenetic tests for prediction of antidepressant drug response have been marketed commercially. These tests typically include the *CYP2D6* and *CYP2C19* genes, but they often also include genes involved in pharmacodynamics, such as the 5-HTTLPR polymorphism of the serotonin transporter gene whose contribution to the modulation of response and side effects from antidepressant medications is less clear [145, 147, 154]. The benefits from the tests have seldom been evaluated in published clinical trials.

Rationale

Despite decades of research, much is still unknown about the heterogenous depressive disorders. To a large proportion we do not know who is at risk of developing the syndromes, why they occur, who will respond to which medication or what adverse reactions the treatment may entail.

Precision psychiatry and the study of biomarkers may be a way to shed light on at least some of these as of yet unanswered questions. This is by many seen as the 'holy grail' of psychiatry, as it could lead to reduced suffering, faster treatment response and possibly even lowered suicide rates in this patient group in the future.

Many different types of biomarkers are being investigated. Markers in peripheral blood have been suggested as desirable as they are easily accessible and more cost-efficient; however, it is not always certain how well these markers correspond to processes in the central nervous system that is protected by the blood-brain barrier.

Serotonin has long been implicated in the treatment of depressive disorders. While serotonin levels do not seem to correspond to depressive symptoms, serotonin levels may contribute to the mechanisms behind treatment effect of certain antidepressive medications. As such, serotonin has been proposed as a possible biomarker to predict treatment response to antidepressant drugs in depressive disorders.

The polymorphic *CYP2D6* gene encodes an enzyme with great functional variability. Decreased function has been implicated to contribute to an increased burden of ADRs. However, there is no clear consensus as to which patients should be genotyped and at what timepoint, and if only patients with a complete lack of enzymatic activity are prone to ADRs or if this may also apply to patients with a reduced enzymatic activity. The ultrarapid metabolizer (UM) CYP2D6 phenotype has been reported to be ten times more common among suicide completers than in the general population, leading to the additional question if CYP2D6 could be a possible biomarker of increased suicide risk.

The rationale behind this doctoral dissertation is to investigate the questions described above, with the hope of taking one small step towards the implementation of precision psychiatry and predictive biomarkers in the treatment of depressive disorders.

Aims

The general aim of this thesis was to investigate whether treatment of depression with antidepressant drugs can be improved by analysing potential biomarkers of treatment effect, including ADRs and suicidality, in peripheral blood.

Specific aims

Papers I and II: To investigate if baseline serotonin levels in peripheral blood differ between responders and non-responders to antidepressant drug treatment in depressive disorders, and how serotonin levels are affected by the antidepressant treatment.

Paper III: To evaluate if the genetic variations of the liver enzyme CYP2D6 are associated with adverse drug reactions in depressed patients, and the effect of drugs that inhibit the CYP2D6 system on the occurrence of adverse drug reactions.

Paper IV: To evaluate if the genetic variations of the CYP2D6 enzyme are associated with a history of suicide attempts in depressed patients.

Overview of this thesis

The four studies included in this thesis are summarized in Table 4.

Table 4. Overview of the studies included in the thesis.

PAPER I	
Aim	To better understand the underlying mechanisms of SSRI treatment response and to investigate plasma serotonin as a marker of treatment prediction
Study design	Open label treatment study
Setting	Psychiatric outpatients with MDD in San Francisco, USA
Data collection	Structured interview, blood sampling at baseline and week 8
Analysis	Independent-samples T-test, one-way ANOVA, paired samples T-test
PAPER II	
Aim	To assess the predictive value of baseline serotonin in peripheral blood for the outcome of antidepressants in patients with depressive disorders
Study design	Systematic review
Setting	Not applicable
Data collection	Systematic search and data collection of relevant published literature
Analysis	Narrative synthesis of results
PAPER III	
Aim	To examine the association between the genetic variation of CYP2D6 and adverse drug reactions in MDD patients with insufficient treatment response
Study design	Cross-sectional observational study
Setting	Patients with depressive disorders and insufficient treatment response referred by their psychiatrist in Skåne, Sweden (GEN-DS)
Data collection	Structured interview, self-rating scales, blood sampling
Analysis	Kruskal-Wallis H-test
PAPER IV	
Aim	To examine the association between the genetic variation of CYP2D6 and previous suicide attempts in MDD patients with insufficient treatment response
Study design	Cross-sectional observational study
Setting	Patients with depressive disorders and insufficient treatment response referred by their psychiatrist in Skåne, Sweden (GEN-DS)
Data collection	Structured interview, self-rating scales, blood sampling
Analysis	Pearson's chi-square test, Fisher's exact test

Paper I

Paper I investigated the association between baseline plasma serotonin levels and SSRI treatment response in depressed subjects.

Materials and methods

Recruitment procedure and participants

MDD subjects and healthy controls were recruited by flyers, bulletin board notices, Craigslist postings, newspaper ads and clinical referrals to a psychiatric clinic in San Francisco, USA. Participants were interviewed with the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (SCID-I) [155] and were diagnosed with MDD without psychotic symptoms. The diagnosis was verified by a board-certified psychiatrist. A current score of ≥ 17 on the 17-item Hamilton Depression Rating Scale (HDRS) [156] was an inclusion criterion. Exclusion criteria were bipolar disorder, psychotic symptoms during the current major depressive disorder, history of psychosis outside of a mood disorder episode, any eating disorder or post-traumatic stress disorder (PTSD) within one month of entering the study, and substance abuse or dependence (including alcohol) within six months of entering the study. The study participants were also excluded in the presence of any acute illness or infection, chronic inflammatory disorder, neurological disorder, or any other major medical condition considered to be potentially confounding. All subjects were free of psychotropic medications (including antidepressants), hormone supplements, and steroid-containing birth control or other potentially interfering medications for at least six weeks prior to inclusion in the study.

SSRI treatment

Patients were treated with open-label outpatient treatment with the SSRI antidepressant sertraline for eight weeks. Treatment was initiated with sertraline 25 mg, and the dosage was increased over the course of treatment to a maximum of 200 mg daily based on efficacy and adverse drug reactions. At week four and eight,

clinical assessment, assessment of drug tolerability and pill count to ensure compliance was performed. HDRS evaluation was repeated at eight weeks.

Response was defined as a reduction of 50 % or more on HDRS ratings at week eight compared to baseline. Remission was defined as a week eight HDRS score of ≤ 7.

Blood sampling and serotonin analysis

Blood sampling was performed at baseline and at eight weeks between 08.00 and 11.00 after fasting (except water) from 22.00 the night before. Serotonin and tryptophan were analysed in plasma using ultrahigh performance liquid chromatography/tandem mass spectrometry (UHPLC/MS/MS2). The plasma concentrations were relative concentrations determined as part of metabolomic profiling.

Statistics

All statistical analyses were performed using IBM SPSS Statistics (version 24). Independent-samples *t*-tests and one-way ANOVAs were used for between-group comparisons. Skewed data were log transformed before analyses. Changes in serotonin levels in responders and non-responders were analysed using paired-samples *t*-test. *P* values were two-sided. A *P* value of < 0.05 was considered statistically significant.

Results

Thirty-seven MDD subjects and 41 healthy controls were recruited. Out of the 37 MDD subjects, 26 participants underwent eight weeks open label sertraline treatment. The decision not to offer all MDD subjects treatment was based on funding. For three of the 26 participants, week eight blood samples were not available.

Demographics

See Table 5. MDD subjects were more likely than healthy controls to be smokers. Non-responders had a higher average BMI (body mass index) and were less likely to be smokers than responders.

Table 5. Demographic characteristics of MDD subjects and controls, and of responders and non-responders to sertraline treatment.

	ALL MDD SUBJECTS (N = 37)	CONTROLS (N = 41)	*P* VALUE	NON-RESPONDERS (N = 9)	RESPONDERS (N = 17)	*P* VALUE
Age (±SD)	38.1 ± 13.5	36.2 ± 13.0	0.52	40.3 ± 15.8	37.8 ± 10.7	0.64
Sex % women (*n*)	64.9 (24)	61.0 (25)	0.72	77.8 (7)	58.8 (10)	0.59
BMI (±SD)	25.0 ± 4.5	24.9 ± 4.9	0.89	28.4 ± 5.1	24.0 ± 3.7	0.02
Smoker % (*n*)	29.7 (11)	7.3 (3)	0.01	11.1 (1)	47.1 (8)	0.16
HDRS at baseline	19.4 ± 2.6	N/A	N/A	19.1 ± 1.8	18.9 ± 2.4	0.86

Plasma serotonin levels at baseline

Plasma serotonin levels were nominally but not significantly lower in MDD subjects than in healthy controls (1.18 ± 0.58 vs 1.42 ± 0.67, $t = 1.7$, $P = 0.095$). Healthy controls and responders had similar baseline plasma serotonin levels ($P = 0.47$), whereas both healthy controls and responders had significantly higher baseline plasma serotonin levels than non-responders ($P = 0.004$ and $P = 0.036$, respectively). For exact values see Table 6. There was a trend towards higher baseline serotonin levels in remitters (n = 10) than in non-remitters (n = 16), but the difference did not reach statistical significance ($t = 1.8$, $P = 0.09$). See also Figure 7.

Baseline serotonin levels did not correlate significantly with baseline HDRS scores in all MDD subjects or when responders and non-responders were analysed separately. Plasma serotonin levels were not significantly correlated with plasma tryptophan levels at baseline or at week eight ($r_S = 0.14$, $P = 0.21$ and $r_S = -0.043$, $P = 0.85$, respectively). Plasma tryptophan levels did not differ significantly between MDD subjects and controls or between responders and non-responders at baseline.

