PHARMACOGENETIC STUDIES IN DEPRESSION:

A FOCUS ON THE SEROTONIN TRANSPORTER GENE

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Voor pap en mam

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1

Introduction

BACKGROUND

Depression is among the top 5 leading causes of disease burden worldwide [1] with a population prevalence of 2-19% [2]. By the year 2020, major depressive disorder is expected to rank second in disease burden measured in Disability-Adjusted Life Years (DALYS) [3]. A major depressive disorder is characterized by a depressed mood for most of the day or a loss of interest or pleasure in (almost) all daily activities for at least 2 weeks. Patients with major depression also experience the majority of the following symptoms nearly every day: significant weight loss or weight gain, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate, indecisiveness, recurrent thoughts of death, recurrent suicidal ideation or a suicide attempt or a specific plan for committing suicide. Depressed persons have greater mortality and impairment in many areas of functioning compared with nondepressed persons [4]. Annually, almost half of the depressive population in the Netherlands receives professional treatment, over 40% receive an antidepressant [5]. In 2004; 5.5 million antidepressant prescriptions were registered in the Netherlands, accounting for 167 million euros. The most frequently prescribed antidepressants belong to the group of selective serotonin reuptake inhibitors (SSRIs) that are responsible for 58% of the prescriptions [Stichting Farmaceutische Kengetallen].

The period until the effect of a treatment with SSRIs is established can take up to 6 weeks and in a large number of the patients, 30 – 40%, a sufficient response to therapy remains absent [6-9]. If an SSRI appears to be ineffective or only partly effective after this period, treatment is altered and another period of 6 weeks is initiated. Many patients are thus exposed to a relatively long period of trial and error before the appropriate antidepressive therapy is established. Within this time, patients are subject to possible antidepressant-induced adverse events and experience a continuous burden of the depression [10]. It would be highly desirable to identify these non-responders prior to initiating therapy in order to avoid prolonged and unnecessary exposure to depressive symptoms and complaints caused by SSRI-induced adverse events. Patients that are not likely to respond to SSRIs could benefit from a different antidepressant with a different pharmacological mechanism. A test to identify such patients could have a large impact on reducing health care costs for the treatment of depression and on improving the overall quality of life for patients with depression by enhancing response rates and minimizing adverse effects [6,11].

Individual variations in treatment response are thought to have, at least partly, a genetic base. Understanding this genetic base could eventually lead to a pharmacogenetic profile that can be used in the identification of patients who are likely to have a unfavourable response to a treatment. Several researchers have pointed out the great potential of pharmacogenetics in the treatment of depression given the lack of knowledge on biological predictors of treatment response and the absence of biologically based treatment guidelines [10,12].

In this introductory chapter we will first explain the role of the serotonergic system and of polymorphisms in the serotonin transporter in depression. We have focused on two polymorphisms in the serotonin transporter, 5-HTTLPR (also named SERTPR) and STin2. Current knowledge on these polymorphisms and their influence on response, adverse events and comorbidity will also be addressed in the introduction. Finally, the study aims and purpose of this thesis are described.

THE ROLE OF THE SEROTONERGIC SYSTEM IN DEPRESSION

The neurotransmitter serotonin (5-hydroxytryptamine or 5-HT) is known to regulate a range of diverse psychological, behavioural and biological functions [13] and is believed to play a crucial part in the pathogenesis of depression [14]. 5-HT is synthesized from the amino acid precursor tryptophan and released into the synaptic cleft following an action potential. Several different pre- and post-synaptic 5-HT receptor subtypes are involved in mediating the physiological actions after the release of 5-HT from the vesicles in the presynaptic neuron into the synaptic cleft [11]. Termination of the serotonergic neurotransmission is accomplished through the uptake of 5-HT from the synaptic cleft back into the presynaptic vesicles. The presynaptic 5-HT transporter (5-HTT of SERT) plays a critical role in this sodium-dependent reuptake of 5-HT and has therefore become the main target of most antidepressants [11,14,15]. In particular, the SSRIs were developed to selectively affect the 5-HT reuptake by inhibiting 5-HTT.

Biological characteristics of 5-HTTLPR and STin2

In 1993, the cDNA for the human 5-HTT was isolated [16,17]. 5-HTT is encoded by a single gene (SLC6A4) located on chromosome 17q11.1-17q12 [18,19]. Subsequently, several variations in the 5-HTT gene were described. One of these variations, a polymorphism in the promoter region of the gene (5-HTTLPR, also called SERTPR), has the potential to regulate the transcriptional activity of the 5-HTT gene promoter [20,21] and eventually the level of the functional transporter [17].

The 5-HTTLPR polymorphism is an insertion/deletion polymorphism consisting of four alleles with 14, 16, 18 or 20 repeated units of approximately 22 base pairs. The most common alleles have 14 or 16 repeated units, the 14 unit allele is therefore designated as the s (short) allele, all other alleles as l (long) alleles [6]. In cells homozygous for the l variant of 5-HTTLPR, the 5-HT uptake was found to be more than 2-fold higher compared to cells with one or two copies of the s variant [21].

A second variation in 5-HTT gene that has been described extensively, is a variable number of tandem repeats (VNTR) in the second intron (STin2). This polymorphism lies outside the coding region for 5-HTT but is thought to change a regulatory element of gene transcription [6,19,22].

The most common alleles of this polymorphism consist of 10 (STin2.10) or 12 (STin2.12) copies of a 17-base pair repetitive element [6] but alleles with 7 (STin2.7), 9 (STin2.9), 11 (STin2.11) copies have also been described [19,22,23]. The STin2.12 is therefore designated as the l (long) allele, all other are designated as the s (short) allele [6].

Current evidence on the association between 5-HTTLPR, STin2 and SSRI response
As SSRIs act directly on 5-HTT, the existence of variations at the 5-HTT locus could result in differences in treatment response. Polymorphisms in the 5-HTT gene are therefore considered as potential predictors for SSRI treatment response [17] and could be important factors in the development of biologically based guidelines for the treatment of depression.

Since the description of the 5-HTTLPR and STin2 polymorphisms, these genotypes have received a lot of attention from research groups attempting to identify predictors of SSRI non-response [17,24-33]. However, there is a great diversity in the methodology and reporting of the results of trials on drug-gene interactions. In addition, possibly due to methodological variation, results from these trials are inconsistent leaving the overall picture diffuse and the question on SSRI-genotype interaction unanswered. As we will show in this thesis, there is a need for methodological criteria for pharmacogenetic research as these studies often encounter specific problems. Since these problems have received little attention up till now, a discussion on pharmacogenetic methodology could be useful in the development of guidelines for studies on drug-gene interactions. Homogeneity in study designs will also facilitate comparability of study results and the inclusion of individual study results in meta-analyses and systematic reviews.

The serotonin transporter and SSRI-induced adverse events
Compared to the earlier antidepressants (such as TCAs), it was initially thought that SSRIs were almost free of adverse events due to their selectivity and to their absence of interaction with other receptors (such as histamine, cholinergic and dopaminergic) [34]. However, since SSRIs were first prescribed, this opinion has changed. A large number, around 75%, of the patients that are treated with SSRIs report one or more adverse events during treatment [35]. Adverse events are also an important reason for early discontinuation of SSRI treatment, previous studies have reported rates of early discontinuation up to 30% [36].

For the most part, occurrence of SSRI-induced adverse events can be attributed to serotonergic effects. 5-HT reuptake is inhibited by the SSRI leading to an increased interaction of the neurotransmitter with the serotonergic receptors or subtype receptors which mediate several functions such as sleep, appetite and sexual function [34]. The most frequently reported SSRI-induced adverse events include gastrointestinal disturbances, anxiety, agitation and insomnia [34]. During long-term SSRI treatment, the most troubling adverse events are sexual dysfunction, weight gain and sleep disturbance. However, the adverse events profile is not identical for all SSRIs, in general citalopram (Cipramil®) appears to be the best-tolerated SSRI and paroxetine (Seroxat®) and fluvoxamine (Fevarin®) are associated with the largest number of adverse events [34].