Table 6. Relative concentrations of plasma 5-HT at baseline and after eight weeks SSRI treatment, and delta 5-HT (measured as 5-HT levels at week eight minus 5-HT levels at baseline) in MDD subjects treated with SSRI, divided by responder/non-responder status.

	NON-RESPONDERS (N = 9)	RESPONDERS (N=17)	*P* VALUE
5-HT at baseline (mean ± SD)	0.78 ± 0.19	1.30 ± 0.62	0.036
5-HT at 8 weeks (mean ± SD)	0.52 ± 0.16	0.48 ± 0.21	0.53
Delta 5-HT	-0.31 ± 0.26	-0.88 ± 0.68	0.047

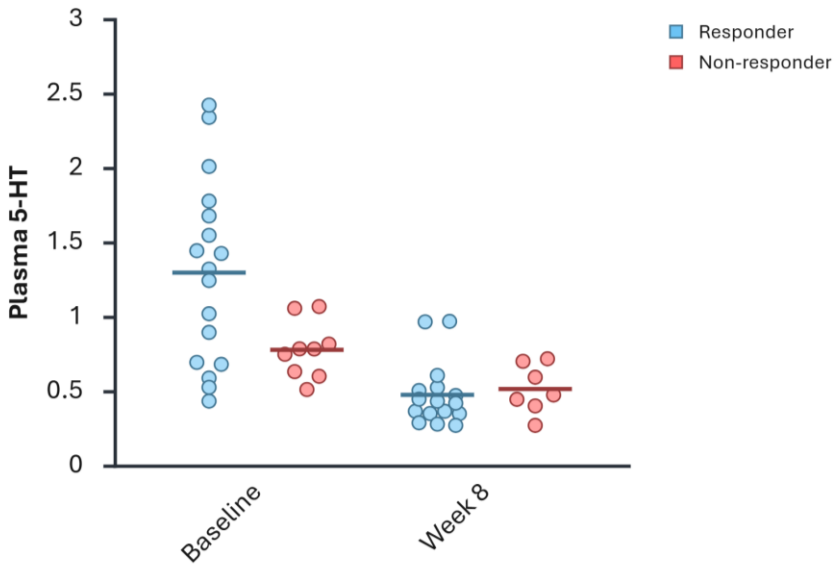

Figure 7. Categorical scatter plot with mean of plasma 5-HT levels at baseline and after eight weeks of sertraline treatment.
5-HT: 5-hydroxytryptamine (serotonin). Created with BioRender.com.

Pre-to-post-treatment changes in plasma serotonin

Sertraline treatment was significantly associated with a decrease in plasma serotonin levels in all treated MDD subjects ($t = 6.2$, $P = 0.000003$). This decrease was more prominent in the responder group than in the non-responder group ($t = 2.1$, $P = 0.047$).

Paper II

Paper II investigated the association between baseline plasma serotonin levels and antidepressant treatment response in the available literature in a systematic review.

Materials and methods

Eligibility criteria

A systematic search of the literature was performed to identify all studies that measured serotonin levels in peripheral blood (platelets, plasma, serum or whole blood) before the initiation of a pharmacological treatment for depressive disorders. Depressive disorders were defined by the Diagnostic and Statistical Manual of Mental Disorders (DSM) [11], the International Classification of Diseases (ICD) [10], or the Research Diagnostic Criteria (RDC) [157]. Included studies were also required to examine the relationship between baseline levels of serotonin and the antidepressant treatment outcome. Additional inclusion criteria required the studies to be full reports of original research, in English and involving human subjects. Studies that only investigated the effect of psychotherapy, ECT or other non-pharmacological treatments were excluded.

Registration

The protocol was registered at PROSPERO (8 April 2023, number CRD42023411877). The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Guidelines were observed [158].

Databases and search strategy

The literature search was conducted in the databases MEDLINE, Embase, PsycINFO, and the Cochrane Central Register of Controlled Trials. The search strategies were created in collaboration with a health sciences librarian specializing in systematic review searching. Search terms included Boolean combinations of the following: depressive disorders, major depressive disorder; SSRIs, SNRIs,

antidepressive agents, antidepressants; serotonin, 5-hydroxytryptamine; antidepressant response, treatment response, treatment outcome, remission. The search was performed on 9 January 2023 including all relevant papers published up to that date, and re-run on 20 November 2023 prior to final analysis.

Selection process and data collection

Two authors independently conducted initial screening of title and abstract. Full-text records were then evaluated for inclusion by two authors independently. Disagreements were resolved through discussion. The screening process is illustrated in Figure 8.

Two reviewers then independently extracted data from each selected report. Collected information included, but was not limited to, information on study aim, design, baseline population characteristics, inclusion and exclusion criteria, withdrawals and exclusions, diagnostic criteria, interventions, outcomes of depression and serotonin levels, study funding and conflicts of interest. Authors were contacted for additional information when necessary.

Assessment of quality

The quality of the research reports was assessed using a modified version of seven criteria proposed by Strawbridge et al [159]. With these criteria, studies can score positively (+1), negatively (- 1) or with no change of score on each criterion resulting in a quality score ranging from -7 to 7. Each study was assessed independently by two reviewers. Disagreements were resolved through discussion. Studies scoring below 1 were classified as having a high risk of bias (poor quality) while those scoring 1 or above were considered at low to moderate risk of bias (average to high quality).

Statistical considerations

Due to between-studies heterogeneity with regards to study design, treatment provision and analyses, it was not possible to perform a meta-analysis.

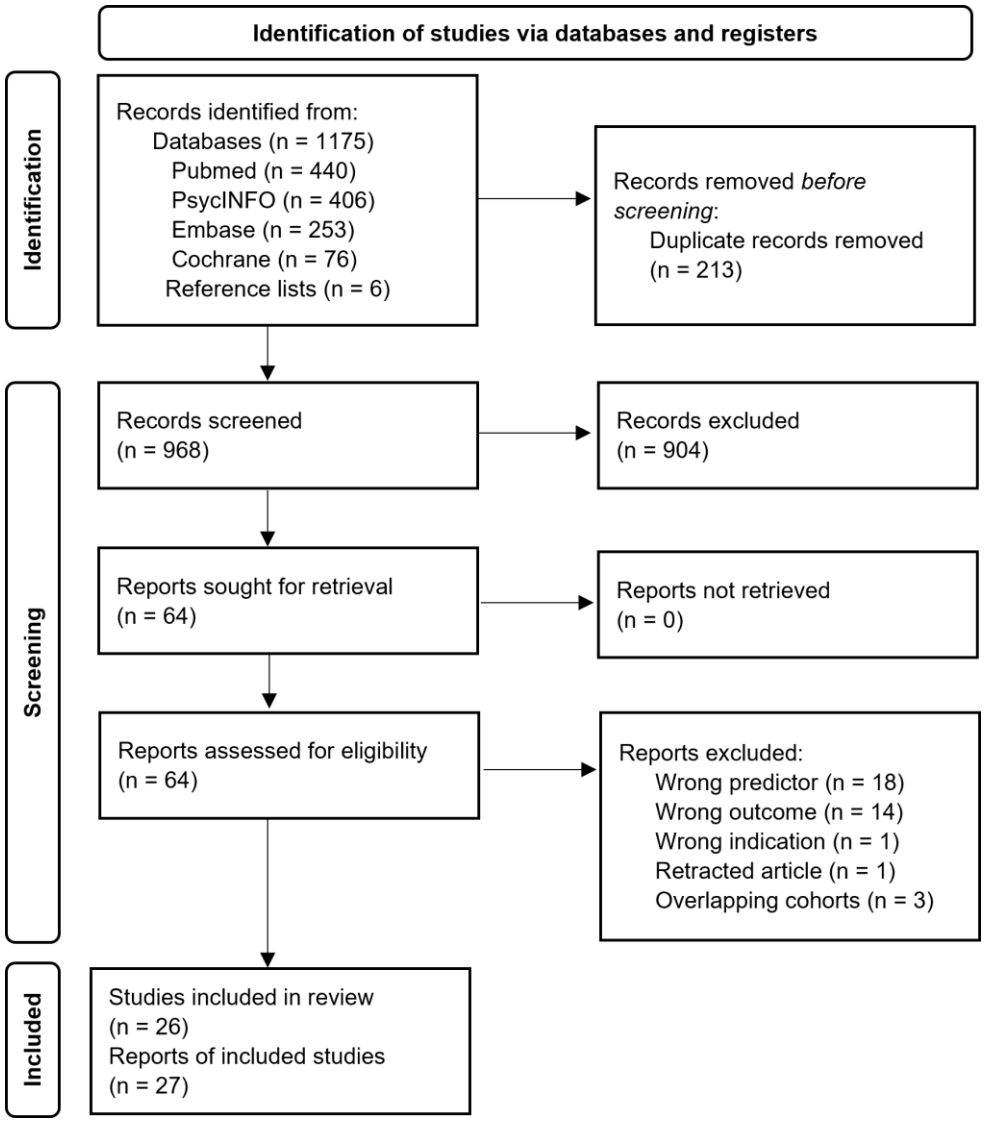

Figure 8. PRISMA flow chart of the identification and screening process.
From "Peripheral serotonin levels as a predictor of antidepressant treatment response: A systematic review" by Holck et al [160].

Results

Study selection

The search yielded 968 articles. Sixty-four articles were reviewed in full text after initial screening of title and abstract resulting in 26 studies reported in 27 different articles that were included in the review.

Study characteristics

Included studies were heterogeneous with regards to treatment provision, length of follow-up, choice of blood component and methods of analysis for serotonin concentrations. All studies included adults aged 18 years or older except for one study that only included adolescents and one study that included patients aged 17 years or older.

Most studies were non-randomized and non-blinded. The included articles most frequently categorized patients as responders or non-responders at the end of the study based on a 50% or more reduction on a depression rating scale score. All included studies are described in the journal article [160].

Results of serotonin levels in different blood components

A total of 16 studies reported on the basal values of peripheral platelet serotonin in relation to treatment response in patients treated with antidepressant medications. Twelve of these studies investigated SSRI treatment. Three of these studies reported that higher platelet baseline serotonin levels were associated with better treatment response [161-163], five studies reported an association between lower serotonin levels and a better treatment response [143, 164-167], and four studies found no association [168-171]. Eight studies investigated the effect of non-SSRI medications (nefazodone, tianeptine, maprotiline, brofaromine, phenelzine, amitriptyline and vortioxetine); none of these studies reported an association between baseline platelet serotonin levels and antidepressant treatment response [161, 167-170, 172-174]. Two studies that reported several medications together, in one case also including ECT treatment, reported no association between baseline platelet serotonin levels and treatment response [175] or an association between lower serotonin levels and better treatment outcome [166].

Ten studies reported on baseline plasma serotonin levels in relation to treatment response. Seven of these studies investigated SSRIs. Three of the SSRI studies reported an association between higher baseline plasma serotonin and better treatment response [144, 176, 177], one small study reported the opposite association [164] and three studies reported no association [143, 165, 178]. Three

studies investigated the effect of baseline plasma serotonin levels and non-SSRIs (phenelzine, brofaromine, tianeptine and desvenlafaxine XR); none of these studies found any association between baseline plasma serotonin levels and treatment response [172, 174, 177], just as no association was found in one study that reported on several antidepressant treatments and ECT treatment together [179].

Six studies reported on baseline serum serotonin levels in relation to treatment response. Five of these studies used SSRI medications. One of these studies reported an association between higher baseline serotonin levels and better treatment outcome [180]; the other four studies found no association [167, 178, 181, 182]. One study investigated the effect of the non-SSRI medication tianeptine and reported no association between serotonin levels in serum and treatment response [167]. Finally, one study investigated several different antidepressant medications separately or in combinations and found an association between higher baseline serum serotonin levels and better treatment outcome [183, 184].

Only one study investigated serotonin levels in whole blood and reported no association between baseline serotonin levels and treatment response with fluoxetine treatment [185].

In conclusion, no studies found an association between baseline serotonin levels and treatment response to non-SSRIs. Higher baseline serotonin was associated with better response in several SSRI studies, including several larger and higher-quality studies. Other studies found no association between baseline serotonin levels, or in a few cases a negative association between higher baseline serotonin and SSRI treatment response.

Changes in serotonin concentrations with treatment

A majority of the included studies also reported on the change in serotonin levels after treatment regardless of treatment response. All studies that reported results on SSRI treatment separately from other treatments reported a treatment-associated decrease in serotonin concentrations in all investigated blood components [143, 144, 161-163, 165-171, 175-178, 181, 182, 185]. A reduction of serotonin levels in plasma was also reported for patients receiving different types of antidepressants and ECT reported together [179] and following treatment with the non-SSRIs amitriptyline or clovoxamine in platelets [175], desvenlafaxine in plasma [177] and vortioxetine in platelets [161]. In contrast, a treatment-associated increase in serotonin was reported for the non-SSRIs brofaromine and phenelzine in both plasma and platelets [172] and for moclobemide in platelets [166]. No significant treatment-associated change in serotonin levels were reported for the non-SSRIs maprotiline, nefazodone or viloxazine in platelets [166, 168, 173, 175], or for tianeptine in platelets, plasma and serum [167, 174].

Some of the studies also reported how treatment-associated changes in serotonin levels related to treatment outcome. Four studies found an association between larger decreases in serotonin levels and better treatment outcome in patients treated

with SSRIs [144, 160, 161, 182]. Contrary to these results, one study reported that treatment with the non-SSRIs brofaromine and phenelzine significantly increased plasma serotonin, more so in responders than in non-responders [172]. The remaining studies found no association between treatment response and change in serotonin levels during treatment with SSRIs [143, 170, 177, 181] or non-SSRIs [161, 174, 177].

Quality assessment

Eleven out of the 26 studies were classified as having a high risk of bias (a quality assessment total score of <1; i.e. low quality). Fifteen studies were classified as having a moderate to low risk of bias (a quality assessment score of 1 to 7; i.e. average to high quality). See Figure 9.

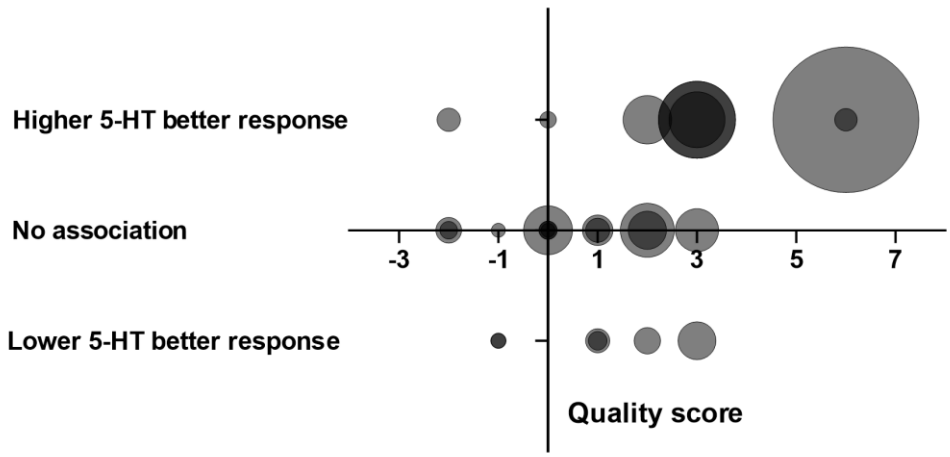

Figure 9. Bubble chart of the quality score and cohort size of the 26 studies.
Shown here in relation to the reported association between baseline serotonin levels and treatment outcome. Some studies with identical quality score overlap resulting in bubbles of darker colours. 5-HT: 5-hydroxytryptamine (serotonin). From "Peripheral serotonin levels as a predictor of antidepressant treatment response: A systematic review" by Holck et al [160].

Papers III and IV

Paper III investigated the association of the polymorphic CYP2D6 enzyme and adverse drug reactions in patients treated for depression.

Paper IV investigated the association of the polymorphic CYP2D6 enzyme and a history of suicide attempts in the same cohort of depressed patients.

Materials and methods

Recruitment procedure and participants

Papers III and IV were based on the GEN-DS study (Genetics, Depression and Suicidality). The GEN-DS project was carried out for ten years between January 2012 and December 2021. Patients were recruited from psychiatric clinics in Region Skåne, Sweden. They were referred to the study by their treating physician based on the referring physician's clinical assessment of the patient as suffering from depression with insufficient treatment response. In this case, depression with insufficient treatment response was defined as the patient being non-responsive to the current and previous treatment attempts of the current depressive episode. Depression with insufficient treatment response included both unipolar depression (MDD with one or recurring episodes, chronic depression or dysthymia) and bipolar depression.

Other inclusion criteria were that all patients were required to be 18 years or older. Exclusion criteria were current liver disease, BMI < 15 and pregnancy.

Diagnostic interview

Semi-structured interview

All recruited patients were subjected to an in-depth, semi-structured interview by a certified psychiatrist or a resident in psychiatry under the supervision of a senior colleague. The interview included questions on current and previous treatments including questions of treatment effect and ADRs of pharmacological treatment and psychotherapy; current somatic illness; family history; social history; childhood

experiences; traumatic events; alcohol and drug use; psychiatric history; and previous self-harm and suicide attempts.

Diagnostic interviews

The interview also included the structured diagnostic interview Mini International Psychiatric Interview (MINI) 6.0 [186] and the Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II) [187]. Patients were diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders 4th ed. (DSM-IV-TR) [188], the version of the DSM that was currently in use when the study was designed. The interview also included the Comprehensive Psychopathological Rating Scale (CPRS) [189], from which the Montgomery-Åsberg Depression Rating scale (MADRS) [190] was extracted.

Assessment of previous suicide attempts

Participants were interviewed about dates and methods of previous suicide attempts, if any were reported. Beck's Suicide Intent Scale (SIS) [191] was used to characterize the suicide attempt that was judged to be the most severe by the participant. A suicide attempt was defined as a non-fatal, self-directed, potentially injurious behaviour with any intent to die as a result of the behaviour, that may or may not have resulted in injury [192]. Suicide attempts were classified as violent or non-violent according to the criteria proposed by Åsberg et al [193], which defines violent attempts as hanging, the use of firearms, jumping from heights, several deep cuts, car crash, burning, gas poisoning, drowning, electrocution and jumping under a train; non-violent suicide attempt is defined as drug overdose.

Self-rating scales

The recruited patients also finished the self-rating scales UKU Side Effect Rating Scale (Udvalgd før Kliniske Undersøgelser) [194], Coping Orientation of Problem Experience (COPE) inventory [195] and Suicide Assessment Scale (SUAS) [196].