It is unknown why some patients experience these events whereas others do not. The ability to identify patients that are at greater risk for particular adverse events would allow clinicians to anticipate on these events in an early phase of treatment by adding prophylaxis against these effects or even change the prescribed SSRI [36].

The 5-HTTLPR genotype has previously been associated with SSRI-induced adverse events. For example, it has been associated with insomnia and agitation [36] as well as with disturbances in circadian rhythms and level of alertness [37]. In addition, it has been suggested that the genotype is related to early discontinuations caused by adverse events such as gastrointestinal complaints, fatigue, agitation, sweating and dizziness [37]. Patients with the s/s genotype are also thought to have a greater severity of adverse events [37]. However, the current evidence on the association between the 5-HTTLPR genotype is too limited to draw any definite conclusions at this moment and empirical evidence on the influence of STin2 on the occurrence of adverse events is absent.

The serotonin transporter and comorbidity in depression

About 50% - 80% of the persons suffering from major depressive disorder also report physical complaints [38-40]. The majority of these complaints, at least 60%, are pain-related [40,41]. It has previously been reported that there might be a genetic link between pain-related complaints and depression. Patients with chronic pain have more first-degree relatives with depression compared to the general population, even if these patients themselves had no history of depression [39]. Earlier studies report that subjects with depression are more than twice as likely to develop chronic pain, such as musculoskeletal pain and chronic back pain [39].

The presence of comorbid physical complaints could also influence the effect of antidepressant treatment. Patients with major depression and medical comorbidity appear to be at greater risk for a chronic course of the depression and are reported to have lower rates of response to antidepressant treatment as compared to patient without comorbidity [42-44]. Physical symptoms, particularly pain-related complaints, adversely influence recognition of depression [38] and compliance to antidepressant treatment [40]. Depressive moods in patients with general aches and pains can be prolonged by more than 6 months [38]. One of the most common causes of chronic pain is fibromyalgia syndrome (FMS) which is frequently seen in patients with major depressive disorder [39]. Previous research has suggested an association between the 5-HTTLPR s/s genotype and FMS [45,46]. In addition, the 5-HTTLPR s/s genotype has also been associated with other illnesses that are often seen in depressive patients such as irratable bowel syndrom (IBS) [47] and migraine [48,49] or the frequency of migraine attacks [50]. STin2 has also been associated with IBS and migraine [51].

If genetic variation in the serotonin transporter is indeed associated with different kinds of comorbidity, such as fibromyalgia, that is frequently occurring in depressive patients, there is a possibility that SSRI non-response cannot be explained by the polymorphisms but instead is a result from the presence of comorbidity.

GENERAL AIM OF THE THESIS

The general aim of the studies in this thesis was to evaluate the usefulness of pharmacogenetics in the treatment of depression. There is a large heterogeneity in methodology of current studies on pharmacogenetics in the treatment of depression which leads to incomparable results from different studies. In addition, there is a lack of evidence on several topics that are also important in the evaluation of the usefulness of pharmacogenetics in depression, such as the effectiveness of a genetic test in psychiatric practice and the influence of comorbidity on SSRI non-response. For this reason the question on whether pharmacogenetics could be a useful tool in improving the treatment of depression is difficult to answer at this moment. In the studies presented in this thesis, we adduce additional evidence that could help elucidating the questions on drug-gene interactions in depression.

Overview of the thesis

In chapter 2 we discuss several methodologic issues that are important when studying drug-gene interactions. In addition, we propose a list with methodological issues for future pharmacogenetic studies. An overview of current pharmacogenetic knowledge in depression on 5-HTTLPR and STin2 is described in chapter 3. Chapter 4 describes a study on the influence of 5-HTTLPR and STin2 genotype on treatment response. In chapter 5 we describe a study of the association between 5-HTTLPR, STin2 and the occurrence of SSRI-induced adverse events. Chapter 6 describes a study of the serotonin transporter genotype and the association with comorbidity. Chapter 7 describes a decision analytical model that evaluates the use of a genetic test on serotonin transporter genotype prior to antidepressant prescription. Finally, in the general discussion section (chapter 8) the evidence adduced in this thesis and the implications for the treatment of depression are discussed and some recommendations for further research are made.

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2

A review on the design and reporting of studies on drug-gene interaction

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ABSTRACT

Objective
Methodological standards for clinical pharmacogenetic studies should be developed to improve reporting of studies and facilitate their inclusion in systematic reviews. The essence of these studies lies within the concept of effect modification.

Study Design and Setting
A narrative review discussing methodological issues in the design and reporting of pharmacogenetic studies.

Results
Studying effect modification within a trial leads to the comparison of subgroups based on genotype. Differences in effect based on genotype should preferably be expressed in absolute terms (risk differences) to facilitate clinical decisions on treatment. Information on the distribution of potential effect modifiers or prognostic factors should be available to prevent a biased comparison of differences in effect between genotypes. Moreover, the distribution of genotypes should be presented and compared to Hardy-Weinberg equilibrium to check for selection bias. Additional points of interest include the possibility of selective non-availability of biomaterial and the choice of a statistical model to study effect modification.

Conclusion
Additional methodological issues should be taken into account when designing and reporting pharmacogenetic studies to assure high study quality. We present several important issues for future studies investigating drug-gene interactions that can serve as a basis for further discussion on methodology in pharmacogenetics.

INTRODUCTION

Standards for the design and reporting of clinical trials have been developed with the aim of improving the reporting of clinical trials and to facilitate their inclusion in systematic reviews [1]. Indeed, the publication of these standards by the CONSORT group has been followed by an improvement in the reporting of trials, although it has been shown that not all recently published trials do meet all these standards [1,2]. The need for well-defined methodological criteria for the design and reporting of research has not only been shown for clinical trials, but also for diagnostic and genetic research [2]. To our knowledge, this issue has not been specifically addressed for clinical pharmacogenetic research. Currently, there is a great diversity in the methodology and reporting of the results of trials in this field [3-8]. Even in reviews of pharmacogenetic studies, relevant methodological issues have received little attention. Clinical pharmacogenetics is a rapidly expanding domain of research, so it would be useful to address key methodological issues to assure not only high quality studies but also adequate analysis and reporting of the results of these studies.

Here we address a number of methodological issues in pharmacogenetic studies that we think researchers should be aware of when designing and reporting on such studies. These issues include the concept of effect modification, issues of study design and confounding and the selection of subjects. In addition, we propose a list with issues for use in future studies on drug-gene interactions.

Effect modification

The clinical relevance of pharmacogenetic studies is based on the idea that, for some drugs, different genotypes are associated with different effect sizes or varying frequencies of side effects. Hence, the efficiency of treatment strategies could be improved by adapting choices of drugs and/or dosages based on genotype [9,10]. Genotyping could especially be advantageous if the magnitude of the difference of treatment effect between genotypes is large. In clinical epidemiology such a difference in treatment effect based on a patient characteristic is called *effect modification* [11]. It should be realized that the interpretation of effect modification can depend on the choice to express treatment effect in relative or absolute terms. This has led some authors to use the term *effect measure modification* [11]. If, for a prognostic factor, relative risks are compared and it appears that effect modification does not occur in the observations, it usually *does* occur when comparing the absolute risk differences, and vice versa [11,12]. The choice of an effect measure depends on the research question [11]. Generally, from the clinician's point of view, and also in clinical decision analyses, a comparison of relative risks is less informative than a comparison of risk differences that can usually be translated to the number-needed-to-treat (NNT) [13,14]. The latter comparison enables the clinician to decide whether the difference in treatment effect is large enough to pursue different treatment policies based on genotype. Often, relative risk or relative odds models (such as logistic regression or Cox proportional hazard models) are used in the analysis. Thus, a translation into an absolute risk difference is usually required in order to decide on the usefulness of genetic testing. For this, it is necessary to be informed on the baseline risk.

Finally, an effect modifier should be distinguished from a prognostic factor. If a certain genotype predicts the outcome it certainly is a prognostic factor, but not necessarily an effect modifier. A genotype is a prognostic factor if it is associated with the clinical course or the natural history of the disease [15]. However, for a genotype to be an effect modifier, it is necessary to be associated with differences in treatment effect.