UKU Side Effect Rating Scale

The UKU Side Effect Rating Scale [194] consists of 42 questions for males and 45 questions for females, and requires participants to assess possible ADRs in the four domains psychological, neurologic, autonomic and other (including sexual) ADRs. The ADRs are rated as none, mild, moderate or severe (corresponding to a score of 0, 1, 2 or 3). The scale has been validated for use in psychiatric samples [197].

For the use in Study III, sexual ADRs were omitted from the overall statistical analyses since the number of scale items varied between males and females. Sexual

ADRs were analysed separately for each sex. Without these items, a total score with a possible range from 0 to 117 was calculated for each participant.

Blood sampling and genotyping

Blood samples were drawn in the morning at 08:00 on the day of the study visit. The participants were instructed to fast for four hours and to avoid nicotine and medications in the morning before the blood sampling.

Blood samples were also analysed for standard clinical blood tests and for serum concentrations of any antidepressant, antipsychotic and mood-stabilizing drugs. Participants were genotyped for CYP2D6 and CYP2C19.

CYP2D6 genotyping

For details of the genetic analysis, see Paper III [198]. In short, the *CYP2D6* single nucleotide polymorphisms (SNPs) **3*, **4*, **6* and **41* were analysed. *CYP2D6* copy number variation was determined to identify gene deletion (**5*) or multiple gene copies. The wild-type allele (**1*) was assigned when none of the SNPs above were identified.

Genotype to phenotype conversion of CYP2D6

The conversion of CYP2D6 genotype into phenotype was performed using activity scores according to the consensus recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group [199]. Alleles with no activity (**3*, **4*, **5* and **6*) were scored as 0; alleles with reduced activity (**41*) were scored as 0.5; alleles with normal activity (**1*) were scored as 1; and **1* alleles with copy number variation were scored as > 1. Each individual's activity score was calculated by adding the score of the two alleles and then converted into predicted CYP2D6 phenotype according to the criterion in Table 7.

Table 7. CYP2D6 phenotype based on activity score.

ACTIVITY SCORE	CYP2D6 PHENOTYPE
0	PM; poor metabolizer
0.25-1	IM; intermediate metabolizer
1.25-2.25	NM; normal metabolizer
>2.25	UM; ultrarapid metabolizer

Adjustment of predicted CYP2D6 phenotype according to phenoconversion (paper III)

For Paper III, CYP2D6 phenotypes as calculated by activity score were adjusted by comedication with CYP2D6 inhibitors. An inhibition factor model proposed in previous studies was used [200, 201], where moderate inhibitors were assigned an inhibition factor of 0.5 and strong inhibitors were assigned an inhibitor factor of 0. The participant's activity score was multiplied by the inhibitor factor to calculate the adjusted activity score, which was in turn converted into an adjusted predicted phenotype according to Table 7. For example, a previous NM with an activity score of 2 who received a strong inhibitor was assigned the adjusted activity score of 2 x 0 = 0, and the adjusted CYP2D6 phenotype of PM.

Drug classification

Drugs classified as CYP2D6 substrates with a clinical annotation level of evidence of 1 or 2 on PharmGKB.com [132, 134, 135] were reported as CYP2D6 substrates. The Flockhart Cytochrome P450 Drug-Drug Interaction Table [133] was used as reference of drugs with moderate to strong levels of CYP2D6 inhibition.

Statistics

Paper III

Total UKU scores and UKU scores in different symptom domains were compared between CYP2D6 phenotypes and adjusted phenotypes using the Kruskal-Wallis *H* test. Means between two groups were compared using the Student *t*-test. To investigate what other factors might be associated with a higher UKU score, a linear regression model was created. All reported *P* values are two-sided, and a *P* value of < 0.05 was considered statistically significant.

Paper IV

Pearson's chi-square test was used to compare proportions, and for sample sizes less than five Fisher's exact test was used. The Mann–Whitney *U* test was used for comparison of age and the Student *t*-test for comparison of MADRS scores.

Results

A flowchart of the recruitment process from the GEN-DS study, including dropout, is shown in Figure 10. A total of 415 patients were included. Genotyping data was available for 407 patients. For demographics, psychiatric diagnoses, history of

suicide attempt and number of current medications see Table 8. The distribution of CYP2D6 phenotypes is shown in Table 9.

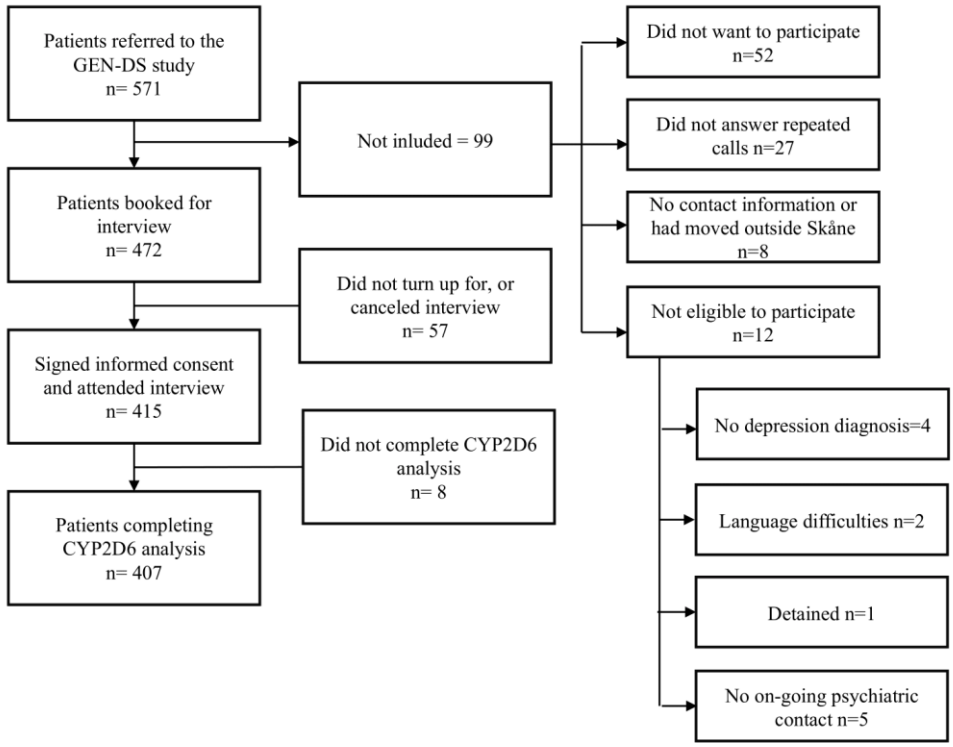

Figure 10. Flow chart of the GEN-DS study.
Reprinted with permission from Marie Asp.

Table 8. Demographics of the patients included in the GEN-DS study (n = 415).

	ALL PATIENTS
Age years	
Mean (SD)	37.6 (13.4)
Range	18-77
Sex n (%)	
Female	273 (65.8)
Male	142 (34.2)
MADRS score mean (±SD)	21.4 (8.5)
Personality disorder n (%)	170 (41.0)
Anxiety disorder n (%)	247 (59.5)
Somatic comorbidity n (%)	270 (65.1)
Bipolar depression n (%)	35 (8.4)
Dysthymia/chronic depression n (%)	206 (49.6)
Previous suicide attempt n (%)	132 (31.8)
Number of current medications mean (±SD)	3.0 (2.1)

Table 9. CYP2D6 genotype in the GEN-DS study.

GENOTYPE	NUMBER OF PATIENTS	PERCENTAGE OF PATIENTS
PM	33	8.1 %
IM	165	40.5 %
NM	198	48.6 %
UM	11	2.7 %

Errata: In the paper "CYP2D6 Genotyping and Inhibition as Predictors of Adverse Drug Reactions in Depressive Disorders" by Holck et al [198], the numbers of patients with specific genotypes were accidentally divided by total number of patients (415) instead of number of patients with available genotyping result (407), leading to different percentages in this table and in the published paper.

Paper III

Out of the 415 patients, 164 were prescribed one or more drugs metabolized by the CYP2D6 enzyme. In 147 of these patients UKU scale and genotyping results were also available.

There were no statistically significant differences between the CYP2D6 phenotypes and overall ADR burden or in any specific symptom domain, see Table 10. There were no apparent differences in UKU scores in the sex-specific items.

Table 10. Median values of UKU score for patients receiving CYP2D6 substrates (n = 147).

	PM (N = 13)	IM (N = 51)	NM (N = 78)	UM (N = 5)	*P* VALUE
Total number[a] median (min-max)	35 (20-62)	35 (4-59)	32 (4-79)	50 (4-54)	0.86
Psychological median (min-max)	16 (7-28)	15 (1-26)	15 (0-27)	8 (2-22)	0.72
Neurological median (min-max)	6 (2-16)	6 (0-18)	7 (0-22)	11 (0-20)	0.99
Autonomic median (min-max)	8 (3-15)	7 (0-18)	6.5 (0-20)	9 (0-16)	0.55
Other[a] median (min-max)	5 (1-11)	4 (0-10)	4 (0-11)	6 (0-13)	0.74

[a]Sex-specific adverse drug reactions not included.

Out of the 147 patients that received treatment with CYP2D6 substrates, 34 patients also received treatment with at least one moderate or strong CYP2D6 inhibitor. There was no statistically significant difference in total UKU score between these patients (mean = 35.9, SD = 15.0) and the patients that did not receive CYP2D6 inhibitors (mean = 32.5, SD = 14.9, $P = 0.27$). There was no difference in total UKU score or in any of the specific symptom domains between the different CYP2D6 phenotypes in this subgroup. When using the adjusted CYP2D6 phenotype, there was only a significant difference in score of "other" ADRs on the UKU scale ($P = 0.044$) with UMs reporting lower median values than the other phenotypes; however, this result did not reach statistical significance when a sensitivity analysis was performed ($P = 0.076$).

An explorative linear regression model indicated that the variable MADRS score made the most significant contribution to the model in predicting total UKU score, followed by female sex and somatic illness (see Table 11).

Table 11. Linear regression model of total UKU score[a].

	B	95 % CI	β	P VALUE
Sex[b]	4.63	[1.77 to 7.49]	0.148	0.002
Number of medications	0.832	[0.110 to 1.55]	0.114	0.024
Age (y)	0.072	[-0.037 to 0.181]	0.064	0.19
Somatic illness	4.50	[1.49 to 7.51]	0.144	0.004
MADRS score	0.724	[0.556 to 0.892]	0.417	<0.001
Any anxiety disorder	1.15	[-1.70 to 3.99]	0.038	0.43
Any personality disorder	1.02	[-1.85 to 3.89]	0.034	0.49

[a] $R^2 = 0.30$.
[b] 0 = male; 1 = female.

Paper IV

Suicide attempters had a higher MADRS score than non-attempters (see Table 12). Bipolar disorder, personality disorder, substance use disorder and lifetime presence of psychotic symptoms were more common among suicide attempters.

Table 12. Patient characteristics of the 407 patients with genotyping results divided by suicide attempters/non-suicide attempters.

	SUICIDE ATTEMPTERS (N = 128)	NON-SUICIDE ATTEMPTERS (N = 279)	*P* VALUE
Gender, men/women, n (%)	34/94 (27%/73%)	105/174 (38%/62%)	n.s.
Age, median (min–max)	36 (18–76)	36 (18–77)	n.s.
Total MADRS score, mean ± SD	23±8	21±9	< 0.05
Unipolar depression	104 (81%)	242 (87%)	n.s.
Bipolar disorder	23 (18%)	29 (10%)	< 0.05
Lifetime presence of psychotic symptoms, n (%)	17 (13%)	9 (3%)	<0.0001
Any comorbid anxiety disorder	78 (61%)	164 (59%)	n.s.
Any comorbid personality disorder	70 (55%)	95 (34%)	< 0.0001
Any comorbid substance use disorder	16 (13%)	11 (4%)	< 0.001

There was no significant difference in the occurrence of suicide attempts for patients with CYP2D6 UM phenotype compared to the other CYP2D6 phenotypes ($P = 0.27$, Fisher's exact test). See Figure 11.

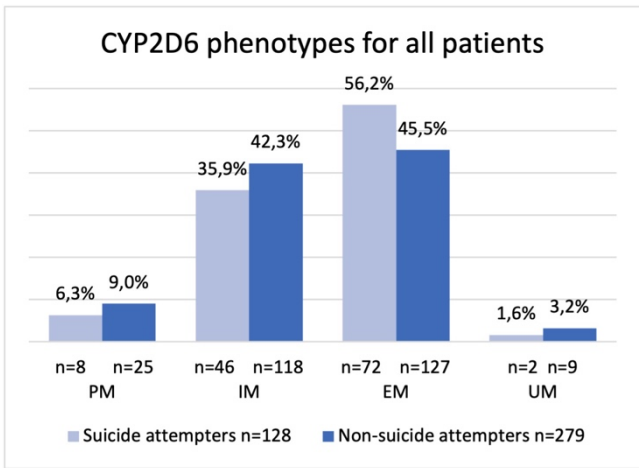

Figure 11. CYP2D6 phenotypes in suicide attempters and non-suicide attempters.
From "CYP2D6 UM phenotype is not related to suicide attempts in depressive patients in secondary psychiatric care" by Asp et al [202].

Of the suicide attempters, 28 (22 %) used a violent method. Both UM suicide attempters used violent methods. The majority of all patients had multiple earlier pharmacological treatments. However, the two UM patients with previous suicide attempts had received no pharmacologic treatments prior to their current medications, none of which were CYP2D6-dependent.

Ethical considerations

The data for paper I was collected in San Francisco, USA. The research was approved by the Committee on Human Research of the University of California, San Francisco.

As the study was performed in another country than Sweden, there are some other ethical aspects that may need to be considered. Patients were recruited to the study by flyers, bulletin board notices, Craigslist postings, newspaper ads and clinical referrals. As healthcare is not subsidized in at all the same way in the United States as it is in Sweden, it is possible that inclusion in this study was the only way for some of the patients to get medical treatment of depression if they did not have insurance that covered it or could not afford to get the medical attention in another way. After the completion of the study, treatment options for the participants were discussed, including whether they were recommended to continue with the medication or try another medication. The participants were offered an additional one to two months of free medication under supervision until they were able to locate a personal psychiatrist or physician. Despite this additional help, it is possible that some patients were forced to discontinue effective treatment due to financial reasons unbeknownst to the research team.

Paper II did not require ethical approval as it was a systematic review.

Papers III and IV were based on data from the GEN-DS study. Patients in the GEN-DS study were provided with oral and written information and gave their written informed consent. The principles of the Declaration of Helsinki were followed, and the study was approved by the Regional Ethical Review Board in Lund, Sweden (dnr 2011/673).

The patients in the GEN-DS study were subjected to in-depth interviews including questions about traumatic events, drug abuse, self-harm and suicidality. This kind of interview may give rise to feelings and questions that could not be thoroughly addressed within the study, as patients only had one session (or sometimes two) with a researcher. This may be seen as unethical; however, all patients had access to standard psychiatric care as well that they were able to contact if necessary. If a clear need arose during the interview, the patients were guided to appropriate care. During recruitment of patients to the study, it also became apparent that referring doctors sometimes motivated the patients to volunteer to the study because the study included an evaluation of psychiatric syndromes such as personality disorders, for which patients sometimes faced long waiting times in their ordinary care. This question was addressed with referring doctors, as it would be

unethical if patients were not offered the same care regardless of their inclusion in a study.

Research on potential biomarkers also raises several general ethical questions. If genetic results from studies are included in the patients' files, this could potentially affect their care in the future. For biomarkers such as CYP2D6 this should hopefully only be a positive thing and may facilitate future drug prescribing; however, research into how different biomarkers can be used is ongoing, and it is possible that new areas of use that we cannot currently predict are discovered in the future.

Discussion

The possible role of serotonin in predicting treatment response in MDD

Peripheral serotonin and response to antidepressant medications

Paper I shows that, in a small cohort of patients suffering from MDD, response to the SSRI sertraline was associated with higher baseline plasma serotonin. Serotonin levels were nominally lower in MDD subjects than in healthy controls, but the result did not reach statistical significance.

Paper II was a systematic review of published papers on peripheral serotonin levels before antidepressant treatment of a depressive episode and its association with treatment outcome. Previous studies have reported both positive, negative and null associations between baseline serotonin levels and antidepressant treatment outcome. To the best of our knowledge, this is the first attempt to systematically summarize the current knowledge about this subject. The results indicated that there was no association between baseline peripheral serotonin levels and non-SSRI antidepressants. For SSRIs the results were inconclusive. However, several larger studies with lower risk of bias reported that higher baseline serotonin levels were associated with greater antidepressant response to SSRIs.

The results from papers I and II also both showed that SSRI treatment was associated with a decrease in peripheral serotonin levels regardless of treatment outcome, which is in line with previous studies [60]. In paper I, the decrease was significantly more pronounced among responders than among non-responders; the results among the studies included in paper II were mixed. In paper I, the lowering of plasma serotonin was reported at 8 weeks. In paper II, 19 studies reported decreased serotonin levels after antidepressant treatment from two to 24 weeks. Based on these results, it seems that the lowering of serotonin levels by SSRI treatment may last for at least several months with continued treatment. It is unknown how serotonin levels change with chronic treatment or with discontinued treatment. A caveat is that although most previous studies, including the findings from papers I and II, suggest that peripheral serotonin levels decrease with SSRI treatment, a few studies have instead reported increases [203-205]. Paper II was not

designed with this question as a primary outcome and will likely not have included all relevant studies.

It is important to remember that antidepressant medications exert their effect in the CNS, and peripheral serotonin levels do not fully mirror central serotonin levels; however, there is evidence of correlations between peripheral and central serotonin levels [56, 57]. Also, previous studies have reported a high agreement between the levels of platelet serotonin content reduction and assessment of SERT occupancy in the brain using neuroimaging during treatment with SSRIs and SNRIs [60].

The results from papers I and II indicate that higher peripheral serotonin levels may be associated with better treatment outcome to SSRIs, although results are inconclusive. In summary, while it is not possible to draw any firm conclusions from these results, future studies to investigate if baseline serotonin levels could be used to predict SSRI treatment response may be merited.

Methodological considerations (papers I and II)

Paper I was based on a small sample of patients. However, it also had several strengths as the sample was well-defined, a wash-out of previous medications by at least six weeks was required, and all patients were prescribed the same medication. Also, a healthy control group was included which enabled us to compare serotonin levels to those of undepressed patients. The study also measured tryptophan levels as a possible confounding factor but found that tryptophan levels in plasma were similar in responders and non-responders.