Design

Randomised clinical trials have become the paradigm by which investigators evaluate the effect of interventions [1]. Randomised clinical trials in which DNA is collected at entry are also to be preferred to non-experimental designs to compare differences in treatment effect between genotypes. Although a randomised clinical trial might have limited generalizability due to a restricted study population, they have the advantage of the availability of randomly assigned control patients that enable the distinction between the genotype as prognostic factor and as a true effect modifier. Current studies in clinical pharmacogenetics are often not prospectively designed but in these studies data from finalised trials are used [3,5,6,8]. In principle, if all biomaterial is available, this strategy will lead to valid results. However, it is possible that non-availability of biomaterial is selective due to more frequent use of biomaterial from exceptional patients. If biomaterial is collected afterwards, low response rates are observed and selection biases are likely to occur due to selective loss of patients if the genotype of interest is associated with the (bad) outcome. Additionally, genotyping should be done unaware of treatment group and clinical outcome since genotyping is also prone to observer bias.

In non-experimental cohort studies in which some patients receive treatment and some do not, the assessment of effect modification is possible. However, the likelihood of treatment could possibly be associated with the genotype of interest, for example if patients with certain genotypes are more likely to show more severe or earlier symptoms [5], causing a biased estimate of the genotype effect. Sometimes studies [16,17] in which all patients receive the treatment of interest are used to investigate whether genotype predicts treatment outcome. Since in this case a control group is lacking, it is impossible to distinguish between the genotype as a prognostic factor or an effect modifier.

Confounding

The purpose of randomisation is to balance extraneous (prognostic and/or effect modifying) variables between treatment and control group so that differences in response between groups are predominantly due to the treatment effect, and not to confounding effects of differently distributed variables. Indeed, if data from a completed randomised clinical trial are used, the advantages of this experimental design are still in effect. However, these advantages do not extend to comparisons of outcomes between groups with different genotypes in the trial. When patients are randomised over treatment groups and subsequently divided according to genotype, the latter division is not random. Although the treatment versus control groups are still randomly divided, genotype is a patient characteristic with a distribution that can be related to other (clinical) variables that are possible effect modifiers or prognostic variables. Hence, confounding between groups defined by genotype can be present and a comparison between genotype groups should consider other differences in patient characteristics.

Therefore, information on the distribution of known effect modifiers and prognostic variables in relation to genotype and treatment group should be given and analyses should be adjusted for potential differences in distribution. Only then, the decision that a difference in treatment effect is due to genotypes can be confidently made.

Selection of subjects

Problems inherent to non-experimental studies could occur in the situation in which only patients referred to a specialised hospital are selected in the pharmacogenetic study. These patients might have been referred because of non-response to drug treatment. This could lead to a selection bias if the non-response to drug treatment is causally related to the polymorphism. To evaluate whether selection in favour of a specific genotype has occurred, the distribution of the genotypes should be reported and compared to the distribution according to the Hardy-Weinberg equilibrium. If the distribution is according to this equilibrium it is less likely that a selection bias has occurred. However, it should be realized that the absence of Hardy-Weinberg equilibrium can be caused by other mechanism than selection bias.

One may also question whether refusal to be genotyped has led to a bias in the results. A way to provide a solid answer to this question is to calculate the effect modification assuming that the missing values have led to a maximum dilution of the difference in treatment effect. If, after this imputation of the missing values, treatment effect differences still exist, the refusal of some subjects to be genotyped has certainly not led to a different conclusion on the presence of effect modification. Similarly, imputation of missing values to create a maximum difference in treatment effect can be used to show the robustness of the absence of a genotype based effect modification. A similar method can also be used in studies that concluded no treatment effect differences between genotype groups. Moreover, a comparison can be conducted of baseline characteristics and the effect of treatment between those who contributed DNA material and those who did not.

Data analysis and presentation

The choice of the effect measure also affects the statistical analysis. If relative risks were chosen to measure the treatment effect, the statistical analysis should be based on a multiplicative model [11]. However, as mentioned before, risk differences, rather than relative risks, are particularly useful for clinicians who want to decide on treatment policy. The statistical analysis for this effect measure is based on an additive model [11]. When investigators have decided on the statistical analysis for their study, they should decide which subgroup to designate as the reference group. When assessing drug-gene effect modification (drug-gene interactions), usually the wildtype genotype in the non-treatment group is chosen to be the reference group to which all other groups are compared. This is not the best method to analyse drug-gene interactions, since it is possible that the homozygous wildtype is associated with a different prognosis. If this occurs, results after treatment in the different genotype groups cannot only be explained by drug-gene interaction but also by the difference in prognosis between patients with different genotypes.

Within one genotype group, comparing effects in the treatment and the non-treatment group assesses treatment differences. Therefore, drug-gene effect modification (drug-gene interactions) are analysed most suitably when outcomes in the treatment and the non-treatment group are compared within one genotype. Consequently, the observed contrasts should be compared within a single model containing the treatment and genotype variable and their interaction term. These models should then be extended to review whether the interaction is maintained, when adjusted for (potential) effect modifiers. However, it should be realized that the interpretation of interaction terms in multivariable models could be difficult.

Point estimates and the corresponding 95% confidence intervals for each genotype group separately should be presented. Not only will this facilitate the use of the results in meta-analyses, but it also helps clinicians to determine the clinical relevance of the study results.

Power considerations
The investigation of effect modification in an RCT requires a higher number of subjects to achieve a sufficient power as compared to the assessment of treatment effect in an RCT. Since the main statistic of interest is an interaction term between two variables, the calculations that are generally used to determine the required power are not suitable for this investigation and alternative calculations have been proposed [18].

SUMMARY

Essential in pharmacogenetic studies is the evaluation of treatment differences between patient groups based on genotype, i.e. the analysis of effect modification. The use of absolute risk differences to illustrate differences in treatment effect size between genotypes should be preferred to facilitate decisions on treatment strategies within certain genotypes. Care should be taken if data from a completed trial or a cohort study are used to investigate whether genotype predicts treatment outcome. Selective non-availability of biomaterial could bias the study results and the lack of a control group makes it impossible to distinguish between the genotype as an effect modifier or as a prognostic factor. Moreover, confounding can be introduced by an unequal distribution of potential effect modifiers of prognostic variables associated with the genotype. Therefore, differences in the distribution of presumed effect modifiers or prognostic variables should be presented and, if possible, taken into account. The choice of a statistical model for analysis should be based on the effect measure that was chosen to measure treatment effect. If risk ratios were used, analyses should be based on a multiplicative model. In addition, due to a possible association between genotype and prognosis, interactions between treatment and genotype should be analysed by calculating the effect of treatment within each of the genotypes and subsequently comparing these effect sizes with each other.

For the use of results in meta-analyses, point estimates and their 95% confidence intervals should be presented for each genotype separately.

After completion of a trial, the question might rise whether selection in favour of a genotype group has occurred. This could be revealed by either calculating effect modification assuming that missing values lead to a maximum dilution of treatment effect differences or by a comparison with the Hardy-Weinberg equilibrium.

Further considerations

Based on our review, we compiled a list that summarizes important methodological issues in pharmacogenetic studies (Table 1). The current list is based on a process of literature review and can be seen as an addition to similar lists, previously published for therapeutic and diagnostic research [19,20]. These earlier checklists have been subject to further discussion since their first publication [19,20]. Likewise, the current list for pharmacogenetic research can be used as a starting point for further debate on methodological guidelines for pharmacogenetic research. In the field of therapeutic and diagnostic research, the use of methodological checklists has not only led to a considerable improvement in the design and reporting of studies, but also to a better performance in therapeutic and diagnostic meta-analyses [1]. This aspect is especially important for pharmacogenetic studies because in this field of research studies often experience power problems. For this reason, the ability to perform an adequate meta-analysis should be regarded as an essential step in the research of drug-gene interactions.

In conclusion, pharmacogenetics is an intriguing new field of research. We have highlighted several problems that can emerge in a pharmacogenetic study. Additionally, we have presented several guidelines for future studies investigating drug-gene interactions that can serve as a basis for further discussion on methodology in pharmacogenetics.