In paper I, serotonin was measured in plasma. Paper II included results from studies that measured serotonin in platelets, serum and whole blood as well as in plasma. Platelet serotonin has been suggested as the most reliable blood component for serotonin analysis, but there is no clear consensus as to whether other blood components are unsuitable for serotonin analysis. Certain studies have however suggested that results from studies of serotonin in platelet-poor plasma may be discrepant, as serotonin levels in plasma are very low and may be contaminated by platelet-derived serotonin [48, 58, 59]. Factors such as food intake and differences in methodology have also been suggested to affect serotonin levels in plasma, and concentrations may be more difficult to compare between studies [56]. In paper II, serotonin analysis in different blood components did not lead to markedly different results when analysing the association between serotonin levels and treatment outcome.

Paper II included some studies that were very small and likely to have been underpowered. Most of the included studies were not RCTs. Some of the included studies were exploratory in nature and examined several possible biomarkers for treatment response without adjusting their results accordingly. Many studies did not control for confounding factors. While most of the included studies had a wash-out period for antidepressant drugs, the length of the wash-out period varied greatly and could be as short as three days. Since our results imply that treatment with SSRIs

may lower peripheral serotonin levels, it is possible that no or short wash-out periods could have led to lower baseline serotonin levels and confounded the results.

Paper II included studies that investigated the association between peripheral serotonin levels and antidepressant response to treatment if the study classified it as possessing antidepressant properties; this led to certain studies being included that reported on the effect of drugs that have not been approved as antidepressive agents (brofaromine and clovoxamine). This might have contributed to negative results.

Although these limitations of the included studies may have influenced the outcome of the systematic review, we deliberately chose to use broad inclusion criteria. Since no previous attempt had been made to systematically summarize research on peripheral serotonin levels as a predictor of antidepressant response, our aim was to obtain a comprehensive overview of the entire field. However, due to the heterogeneity of included studies it was not possible to perform a meta-analysis.

Hypotheses on SSRI effects

It is interesting to speculate on what the results from studies I and II may say about the mechanisms of SSRI action. Responders to SSRI treatment may have higher baseline serotonin levels, and SSRI treatment seems to lower serotonin levels during at least the first few weeks of treatment [144, 160, 176]. Previous theories about low brain serotonin levels being an explanation of the pathophysiology of depression have been debunked and previous positive results are likely to be influenced by the effect of prior antidepressant treatment on the serotonin system [74]. Although it is uncertain whether serotonin is involved in the pathogenesis of depression, it is reasonable to assume that serotonin may modulate depressive symptoms as antidepressant medications with serotonergic effect are effective in a percentage of patients. It has been suggested that serotonin plays an essential role in complex brain systems that are affected in MDD and in response to antidepressant treatment [206]. Systems proposed to be modulated by serotonin include negative affective bias, motivation, decision-making, and the cognitive appraisal of positive and negative experiences, and serotonin may mediate therapeutic response in depression through systems like these regardless of pathophysiology [206]. Other psychiatric treatments such as rTMS (repetitive transcranial magnetic stimulation) and ECT have also been suggested to exert part of their clinical effect through effects on the serotonergic system [207-209].

Our results from paper I implicate that higher serotonin levels may be associated with better treatment response to sertraline. If the serotonin system is indeed involved in antidepressant response, this may imply that a certain level of activity in the serotonergic system is necessary for SSRIs to be able to exert their effects. If there is not enough serotonin in the synapses, the blockade of SERT may not lead to a clinically relevant increase of available serotonin.

While interesting to speculate about, the biological effects of SSRIs are likely to be complex and involve many different systems in the CNS. Even though SSRIs are

presumed to exert their effect mainly through the serotonergic system, there is evidence of interplay with the HPA axis and inflammatory systems [210-212]. Neuroplastic effects through down-stream effects of compounds such as brain-derived neurotrophic factor (BDNF) and glutamate have also been proposed [93, 96, 98].

CYP2D6 as a potential biomarker

CYP2D6 and ADRS

The GEN-DS study was performed in a naturalistic setting where the patients were already being treated with various psychotropic medications. Many of the patients reported several ADRs from different symptom domains. Paper III did not find evidence of an effect of the different CYP2D6 phenotypes on reported ADRs in patients with depressive disorders, neither by genetic prediction nor by phenoconversion by comedication with CYP2D6 inhibiting drugs.

The results from paper III were mainly in line with previous research; several studies have been performed that did not find that CYP2D6 gentoype predicted ADRs in patients with pharmacological treatment of depression [213-215]. However, other studies have reported an overrepresentation of patients with the PM phenotype in patients referred to a study because of ADRs or non-response to CYP2D6-dependent antidepressants [216] and a higher risk of ADRs among PMs and IMs [217].

The investigation into phenoconversion in paper III is a strength, as a systematic review that have reported inconclusive results from previous studies have suggested interactions with comedication as a possible explanation for the inconsistent results [113]. Most previous studies have not taken the risk of phenoconversion into account. The few studies that have examined phenoconversion for CYP2D6 in treatment of depression have reported high frequencies of phenoconversion into PMs or increased drug levels [218, 219], but have generally not found any certain clinical effect [201]. This is not altogether unexpected, as many frequently used antidepressant and antipsychotic medications have wide therapeutic ranges where the effect is not typically dependent on the blood drug concentration [123, 142].

When considering our results from paper III together with the previous literature, it does not seem that routine CYP2D6 testing for patients that are treated with psychotropic medications and experience ADRs is warranted in naturalistic cohorts of patients with depressive disorders and inadequate treatment response. We did not find that IM patients reported more ADRs than EMs as was our hypothesis, and the extreme genotypes that are most likely to lead to high drug concentrations or decreased effect (PMs and UMs, respectively) have relatively low frequencies in Sweden [121]. Before CYP2D6 testing is performed, other factors such as

depressive symptom burden and somatic illness may need to be considered, as these were factors that were found to contribute to higher scores on the UKU scale in the linear regression model that was performed. Previous literature also supports the notion that ADRs may be more related to the severity of depressed mood in patients than to antidepressant dose [220]. Somatic examinations are also relevant, as symptoms of other medical conditions may be mistaken for ADRs; the same is true for symptoms of the depressive episode itself. Many other aspects may contribute to the experienced treatment difficulty, which is exemplified by the high frequency of personality disorders that were largely undiagnosed before inclusion in the GEN-DS study.

However, the results from paper III should not be interpreted to mean that genetic testing of CYP2D6 is not relevant. There are many different situations where CYP2D6 testing may be pertinent, such as with severe ADRs or repeated complaints of ADRs with multiple medications that are metabolized through the CYP2D6 system. However, it may be reasonable to suspend genotyping until the patient has experienced ADRs or a lack of treatment effect specifically when starting treatment with a CYP2D6-dependent drug and not to test all patients pre-emptively.

CYP2D6 and suicide attempts

In paper IV, we hypothesized that the CYP2D6 UM phenotype would be overrepresented in patients with treatment-resistant depression and a history of one or more previous suicide attempts as compared to in patients without a previous suicide attempt. We investigated this in the GEN-DS cohort but did not find an overrepresentation of CYP2D6 UMs among suicide attempters.

Women were overrepresented both among suicide attempters and among suicide non-attempters, which is excepted as women are overrepresented in psychiatric care in general and suicide attempts are more common among women [38, 39]. Higher MADRS score, bipolar disorder, personality disorders, substance use disorders and lifetime presence of psychotic symptoms were significantly more common among suicide attempters. Bipolar disorder, personality disorder and substance use disorders are all known risk factors of suicide [221].

Paper IV focused on a cohort of treatment-resistant depressive patients in secondary psychiatric care, and the results may not be generalizable to depressive disorders in general. However, other studies have investigated the genetic variation of CYP2D6 and suicide attempts or suicidal ideation, and have reported a higher frequency of UM phenotype in eating disorder patients with a lifetime history of suicide attempt [127], in patients with higher scores on the Suicidal Intent Scale after suicide attempts [128] and in patients with higher suicidality scores in the Mini International Neuropsychiatric Interview (MINI) [222]. However, there are also previous studies that have not found any association between the UM phenotype and suicide attempts [129, 131].

The hypothesis that CYP2D6 UMs would be overrepresented among suicide attempters was based on a Swedish sample of suicide completers where the UM phenotype was ten times more common than among patients who died of natural causes [125]. The study by Zackrisson et al [125] reported that the UM phenotype was overrepresented among suicide completers excepting intoxication cases. Intoxication cases were excluded due to the difficulty of drawing conclusions from results of postmortem toxicology. The assessment that a death is caused by a drug intoxication is based on the entire death investigation and not only the analytical results, as other causes of death first need to be excluded, and it is often not possible to assess whether a lethal intoxication was intentional or not [125]. This means that the suicide case cohort consisted of patients who had committed suicide through violent means. In the GEN-DS cohort, only 22 % of suicide attempters had ever used a violent method (hanging, strangling/suffocation, cutting, jumping from a height or other), and a majority had made suicide attempts through intoxication. Since suicide attempts and completed suicides have also been reported to be distinct groups [33], it is difficult to compare results from paper IV with the study by Zackrisson et al.

Hypotheses as to why the CYP2D6 UM phenotype should be overrepresented among suicide attempters have mainly focused on the increased metabolic activity of the enzyme. UMs may be more likely not to reach the desired therapeutic concentrations of prescribed medications and thus not be effectively treated [125]. In paper IV, only two patients with a history of one or more suicide attempt were CYP2D6 UMs. Neither of these patients had received any pharmacological treatment that was CYP2D6 dependent, which excluded ultrarapid metabolism of drugs due to their CYP2D6 phenotype as an explanation of treatment resistance. Another previous hypothesis has been based on reports that CYP2D6 is expressed in the brain and affect personality traits [223, 224].

In conclusion, the results from paper IV together with the existing literature do not lend any strong support to future studies of CYP2D6 genotype and suicide attempts. It is unlikely that the expression of a single gene would have a significant impact on suicidality. Continued research in the field of CYP2D6 and completed suicides due to lack of treatment effect may show more promise.

Methodological considerations (papers III and IV)

Papers III and IV were based on the GEN-DS cohort, a cross-sectional, naturalistic study of patients with treatment-resistant depression in psychiatric secondary care. Patients were thoroughly interviewed and rigorously assessed and diagnosed with regard to both depressive disorders and comorbid disorders such as anxiety syndromes and personality disorders. Information about ongoing and previous treatments was compiled from patient journals and the interview with the patient.

Paper III focused on the genetic variation of CYP2D6 and ADRs. As with all reporting of ADRs, it is possible that patients mistook symptoms from somatic or

psychiatric illnesses as side effects of their current medications. Many reported drugs were metabolized through other pathways than through the CYP2D6 enzyme, and ADRs from these drugs may have influenced the results.

ADRs were measured through the patient-rated UKU scale [194]. While this scale has been validated for use in psychiatric samples, is has also been stated that patients tend to report ADRs more frequently and rate symptoms as more severe than clinicians [197]. It is conceivable that this influenced the results and weakened a possible association between CYP2D6 phenotype and ADRs.

The naturalistic nature of the GEN-DS study leads to certain limitations but is also a strength as it in many ways mirrors the clinical reality. A potential limitation is that ADRs were assessed at a single time point, despite patients having received various medications for differing lengths of time, likely making it even harder for the patients to differentiate between ADRs and other symptoms. Drugs that were metabolized by CYP2D6 may already have been tested and discontinued due to ADRs or lack of effect before inclusion in the study. However, the sample was in many ways characteristic of patients in secondary psychiatric care, in that many of the patients were treated with several medications, had other diagnoses in addition to depressive disorders and that patients with varying degrees of suicidality were included.

More *CYP2D6* alleles have been discovered over the past few years, and new variants are continuously discovered. The alleles that were analysed in the GEN-DS study were the ones that were considered most relevant at the time, as it was not deemed possible to test for all known variants. The frequencies of the different alleles vary between different ethnic groups [119, 120]. However, our sample consisted mostly of patients with European lineage. Another caveat when comparing our results to other studies is that different classifications of the CYP2D6 phenotypes are sometimes used; we have used the classification recommended in current guidelines [199].

The power calculation for the GEN-DS study was based on the previous study by Zackrisson et al [125]. According to the power calculation, the GEN-DS study was planned to include 516 patients; however, due to the COVID-19 pandemic, we were prevented from recruiting patients into the study for a long time and were only able to include 415 patients. The power calculation was based on the presumption that UMs would be ten times more common in patients with a previous suicide attempt than in patients without suicide attempts in the sample. The UM phenotype is rare in Scandinavia, only occurring in about 1 % of the population [121]. The frequency was somewhat higher in our sample (2.7 %). However, as our results in paper IV showed that UMs were in fact only half as common among suicide attempters as among non-suicide attempters in our sample, it is highly unlikely that we would have been able to reject the null hypothesis even if we had been able to include 516 patients.

Biomarkers in psychiatry: Possibilities and limitations

Limitations of biomarkers in mood disorders

The search for biomarkers in psychiatry in general, and in mood disorders specifically, has proven more difficult than initially hoped for. Some reasons for this have been described as problems with the current diagnostic classifications, underpowered studies and methodological problems [225]. Even if a relevant biomarker is found, it needs to have very high specificity and sensitivity, in addition to being cost-effective and simple to implement, in order to be relevant for clinicians [225].

The heterogeneity of psychiatric disorders in general, and MDD in particular, is most likely one of the underlying reasons of the difficulties in finding biomarkers for these states [140]. It is unlikely that all of the different states that are currently grouped together as MDD, including melancholia, would have the same underlying biological alterations [226]. For example, the dexamethasone suppression test that was proposed for diagnosing melancholic depression in the 1980s and yielded promising initial results was later abandoned. The test showed that a high proportion of patients with affective disorders, melancholia in particular, had elevated cortisol levels that were not reactive to the suppressing effect of the synthetic glucocorticoid dexamethasone [227]. The inconclusive results from later studies have been suggested to be at least in part due to the implementation of the DSM-III criteria where the broad diagnosis MDD was introduced, which led to a large decrease in sensitivity for the test [228]. Also, patients with certain characteristics are frequently excluded from studies, such as MDD patients with an increased suicide risk or severely paranoid patients. Consequently, the collected data will commonly be from moderately ill patients, which is a limitation for the generalizability of findings to other, possibly more ill, patients [229].

Limitations of evaluating treatment response in mood disorders in research

Papers I and II investigated peripheral serotonin as a possible predictive biomarker for treatment response in depressive disorders. The evaluation of depressive symptoms is not necessarily as straightforward as may first be believed.

A challenge in depression research is how treatment effect is measured. Rating scales such as the HDRS [156] or MADRS [190] are commonly used. Fried (2017) investigated seven common depression scales and concluded that overlap among all scales was low, leading him to conclude that this may pose a threat to the replicability and generalizability of depression research [230]. Some scales, such as the HDRS, also include somatic symptoms that closely resemble adverse effects caused by common antidepressant treatments. It has been suggested that certain

items on rating scales such as depressed mood may better reflect changes due to antidepressant treatment than whole-scale comparisons [231]. The included studies in paper II used various different measurements of treatment outcome, although the use of the HDRS was the most common. Even when using the same rating scales, different definitions of response and remission may be used. The comparison of studies that have used different rating scales may influence results.

The results of studies on depressive disorders are inevitably dependent on which patients are included in the studies. Common exclusion criteria in antidepressant treatment studies include a history of manic or hypomanic episodes, suicide risk, alcohol or drug abuse, illness duration of less than four weeks or more than two years, presence of comorbid psychiatric disorders and presence of borderline personality disorder [232]. Zimmerman et al applied inclusion and exclusion criteria from 158 antidepressant efficacy trials to a large sample of psychiatric outpatients with a principal diagnosis of MDD [233]. They found that a high percentage of clinical MDD patients are likely to be excluded from this kind of study, ranging from 44.4 to 99.8% in the included studies. The conclusion of the authors was that the generalizability of results from studies is low. The STAR*D sample has also been analysed for comparisons between the part of the sample that would have been likely to be included in phase III clinical trials (22.2 %) and those that would likely have been excluded (77.8 %) [234]. The results showed that patients that might be included in phase III clinical trials were more likely to be younger, more educated, employed, married, have a shorter duration of illness and lower rates of previous suicide attempts. They were also less likely to have ADRs and had higher remission rates. While studies that are not performed by medical companies may have less strict exclusion criteria, there is still a risk that patients with more severe psychiatric symptoms are excluded from treatment trials that strive to find predictive biomarkers. This might decrease the chance of positive findings of biological anomalies, as these could be hypothesized to be more pronounced in patients with more severe symptoms. It may also decrease the generalizability of any positive findings to a population of patients in secondary psychiatric care. A strength of the GEN-DS study was that patients with severe symptomatology, including previous suicide attempts, psychotic symptoms and personality disorders, were included. Paper I primarily included patients from primary care level. The illness severity of the patients in the studies that were included in the systematic review (paper II) varied.

Another example is that the occurrence of personality disorder has been reported to be common in psychiatric samples, in most studies ranging from 40-60 % [235, 236]. In the GEN-DS material, where patients with treatment-resistant depression were included, 41 % fulfilled the criteria for any personality disorder. Most of these were not diagnosed prior to inclusion in the study. Most treatment studies do not evaluate patients for possible personality disorder. It has been reported that the occurrence of personality disorder leads to a poorer outcome in treatment of depression [237]. If the occurrence of a personality disorder in patients with

depressive disorders does influence treatment response, that would most likely also affect the association between treatment response and biological markers.

Since there are also many problems related to the overinclusion and heterogeneity of psychiatric diagnosis, other groupings of patients may be necessary to be able to find robust evidence of predictive biomarkers. Strategies that are sometimes already used include the Research Domain Criteria (RDoC) [238] and the Hierarchical Taxonomy of Psychopathology (HiTOP) [239], but new methods of classification may be necessary to increase the likelihood of clinical utility in the future.

These are just a few examples of factors that may influence the results of studies of possible predictive biomarkers; there are likely to be many more.

Pharmacogenomics

Previous studies as to the benefit and cost effectiveness of pharmacogenomics are inconclusive. For example, two similar systematic reviews and meta analyses that were performed in 2022 and 2023, respectively, analysed clinical trials that compared the use of PGx-guided antidepressant treatment and treatment as usual for MDD came to quite different conclusions [147, 148] (for details of the studies see background). While the authors of one of the studies concluded that their results suggested that PGx-guided antidepressant prescribers should expect modest but significant increases in depressive symptom remission [147], the other study reported that while the PGx-guided group showed an increased remission rate at week 8 and 12, the test-guided prescribing made no difference in final response or remission rates (week 24) [148]. However, while testing may not have improved long-term outcome as measured by remission status, a decreased time until remission may help reduce avoidable suffering, diminish ADRs and increase compliance.