Table 1: Methodological issues in the design and reporting of pharmacogenetic studies

	Design
1	Was the study design experimental?
2	Was selective non-availability of biomaterial avoided?
3	Was genotyping performed unaware of treatment group and treatment result?
4	Were appropriate power calculations for the study of effect modification used?
5	Were baseline characteristics from participants and patients that refused to be genotyped compared to check for selection bias?
6	Were genotype frequencies presented and compared to Hardy-Weinberg equilibrium?
7	Were the distributions of possible effect modifiers or prognostic factors between genotypes presented?
	Reporting
8	Was treatment effect expressed in absolute terms and were statistical analyses based on an additive model?
9	Were treatment versus non- (or other-) treatment comparisons made for each genotype?
10	Were point estimates and corresponding 95% confidence intervals presented for each genotype separately?

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3

Influence of SERTPR and STin2 in the serotonin transporter gene on the effect of selective serotonin reuptake inhibitors in depression: a systematic review

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ABSTRACT

Large differences in clinical response to selective serotonin reuptake inhibitors (SSRIs) are observed in depressive patients with different genotypes. Quantification of these differences is needed to decide if genetic testing prior to antidepressant treatment is useful. We conducted a systematic review of the literature on the influence of polymorphisms in the serotonin transporter gene (SERTPR [or 5-HTTLPR] and STin2) on SSRI response. Studies were identified by use of MEDLINE, EmBase and PsycINFO, references of articles, reviews and information from pharmaceutical companies. Nine studies assessing the influence of SERTPR or STin2 on treatment response were included. Outcome was expressed as the percentage of decrease in depression score (HAM-D or MADRS), or as the percentage of responders (≥ 50% reduction on depression scale). Both study methodologies and study outcomes showed large heterogeneity. Weighted mean decreases in depression score for patients with the s/s, s/l and l/l genotypes were 35.4%, 46.3%, and 48.0% at week 4, respectively, and 53.9%, 54.6%, and 48.3% at week 6. Among Caucasian patients, both mean decrease in depression score and response rate were lowest in the s/s group, while among Asian patients, results were inconsistent. Weighted response rates were 36.1% for the 10/12 genotype of the STin-2 polymorphism and 80.7% for the 12/12 genotype ($\chi^2 = 27.8$, $p < 0.001$) (only Asians). The available evidence points to a less favourable response to SSRI treatment among Caucasian patients with the SERTPR s/s genotype and among (Asian) patients with the STin2 10/12 genotype. In view of the scarcity and heterogeneity of the studies, however, current information is insufficiently reliable as a basis for implementing genetic testing in the diagnostic work-up of the depressive patient.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are used for the treatment of a number of conditions including depression [1,2]. It has been previously suggested that the response to SSRIs is, at least partly, under genetic control [3,4]. Two polymorphisms have been proposed as possible explanations for the observed interindividual differences in SSRI response; an insertion/deletion polymorphism in the promoter region (SERTPR, also named 5-HTTLPR) and a variable number of tandem repeats (VNTR) polymorphism in intron 2 (STin2) of the serotonin transporter gene which is the primary target for antidepressants [1,3,5].

Since approximately one-third of all depressive patients does not express an adequate positive response to initial treatment with antidepressants, and the duration of medication needed to evaluate treatment effect is long (4-6 weeks)[6], it might be cost-effective to incorporate testing on genetic polymorphisms affecting treatment response into the diagnostic work-up of the depressive patient. In order to analyse the cost-effectiveness of such a procedure, valid and reliable quantifications of the differences in clinical response to SSRIs between depressive patients with different genotypes are needed. We carried out a systematic review of the literature regarding the clinical response to SSRIs in depressive patients in relation to genetic polymorphisms in the serotonin transporter gene (SERTPR and STin2).

METHODS

Search strategy

A literature search was conducted in Medline, EmBase and PsycINFO to identify studies on SSRI treatment in relation to the SERTPR and STin2 polymorphisms in the serotonin transporter gene. This search was performed in November 2002 for all papers published from 1966 and updated on January 2 2003 and April 29 2003. The keywords 'depression AND serotonin transporter AND gen*, depression AND medication AND gen*, antidepressants AND genotype' were used. Moreover, references of retrieved articles and relevant previously published reviews were hand-searched to identify additional studies. Finally, the five pharmaceutical companies producing SSRIs were kindly requested to provide any unpublished information on the subject.

Studies were included if they assessed the association between response on SSRIs and a genetic polymorphism of the serotonin transporter gene in patients diagnosed with Major Depressive Disorder according to DSM criteria. Studies were excluded from the review if study outcome was not assessed as a reduction on a depression scale, if the study population had been used to analyse the same polymorphism more than once and if the article was not written in English, German, French, Spanish, Italian, Norwegian, Swedish, Danish or Dutch. Authors were contacted in case of confusion about potentially overlapping study populations.

Data extraction
Data were collected from all included studies by two investigators independently (KS and LS). The following study characteristics were extracted: inclusion and exclusion criteria, polymorphism (type and frequency of occurrence), treatment (type of SSRI, dose and dose escalation schedule, duration, presence of pindolol addition), blinding procedures (blinded for clinical course of depression or for genotype), evaluation of confounding (tested for differences at baseline or confounders included in analyses) and population descriptives, i.e. number of patients, sex, age and ethnicity. Outcomes were extracted for three points in time: at baseline, and at 4 and 6 weeks after start of medication. Differences in extracted data were resolved by discussion. In case of any unresolved dispute, a third investigator (MP) was available for consultation.

Analyses
In order to enhance the comparability of individual study results, we transformed mean depression scores on the Hamilton Rating Scale for Depression or the Montgomery and Ashberg Depression Rating Scale as presented in the individual studies into mean decrease on HAM-D or MADRS if possible. This was achieved by comparing the mean scores with the mean baseline scores for the different genotypes. When specific values were not available elsewhere in the article, we estimated mean HAM-D or MADRS scores from presented figures. Additionally, we transformed the number of responders into the percentage of responders. Differences of genotype frequencies for Caucasians and Asians were tested by use of one-way ANOVA. Deviations from Hardy-Weinberg equilibrium were analysed for all included studies using the χ^2 test. Pooled estimates for treatment effect weighted for individual study size were calculated for all patients and for Caucasians and Asians separately. Differences in response rates between genotypes were tested by use of a χ^2 test. Relative Risks (RR) were then calculated for the pooled response rates using the random effects model.

RESULTS

Twelve studies on SSRI response and genotype were identified by use of the literature search. Further hand search and requests for information from SSRI-producing companies yielded no additional studies. One study was excluded from the review because it addressed a manic switch possibly related to antidepressant use rather than a reduction on a depression scale [7]. Another study [8] was excluded because the study population had already been published in another study that was included in the review[9]. A third study was excluded because HAM-D scores were not reported in the text or in a figure [10]. Finally, nine studies were included in the review [9,11-18].

Patient characteristics
Table 1 shows the characteristics of the studies included in the review. Seven studies addressed the SERTPR polymorphism [9,11-16], one study analysed the influence of the STin2 polymorphism [18] and one study addressed the SERTPR polymorphism as well as the STin2 polymorphism [17].

Studies varied with respect to type of drug (fluoxetine, fluvoxamine and paroxetine); dose (variation between and within studies), duration of treatment at outcome evaluations (2 to 18 weeks), the inclusion of in- or outpatients, bipolar patients or delusional patients, and the evaluation of potential confounders. Mean age in all studies was similar (range: 44.7-54.2), except for one study that only included patients over 60 years old [15], and was not reported in another study [13]. Four studies included Asian patients [12,14,17,18], three other studies only included Italian patients [9,11,16] and two studies did not explicitly report the ethnicity of the patients but probably included predominantly Caucasian subjects [13,15]. Population size of the included studies varied between 51 and 121 patients, and studies reporting the sex of the subjects included more women except for one study [12]. All studies excluded patients with other serious Axis I or II disorders, other serious medical disorders and patients using psychotropic drugs during the last two or four weeks before inclusion in the study. Some studies also excluded patients with a serious suicide risk, with a history of substance abuse or with pregnancy [12,13,15,17].