There are examples of studies that report that the use of pharmacogenetic testing would lead to a reduction of health costs [149, 150] as well as studies that conclude that cost-effectiveness remain unclear [148]. Many of the clinical trials that have been performed are funded by the industry, which introduces the relevant question of bias [147, 148]. Many commercially marketed pharmacogenetic tests have not been evaluated at all in clinical trials, and include testing of genes whose contribution to response and side effects to antidepressants are mostly unknown [145, 147, 154].

A limitation in pharmacogenomics is the lack of consensus between laboratories on a minimum testing panel for different alleles with clinical validity for polymorphisms in genes such as *CYP2D6*, and on a genotype to phenotype translation (although consensus recommendations for standardizing the translations have been published, these are not always followed) [152, 199]. Polygenetic risk scores (PRS) have been proposed as another means to predict treatment response but have only been found to explain about 1.5 % of the variance of antidepressant efficacy [152].

In conclusion, while much research has been made into the possible benefits of pharmacogenetic testing to improve the results of drug treatment in depressive disorders, more research is still needed to ascertain the positive effects and elucidate in which situations testing should be performed. Studies that have investigated the effect of PGx guided prescribing typically only have a shorter follow-up, and longer follow-ups are necessary to provide stronger evidence of PGx impact on therapy management and outcomes [152]. Research also needs to investigate whether testing should be performed pre-emptively for all or certain patient groups, or if testing would be more appropriate as a reaction when a patient shows inadequate response or troublesome ADRs [145]. Limitations to the use of PGx testing in clinical settings currently also include the lacking education of physicians, who are usually not accustomed to the use. Support by a clinical pharmacist or a genetic counsellor may be necessary.

Biomarkers for suicidality and suicide attempts

Non-genetic biomarkers that have been implicated in suicidality include increased peripheral cortisol levels, lower tryptophan levels, endocannabinoid levels and increased CRP levels [240]. An association between lower plasma serotonin and current suicidal ideation has also been reported [241]. However, it is often unclear whether the abnormalities in biological tests are a cause or effect of suicidal behaviour. Another challenge is differentiating biomarkers specific to suicidality from those associated with psychiatric disorders, as suicidality is most common in people that suffer from disorders such as MDD. For example, in a recent study the authors used blood samples and the whole genome as a starting point to discover, validate and test possible biomarkers of suicidality; they reported that over 80 % of top biomarkers overlapped with genes implicated in alcoholism, depression and stress [242]. Also, treatment with psychotropic medications may influence levels of certain potential biomarkers for suicidality [243].

A problem with biomarkers in patients that have a history of one or more suicide attempt is that suicide attempts seem to be a different phenotype from completed suicides, and biomarkers in people with attempted suicide may not actually predict death by suicide [244]. This was also a limitation in paper IV, where we did not find an association between the CYP2D6 UM phenotype and previous suicide attempts, as the hypothesis was based on a cohort of suicide completers [125]. Another issue is that patients were included in the GEN-DS study at different ages and stages of their lives; some patients may go on to develop suicidal behaviours even if they had not done so at the time of the study, which also makes the exploration of CYP2D6 as a biomarker of “trait” suicidality difficult to interpret. However, an advantage with genetic markers such as CYP2D6 compared to other types of biomarkers is that genetic markers remain unchanged throughout a person’s lifetime and could not be attributable to biological changes due to suicidal behaviour.

Another problem with suicide prediction is that suicide is a rare event, which means that prediction models will always have a low positive predictive value as most people who are classified as having a high risk will not die by suicide [245].

In order to be implemented, biomarkers need to be clinically relevant and contribute to optimizing decision-making beyond clinical data; in cases where the information from the biomarker is likely to be overridden by clinicians on the basis of the patient's complaints and clinical presentation, it may not serve its purpose [140]. This aspect may be particularly relevant in biomarkers for suicidality. It is unlikely that a positive test for a biomarker associated with suicidality would lead to specific measures being taken if there is no other evidence of an increased suicide risk, especially considering the probable low positive predictive value. Conversely, a clinician would be obligated to act on clinical information about an increased suicide risk regardless of the result of a biological test. Unless the biomarker was related to a specific biological abnormality that could easily be treated, the clinical utility is not clear. The question of clinical utility seems to be mostly avoided in the literature about potential biomarkers for suicidal behaviour.

Conclusions

There is a great need for further knowledge about the biological systems that underpin psychiatric disorders and symptoms such as major depressive disorders and suicide attempts. There is also a lack of knowledge about which patients will respond to which antidepressant, and which patients will develop unmanageable adverse drugs reactions. Further research into potential biomarkers could be of great importance in all these areas.

However, as of today, there are still not any biomarkers of this kind that are routinely used in the clinic. Repeated studies have often failed to reproduce previous results, or the explanatory power of a certain biomarker is too low to be able to add any clinically relevant information. So far, there is more evidence for the use of CYP2D6 and CYP2C19 to help guide psychiatric prescription than for other biomarkers, but there remains an uncertainty as to which patients should be tested and when. Based on our results, there does not seem to be an association between predicted CYP2D6 phenotype and the number of reported ADRs among depressed patients with various different medications in a naturalistic setting. Serotonin is an interesting potential biomarker as higher baseline peripheral serotonin levels may be associated with treatment response to SSRIs, but more studies are needed as there is not convincing evidence of this association in published studies so far. Future studies would likely benefit from more standardized methodologies, and platelets would seem to be the most appropriate blood component for the analysis of serotonin.

While the rationale for further research into biomarkers of suicidal behaviour from a clinical perspective remains uncertain, biological suicide research is still important to advance the knowledge into biological systems that may contribute to suicidality in patients, and to possibly help reduce suicidality world-wide as a result. We did not find any overrepresentation of the CYP2D6 UM phenotype in patients with a history of suicide attempts in a cohort of patients with depressive disorders. Based on the current knowledge, the genetic variation of CYP2D6 does not seem to be a good candidate to predict suicide attempts.

In summary, from the papers included in this thesis we can conclude that the use of biomarkers to predict antidepressant treatment response, including ADRs and suicidality, is a complex research area that requires further study. Future studies may help decrease time until treatment response is achieved, reduce ADRs and increase the knowledge about biological correlates of pathophysiology and drug effects in depressive disorders and suicidality.

Clinical implications

As of today, there are no biomarkers that are routinely analysed in the clinic for the treatment or evaluation of depressive disorders. Pharmacogenetic testing of CYP2D6 and CYP2C19 are sometimes performed, although there is no clear consensus as to which patients should be tested or when. Even though international clinical guidelines have been published for adjustment of dosages of certain CYP2D6-dependent medications, the scientific base for this is still somewhat weak.

If the use of biomarkers should be implemented into psychiatric clinical care, medical staff need to be educated in pharmacogenetic testing to better be able to decide when testing may be relevant and how to interpret the results. There is currently also a lack of a consistent system for documentation of such testing, to ensure that test results are available to current and future prescribers.

Based on the results from paper III, there is no indication for routine CYP2D6 genotyping in patients with treatment-resistant depression and ADRs in the clinic. Testing should however be considered if patients experience severe ADRs or if there is a clear temporal connection between ADRs and the start of CYP2D6 dependent medication or CYP2D6 inhibiting drugs, and when TDM does not provide sufficient information. There is no indication for the use of CYP2D6, or any other putative biomarker, to help assist in suicide risk assessment as of today.

As described in paper I and II, further research into serotonin as a possible predictive biomarker of SSRI response may be warranted, but there are currently no clinical implications for the testing of peripheral serotonin concentrations.

Future perspectives

During the last decades, much research has been made into personalized psychiatry, precision psychiatry, and the prediction of treatment response to antidepressant treatment. Research in these areas is still very much ongoing.

However, when using biomarkers to predict treatment outcome is considered, future studies also need to evaluate how such test results are handled, and what the results of tests should lead to. For such prognostic biomarkers to be relevant, a test result that for example indicates low probability of response to SSRIs needs to lead to a suggestion of a different treatment approach. If future studies were able to produce robust evidence of an association between serotonin levels and treatment response, the next necessary step would be to produce research that stratifies patients to different treatments depending on test results and explore if this strategy leads to increased response and remission rates as compared to treatment as usual.

Current research has not been able to produce results of any single biomarker that can be used to predict treatment response in depressive disorders. It is possible that future research will need to focus on panels of putative biomarkers instead of single biomarkers to yield success. Even this may not be enough, and it may be necessary to combine clinical characteristics with tests such as genetic profiling, genomics and metabolomics results, structural and functional brain imaging and data from electronic health records. Digital tools such as mobile phone apps and information from wearable devices could also be used. This data could then be compared to large datasets from previously treated patients, and personalized clinical recommendations could be created through machine learning. Examples such as this are so far highly speculative, as the potential for machine learning in psychiatry has only just begun to be explored.

If future research could find biomarkers that help predict treatment response in depression, the clinical implications would be immense. Time and money would likely be saved, but most importantly, the suffering of psychiatric patients could be reduced. Instead of a trial-and-error approach, the right drug could be prescribed at the right dose the first time around.

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This thesis explores whether the treatment of depression with antidepressant drugs can be improved by analysing candidate biomarkers of treatment response, including adverse drug reactions and suicidality. The biomarkers examined are peripheral serotonin levels and genetic variation in CYP2D6 expression.

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