Study outcomes

Table 2 shows frequencies of genotypes and study outcomes at baseline, for 4 and 6 weeks. Outcome measures included mean decrease in HAM-D or MADRS score and percentage responders for various moments during treatment. In two studies, a number of patients were also taking pindolol as an augmentation to SSRI treatment [9,16]. In one of these studies it was not possible to extract data on patients without pindolol addition [9].

SERTPR

Frequencies of the different genotypes of SERTPR were reported in six studies [11-14,16,17]. Caucasian patients appeared to have different gene frequencies as compared to Asian patients. Frequencies for the s/s genotype varied from 21.6% to 28.3% in the studies predominantly including Caucasian patients [11,13,16], for the Asian studies these frequencies varied between 55.6% and 60.0% ($F = 159.29$; $p = 0.000$).

For the s/l genotype frequencies varied between 43.4% and 51.0% for Caucasians and 30.0% and 39.2% for Asians ($F = 9.20$; $p = 0{,}039$), for the l//l genotype this range was 27.4% and 28.3% for Caucasians and 4.2% and 10.0% for Asians ($F = 146.48$; $p = 0.000$)[12,14,17]. The mean decrease in HAM-D score was reported by five studies [11-13,15,16]. One of these studies reported this outcome measure however solely for patients carrying an s allele compared to patients carrying the l/l genotype [15]. The mean decrease in HAM-D and MADRS scores per genotype varied largely between studies (e.g., mean 4-week decrease in HAM-D/MADRS scores for the l/l genotype: range 9.8%-70.6 %). The weighted mean decrease in HAM-D/MADRS score after 4 weeks was 35.4% for the s/s genotype, 46.3% for the s/l genotype, and 48.0% for the l/l genotype [11-14,16]. For 6 weeks, these values were 53.9%, 54.6%, and 48.3%, respectively [13,14,16]. Within the studies comprising Caucasians only, however, the s/s genotype showed a less favourable response at both 4 weeks (weighted mean decrease in HAM-D score: 26.2 % (s/s) vs. 51.5% (s/l) and 50.2% (l/l) [11,13,16]) and 6 weeks (40.7% (s/s) versus 56.0% (s/l) and 52.4% (l/l) [13,16]).

Table 1: Characteristics of studies assessing the influence of SERTPR and STin2 polymorphisms on the effects of SSRIs in depressive patients

Author and year of publication	Poly-morphism	SSRI	Dose	Bipolar patients in analyses	Inpatients/ outpatients in analyses
Yu YW 2002 [10]	SERTPR	Fluoxetine	Mean dose 29.4 mg/day (20-60mg/day)	Not reported	Not reported
Rausch JL 2002 [11]	SERTPR	Fluoxetine	1st 6 weeks: 0; 1.25; 2.5; 5.0 or 10.0 mg/day 2nd 6 weeks: random one dose increment 3rd 6 weeks: one dose increment	N	Not reported
Yoshida K 2002 [12]	SERTPR	Fluvoxamine	Escalation dose to 200 mg/day.	Not reported	Not reported
Zanardi R 2001 [13]	SERTPR	Fluvoxamine	Escalation dose to 300 mg/day	Y	Inpatients
Pollock BG 2000 [14]	SERTPR	Paroxetine	20 mg/day. After 5 weeks 30 mg/day to non-responders.	N	Inpatients and outpatients
Zanardi R 2000 [9]	SERTPR	Paroxetine	40 mg/day	Y	Inpatients
Smeraldi S 1998 [15]	SERTPR	Fluvoxamine	300 mg/day	Y	Inpatients
Kim DK 2000 [16]	SERTPR, STin2	Fluoxetine, paroxetine	Mean dose fluoxetine 29,8 mg/day 57(20-50 mg/day). Mean dose paroxetine 31.5 mg/day (20-60 mg/day).	N	Not reported
Ito K 2002 [17]	STin2	Fluvoxamine	Escalation dose to 200 mg/day.	Not reported	Not reported

The results of another study combining the s/s and s/l genotype pointed into the same direction [15]. For Asians, pooled estimates could only be calculated for 4 weeks (mean decrease: 39.2% (s/s), 39.3% (s/l), and 42.4% (l/l) [12,14]). At 6 weeks, a mean decrease in MADRS score was observed of 65.3% (s/s), 51.4% (s/l), and 18.0% (l/l) [14].

The percentage of responders, in all studies defined as a 50% decrease on the HAM-D or MADRS scale, was reported by five studies [9,12,14,15,17], one of which only reported these values including subjects receiving pindolol augmentation [9] and another reported these values only after 2 weeks of medication [15]. The one study comprising Caucasian patients showed poorer response rates at 6 weeks of medication for patients with the s/s (70.4%) and s/l (75.5%) genotype than those with the l/l genotype (87.5%) ($p = 0.029$) [9]. RR's could not be calculated for these response rates because the number of responders was not mentioned in the article.

Deviations from Hardy-Weinberg equilibrium were analysed using a χ^2 test. All studies appeared to be in Hardy-Weinberg equilibrium except for one ($\chi^2 = 6.01$; $p = 0.05$) [12]. The weighted mean decrease in HAM-D/MADRS score after 4 weeks for the studies in Hardy-Weinberg equilibrium solely was 38.1% for the s/s genotype, 50.0 % for the s/l genotype and 46.9% for the l/l genotype [11,13,14,16].

Table 1: continued

Number of patients	Mean age	Number of men/women	Ethnicity	Blinding	Inclusion criteria
121	44.7	70/51	Asian	Genotype	- Diagnosis according to DSM-IV - HAM-D at least 18 - Presence of depressive symptoms 2 weeks before entry without antidepressant therapy
51	Not reported	Not reported	Not reported	Not reported	- Diagnosis according to DSM- IV criteria
54	51.2	22/32	Asian	Depression Genotype	- Diagnosis according to DSM-IV criteria. - MADRS at least 20. - Age 20-69.
82	51.7	25/63	Italian	Not reported	- Diagnosis according to DSM-IV criteria
57	72.0 (whole group)	Not reported	Not reported	Not reported	- Diagnosis according to DSM-IV criteria. - Over 60 years - Baseline HAM-D at least 15 - MMSE at least 18.
58	47.7	15/43	Italian	Depression Genotype	- Diagnosis according to DSM-IV criteria
53	49.0	16/37	Italian	Depression Genotype	- Diagnosis according to DSM-IV criteria
120	54.2	42/78	Asian	Genotype	- Diagnosis according to DSM-IV criteria
54	51.2	22/32	Asian	Depression Genotype	- Diagnosis according to DSM-IV criteria - MADRS at least 20. - Age 20-70.

The picture was reversed for Asians; the pooled response rates at 6 weeks were 81.6% for the s/s genotype, 58.2% for the s/l genotype and 33.3% for the l/l genotype ($\chi^2 = 16.56$, $p < 0.001$) [14,17]. The RR for being a responder when having the s/s genotype compared with the l/l genotype was 2.48 (95% CI 0.30 – 32.32). For the s/l versus the l/l genotype, this RR was 1.70 (95% CI 0.24 – 11.76). However, another study among Asian patients, reporting response rates after 4 weeks of medication, found 29.2% responders among those with the s/s genotype, 27.8% among those with the s/l genotype, and 69.2% among those with the l/l genotype [12]. RR for the s/s versus the l/l genotype is 0.42 (95% CI 0.25 – 0.70), OR for s/l versus l/l is 0.40 (95% CI 0.21 – 0.76).

STin2

The STin2 polymorphism was addressed by two studies, both among Asian patients. The number of patients with the 10/10 genotype was very small in both studies (1 and 2) [17,18]. The pooled response rate for the 10/12 genotype was 36.1%, and 80.7% for the 12/12 genotype (0 of 3 for the 10/10 genotype) ($\chi^2 = 27.8$, $p < 0.001$) [17,18]. The RR for the 10/12 genotype versus the 12/12 genotype is 0.46 (95% CI 0.07 – 3.05). Mean decrease in MADRS score (reported in one study) was equal for patients with the 10/12 and 12/12 genotype (46.6% versus 48.4% at 4 weeks, and 55.5% versus 55.5% at 6 weeks) [18]. All studies on STin2 were in Hardy-Weinberg equilibrium.

Table 2: Depression scale results in relation to genotype among SSRI users

	Yu YW 2002 [12]	Rausch JL 2002 [13]	Yoshida K 2002 [14]	Zanardi R 2001 [9]
Frequencies of polymorphism	SERTPR: s/s: 72/121 (60.0%) s/l: 36/121 (30.0%) l/l: 13/121 (10.0%)	SERTPR: s/s: 11/51 (21.6%) s/l: 26/51 (51.0%) l/l: 14/51 (27.4%)	SERTPR: s/s: 30/54 (55.6%) s/l: 20/54 (37.0%) l/l: 4/54 (7.4%)	
Baseline values	21-item HAM-D score*: s/s: 31.0 s/l: 29.3 l/l: 28.2	24-item HAM-D score *: s/s: 32.6 s/l: 28.0 l/l: 29.3	MADRS score *: s/s: 28.8 s/l: 28.8 l/l: 24.4	21-item HAM-D score for the total population 28.5
Study outcome 4th week	Mean decrease in HAM-D score*: s/s: 32.7% s/l: 36.4% l/l: 52.4% p = 0.013 Response: s/s: 21/72 (29.2%) s/l: 10/36 (27.8%) l/l: 9/13 (69.2%) p = 0.019	Mean decrease in HAM-D score*: s/s: 15.3% s/l: 19.3% l/l: 14.7% p < 0.02	Mean decrease in MADRS score *: s/s: 54.9% s/l: 44.4% l/l: 9.8%	
Study outcome 6th week		Mean decrease in HAM-D score*: s/s: 19.3% s/l: 23.9% l/l: 22.9%	Mean decrease in MADRS score *: s/s: 65.3% s/l: 51.4% l/l: 18.0% Response: s/s: 24/30 (80.0%) s/l: 11/20 (55.0%) l/l: 0/4 (0.0%) p = 0.004‡	Response †: s/s: 70.4% s/l: 75.5% l/l: 87.5%. p = 0.029

Table 2 continued

Pollock BG 2000 [15]	Zanardi R 2000 [11]	Smeraldi E 1998 [16]	Kim DK 2000 [17]	Ito K 2002 [18]
	SERTPR: s/s: 16/58 (27.6%) s/l: 26/58 (44.8%) l/l: 16/58 (27.6%)	SERTPR: s/s: 15/53 (28.3%) s/l: 23/53 (43.4%) l/l: 15/53 (28.3)	SERTPR: s/s: 68/120 (56.7%) s/l: 47/120 (39.2%) l/l: 5/120 (4.2%).	
			STin2: 10/10: 2/120 (1.7%) 10/12: 22/120 (18.3%) 12/12: 96/120 (80.0%)	STin2: 10/10: 1/54 (1.9%) 10/12: 14/54 (25.9%) 12/12: 39/54 (72.2%)
17-item HAM-D score not reported	21-item HAM-D score: s/s: 27.7 s/l: 28.4 l/l: 25.1	21-item HAM-D score *: s/s: 34.4 s/l: 31.9 l/l: 32.5	17-item HAM-D score for the total population 22.3	MADRS score *: 10/10: 23.1 10/12: 28.1 12/12: 28.1
Mean decrease in HAM-D score *: s/s + s/l: 40.0% l/l: 50.0%	Mean decrease in HAM-D score: s/s: 25.7% s/l: 63.9% l/l: 70.6 % p = 0.0001	Mean decrease in HAM-D score *: s/s: 34.6% s/l: 74.0% l/l: 61.5%		Mean decrease in MADRS score *: 10/10: 17.7% 10/12: 46.6% 12/12: 48.4%
Mean decrease in HAM-D score *: s/s + s/l: 50.0% l/l: 58.0%		Mean decrease in HAM-D score *: s/s: 56.4% s/l: 92.2% l/l: 80.0 % p = 0.0346		Mean decrease in MADRS score *: 10/10: 17.7% 10/12: 55.5% 12/12: 55.5%
			SERTPR response: s/s: 56/68 (82.0%) s/s: 28/47 (60.0%) l/l: 3/5 (60.0%) p = 0.022‡	
			STin2 response: 10/10: 0/2 (0.0%) 10/12: 4/22 (18.0%) 12/12: 83/96 (86.0%) p < 0.001‡ π	Response: 10/10: 0/1 (0.0%) 10/12: 9/14 (64.0%) 12/12: 26/39 (67.0%) p > 0.999§

* values were obtained from presented figures

† Patients taking pindolol included

‡ p-values were calculated for this review

§ p-value excluding the 10/10 genotype, if 10/10 genotype included p-value = 0.386

π p-value identical if 10/10 genotype is included

DISCUSSION

This review systematically summarizes the available empirical evidence concerning the influence of two polymorphisms in the serotonin transporter gene, SERTPR and STin2, on the response to SSRI treatment in patients diagnosed with Major Depressive Disorder. We were able to retrieve 9 articles reporting on either SERTPR or STin2. Although all 8 studies addressing the SERTPR polymorphism reported at least some influence of genotype on the response to SSRIs, the overall picture appeared to be more diffuse, since the reported effects showed opposite directions. Ethnicity might, however, have played a role in determining the direction of the effect. In Caucasian patients, response to SSRIs seemed less favourable for patients with the s/s genotype than for those with the s/l and l/l genotype. On the other hand among Asian patients effects in both directions were observed. Patients with the 10/12 variant of the STin-2 polymorphism showed a less favourable response to SSRI treatment than those with the 12/12 variant. However, data of only two studies were available, both among Asian patients. The studies conducted among Caucasian patients report an s/s SERTPR genotype frequency around 25%. This is in accordance with frequencies previously reported in studies on this polymorphism [4]. On the other hand, Asian studies report a frequency of the s/s genotype of around 57%. It is unclear how this ethnic difference in the distribution of the s allele relates to the difference in influence on the effect of SSRIs. After analysis, one study [12] showed a significant deviation from the Hardy-Weinberg equilibrium, even though the article from this study mentioned no deviations.

Several versions of the Hamilton Rating Scale for Depression were used in the individual studies. It is possible that the version that was used or the experience of the interviewers using the HAM-D have influenced depression scores [19], which might have hampered the comparison of depression scores among individual studies.

It should be emphasized that, even though studies were only included if they met our inclusion criteria, there was considerable heterogeneity between individual studies with respect to population characteristics, type of intervention, outcome measurement and validity. Although all studies only included patients if diagnosis was confirmed according to DSM-IV criteria, differences regarding the inclusion of inpatients or patients with bipolar depression could have affected individual study results by influencing treatment effect. A manic relapse in patients with bipolar depression could lead to an apparent 'improvement' in depression scores and therefore alter study outcome [20]. Likewise, differences in diagnosis could affect inter-patient comparability. Different SSRIs, fluoxetine, fluvoxamine and paroxetine, were used in the included studies and some studies used multiple dosages of SSRIs or did not stipulate the dosage to be used. Using different SSRIs and varying dosages impedes the comparison of study results. Subjects did not all receive an identical treatment and non-response could be caused by the received dosage that was too low rather then the genotype of interest. Furthermore, it is unclear whether the possible influence of genotype is equal for each of the SSRIs [1]. One study was designed to increase the dose of the SSRI after 5 weeks if patients did not respond to the treatment [15]. This strategy could reduce the differences in response between the genotypes by the increase in dose.

Although such a strategy is appropriate in daily psychiatric practice, it limits the ability to gain a valid quantitative insight into the mechanisms of SSRI response. Likewise, the administration of pindolol in addition to SSRI treatment may have distorted study results.

A distortion of individual study results could also have been caused by the assumption of a dominant model for the s-allele as was done in one of the studies [15]. Even though research in human cell lines suggests a dominant s-allele [4] and this model was confirmed in some studies [9,12], other study results point to a model in which the l-allele functions as the dominant allele [11,13,16]. Larger samples are probably needed to determine which allele brings about a dominant influence [9].

Information on blinding procedures and correction methods was lacking in some studies. Although previously suggested as possible influences on the course of depression and treatment effect, age and sex of the study population was not described in all articles. Other factors possibly influencing treatment effect, such as history of depression and of medication, were not reported in any of the studies. Finally, the numbers of potential confounders considered in the analyses, and the way in which their influence was evaluated, were marginal in some studies.

The diversity of designs is likely to have contributed to the observed heterogeneity of study results. Moreover, it cannot be excluded that other factors, such as differences in background genes between Asian and Caucasian subjects or differences in the cultural context of the diagnosis of depression and selection for treatment in different countries, have also influenced responsiveness to SSRI treatment. In view of the small number of studies, however, it is not possible to reliably identify specific determinants of study outcome.

In summary, the available evidence points to a somewhat less favourable effect of SSRIs among Caucasian patients with the s/s variant of the SERTPR polymorphism (as opposed to those with the s/l and l/l variant) and among Asian patients with the 10/12 variant of the STin2 polymorphism (as opposed to those with the 12/12 variant). However, considering the heterogeneity of the studies with respect to population characteristics, type of intervention, and validity as well the broad confidence intervals corresponding with the calculated RR's for the pooled response rates, accurate quantitative conclusions are presently out of reach. Therefore, it can currently not be recommended to implement testing on these polymorphisms prior to antidepressive treatment for selection of type or dose of medication. Future research, in order to be relevant for clinical practice, should report clearly on patient history (with respect to both medication and disease), patient recruitment, age, gender, ethnicity, and type of blinding, and should evaluate the confounding influence of important determinants of treatment outcome. In the meantime, it would be worthwhile to evaluate what would be the minimum difference in treatment effect between genotypes to cost-effectively influence clinical practice; statistically significant, but relatively small effects could be irrelevant for practice.

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4

The influence of 5-HTTLPR and STin2 polymorphisms in the serotonin transporter gene on treatment effect of selective serotonin reuptake inhibitors in depressive patients

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ABSTRACT

Background
Serotonin transporter gene (SLC6A4) variations have been proposed as explanation for inter-individual differences in selective serotonin reuptake inhibitors (SSRIs) effects. Quantitative assessment of genetic influences is necessary to evaluate whether genetic testing prior to antidepressant prescription would lead to earlier treatment effects. This study evaluates the influence of two polymorphisms (5-HTTLPR and STin2) on SSRI treatment outcome in depression.

Methods
We included 50 SSRI non-responders (cases) and 164 referents meeting DSM-IV criteria for major depression and using an SSRI for at least 6 weeks. Blood samples or buccal swabs were gathered to determine 5-HTTLPR (N=48 for cases and 161 for referents) and STin2 (N=50 for cases and 162 for referents) genotypes. The association between genotype and SSRI response was assessed by use of logistic regression.

Results
Patients with the 5-HTTLPR s-allele had a non-significantly increased risk of SSRI non-response; Odds Ratio (OR) 1.60 (95%-CI: 0.66-3.89). 5-HTTLPR effects were strongest in female patients (OR 3.54, 95%-CI: 1.05-11.92), for male patients 5-HTTLPR seemed to have no effect (OR 0.29 (95%-CI 0.04-2.34). An age-dependent effect of 5-HTTLPR was observed; patients under 44 years old had an increased non-response risk (OR 9.34, 95%-CI 1.41-61.98). STin2 genotype had no clear influence on treatment outcome.

Conclusions
Our findings indicate that women with the 5-HTTLPR s-allele have a less favourable response to SSRI treatment. To our knowledge, this is the first time that a gender-dependent influence of 5-HTTLPR is reported. More research is needed, particularly in subgroups of patients, before implementation of genetic testing can be recommended.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are the first-choice pharmacological treatment for depression almost everywhere throughout the world. Nevertheless, 30 – 40% of the patients do not respond sufficiently to SSRI treatment [1], other studies have even reported higher numbers of non-response [2]. It has previously been suggested that SSRI response is partly under genetic control, and two polymorphisms in the serotonin transporter gene (SLC6A4), 5-HTTLPR and STin2, have been proposed as possible factors that explain the observed differences in clinical response [3-13]. 5-HTTLPR (also named SERTPR) is an insertion/deletion polymorphism in the promoter region, and STin2 is a variable number of tandem repeats (VNTR) in the second intron of SLC6A4 [1,3,14]. Since the serotonin transporter protein (5-HTT or SERT) is the primary target for antidepressants such as SSRIs, variations in expression and/or regulation of SLC6A4 could lead to individual differences in treatment response.

If DNA-polymorphisms indeed are (partly) involved in SSRI non-response, the implementation of genetic testing prior to antidepressant prescription might help health care professionals in achieving earlier treatment effects. However, in a recent systematic review, we concluded that current information on genetic modification of SSRI response is insufficiently reliable to implement a genetic test in the diagnostic work-up of the depressive patient [13]. In addition, several studies previously reported differences in treatment outcome, but also in treatment dosage and duration, between men and women [15]. Previous studies have also mentioned serotonin transporter genotype influences that were gender-dependent in, for example, suicide attempts or certain personality traits [16 17]. In addition to gender, age has been proposed as a possible moderator for antidepressant response [18] and there are indications that serotonin transporter affinity is modified by age [19]. Knowledge on the influence of serotonin transporter genotype on SSRI treatment outcome in these subgroups is not available at this moment.

In order to expand the knowledge base relevant to this issue, we examined the influence of 5-HTTLPR and STin2 polymorphisms on treatment response among patients using SSRIs for depression. To assess whether 5-HTTLPR or STin2 influence is different for men and women and for different age categories, we also examined genotype influence in these subgroups separately.

METHODS

Patient population

The influence of polymorphisms in this study was evaluated in a case-referent design [20]. This design allows unbiased estimation of relative risks without the need of a rare-disease assumption [20]. We compared frequencies of 5-HTTLPR and STin2 genotypes among all patients treated with SSRIs (referents) with genotype frequencies among patients without clinical response to SSRI treatment (cases).

Cases were defined as outpatients that used an SSRI for at least six weeks without a satisfactory clinical response as assessed by the treating psychiatrist. They were recruited in the regional mental health services in the area of South-Limburg in the Netherlands. This recruitment strategy allows for the selection of non-responders, given the fact that GPs in this region generally refer to mental health services if no response has been obtained after a course of antidepressant treatment. Referent patients each originated from one of 16 general practitioner practices affiliated to the Registration Network Family Practices of Maastricht University [21] that agreed to collaborate in the study. All practices were situated in the province of Limburg in the Netherlands. Referent patients were defined as patients that had used an SSRI for at least six weeks. They were recruited irrespective of treatment outcome within the source population from which the cases originated.

A total of 50 cases and 164 referent patients meeting the DSM-IV criteria for major depressive disorder were included in the study. Inclusion criteria were: between 18 and 64 years old, Caucasian, diagnosis of major depression according to the DSM-IV, SSRI use for at least 6 weeks and living in the province of Limburg. Exclusion criteria for the study were: additional diagnoses on axis-I other than an anxiety disorder, diagnosis of bipolar disorder, pregnancy and inability to complete an interview in Dutch. Informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of Maastricht university/Academic Hospital Maastricht.

Sample collection
Blood samples were taken from all participants using the BD Vacutainer system with EDTA tubes. If it was not possible to take blood samples from a participant, buccal cells were collected by use of Sterile Omniswabs from Whatman. To test for selection bias ABO blood group was assessed for all participants according to the standard procedure. Additional information on treatment, adverse effects, compliance and work-related items was collected through a face-to-face interview. Interviews were performed unaware of genotype status.

Genotyping
Genomic DNA was isolated either from 8 ml whole blood (EDTA tubes) using the Wizard Genomic DNA purification kit (Promega, Leiden, the Netherlands) or from buccal cells (2 Omniswabs per sample, Whatman) with the QIAamp DNA Mini Kit (QIAgen, Venlo, The Netherlands). For both procedures the manufacturers protocols were followed.

Determination of the 5-HTTLPR genotype was performed as previously described [22], with some modifications. A FAM-labeled forward primer: 5'-GGCGTTGCC GCTCTGAATGC-3' was used together with the following reverse primer: 5'-GAGGGACTGAGCTGGACAACCCAC-3'. Polymerase chain reaction (PCR) was carried out in 96-well microtiterplates on a Biometra T1 Thermocycler (Westburg, Leusden, The Netherlands).

We used approximately 10 – 100 ng of genomic DNA in a 25 µl reaction mixture containing 1 x PCR buffer (Invitrogen, Breda, The Netherlands), 0.2 mM dNTPs, 0.4 µM of each primer, 0.75 mM $MgCl_2$, and 1U Taq DNA polymerase (Invitrogen, Breda, The Netherlands). Cycling conditions were: initial 3 min. denaturation at 95°C; 5 cycles of denaturation at 94°C for 30 sec., annealing at 65°C (touch down 0.3°C) for 1 min. and extension at 72°C for 1 min.; 35 cycles of denaturation at 94°C for 30 sec., annealing at 63°C for 1 min. and extension at 72°C for 1 min.; and a final extension for 10 min. at 72°C.

Determination of the STin2 genotype was performed as previously described [23], with some modifications. A FAM-labeled forward primer: 5'- GTCAGTATCACAGGCTGCGAG -3' was used together with the following reverse primer: 5'-TGTTCCTAG TCTTACGCCAGTG -3'. Polymerase chain reaction (PCR) was carried out in 96-well microtiterplates on a Biometra T1 Thermocycler (Westburg, Leusden, The Netherlands). We used approximately 10 – 100 ng of genomic DNA in a 25 µl reaction mixture containing 1 x PCR buffer (Invitrogen, Breda, The Netherlands), 0.2 mM dNTPs, 0.4 µM of each primer, 0.75 mM $MgCl_2$, and 1U Taq DNA polymerase (Invitrogen, Breda, The Netherlands). Cycling conditions were: initial 3 min. denaturation at 94°C; 35 cycles of denaturation at 94°C for 30 sec., annealing at 60°C for 45 sec. and extension at 72°C for 45 sec.; and a final extension for 8 min. at 72°C.

For each sample, PCR products were pooled (1 µl each) and subsequently size-resolved on an ABI3100 Genetic Analyzer, using 36 cm capillaries filled with POP6 polymer. The peaks corresponding to the different alleles (STin2.9: 248 bp, STin2.10: 265 bp, STin2.12: 299 bp, 5HHTLPR Short: 478 bp and 5HHTLPR Long: 520 bp) were identified using Genescan Analysis software version 3.7 (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Two researchers independently scored genotypes, and a third expert judged discordant results before entering final values in the database.

Data Analysis

Exact 95% confidence intervals were calculated around observed ABO bloodgroup frequencies and compared to the frequencies in the general population (11/2005, Sanquin, www.sanquin.nl). Deviations from Hardy-Weinberg equilibrium were analysed using the chi-square test. The association between polymorphism and SSRI response was evaluated by use of logistic regression analysis. The 1/l genotype group was designated as the reference group as this group was expected to have the best response to SSRI treatment [4,6,13,24]. For STin2, the 12/12 genotype was designated as the reference group. To adjust for potential confounders, all possible confounding variables that were measured in the study were included as covariates in the logistic regression model. Variables that were considered as potential confounders were age, gender, type of SSRI, level of education and marital status.

In addition to the genotype analyses, we evaluated the influence of the separate alleles on treatment effect. This was also assessed for men and women separately and for two age categories.

Age was divided in tertiles and the youngest tertile (≤ 44) was compared to the other two tertiles (>44). In the allele analyses, we assumed that the 5-HTTLPR s-allele was the dominant allele. Patients carrying one or two s-allele(s) were compared to patients homozygous for the l-allele. The l-allele was designated as the reference groups as it was expected to have the best response on SSRI treatment [4,6,13,24]. We included an interaction term between the separate alleles and gender and between the alleles and age in the logistic regression model to test for interaction. All statistical analyses were performed by use of the SPSS package.

RESULTS

For the 5-HTTLPR, we were able to determine the genotype of 48 cases and 161 referent patients. For STin2, these numbers were 50 and 162, respectively. Genotyping was not possible for all patients due to problems with DNA quantity or quality. Table 1 presents baseline characteristics of cases and referents. Referent patients were older and more often had a positive family history of depression as compared to cases. The type of SSRI that was used also differed between cases and referents, paroxetine was the most frequently prescribed antidepressant in both groups. The larger part of the population was born in the Netherlands, the other countries of birth that were reported by the participants included Belgium (1), Germany (4), the United Kingdom (1), Poland (1), Romania (1), Greece (1) and Indonesia (1). Gender, previous treatments as well as level of education (not shown here) were not different between cases and referents. Exact 95% confidence intervals around observed ABO blood group frequencies did not exclude ABO blood group frequencies in the general population. In addition, ABO blood group frequencies in cases did not differ significantly from frequencies in referents (chi-square = 3.17, p = 0.87, df = 2). Genotype frequencies for 5-HTTLPR and STin2 in cases were in Hardy-Weinberg equilibrium, in referents genotype frequencies for 5-HTTLPR were not in Hardy Weinberg equilibrium (p = 0.03). For the total group frequencies for STin2 were in Hardy-Weinberg equilibrium, deviations from Hardy-Weinberg equilibrium were borderline significant for 5-HTTLPR (p = 0.05).

Table 1: Baseline characteristics of non-responders and referent patients

		Cases (n=50)		Referents (n=164)	
		% (N)	Mean (SD)	% (N)	Mean (SD)
Age			43.12 (10.15)		50.12 (9.46)
Gender	Men	36.0 (18)		28.7 (47)	
	Women	64.0 (32)		71.3 (117)	
SSRI	Paroxetine	46.0 (23)		61.6 (101)	
	Fluoxetine	14.0 (7)		17.1 (28)	
	Fluvoxamine	8.0 (4)		8.5 (14)	
	Sertraline	14.0 (7)		7.9 (13)	
	Cipramil	18.0 (9)		4.9 (8)	
Country of birth	Netherlands	96.0 (48)		95.1 (156)	
Family history	Yes	70.0 (35)		81.7 (134)	
	No	30.0 (15)		18.3 (30)	
Previous treatments	Yes	54.0 (27)		48.2 (79)	
	No	46.0 (23)		51.8 (85)	

The results of the logistic regression analysis are presented in table 2. The 5-HTTLPR l/l and the STin2 10/12 genotype were found more often in referent patients as compared to cases. Cases more often had a s/l or 10/10 genotype as compared to referent patients. Although the ORs for 5-HTTLPR suggest a slightly increased risk on non-response on SSRI with the s/l and s/s genotype, 95%-confidence intervals included 1; OR 1.86 (95%-CI 0.71-4.88) and 1.27 (95%-CI 0.44-3.72) respectively. For STin2, results show no increased risk on non-response with the 10/10 genotype or the 12/10 genotype; OR 1.07 (95%-CI 0.40-2.87) and OR 0.73 (95%-CI 0.32-1.68) respectively. Because STin2 genotype shows no influence on SSRI treatment outcome, additional analyses were only performed for 5-HTTLPR genotype.

Table 2: Results of logistic regression analysis of serotonin transporter genotype and non-response to SSRI-treatment

Genotype	Frequency % (n†)		OR (95% CI)	Adjusted OR*
	Cases	Referents		
5-HTTLPR				
l/l	29.2 (14)	36.0 (58)	1 (reference)	1 (reference)
s/l	47.9 (23)	39.1 (63)	1.51 (0.71-3.21)	1.86 (0.71-4.88)
s/s	22.9 (11)	24.8 (40)	1.14 (0.47-2.76)	1.27 (0.44-3.72)
STin2				
12/12	44.0 (22)	43.2 (70)	1 (reference)	1 (reference)
10/12	34.0 (17)	41.4 (67)	0.81 (0.40-1.65)	0.73 (0.32-1.68)
10/10	22.0 (11)	15.4 (25)	1.40 (0.60-3.30)	1.07 (0.40-2.87)

* adjusted for age, gender, type of SSRI, level of education and marital status

† numbers do not add up to 50 (cases) or 164 (referents) due to missing values in genotype data

The results from the logistic regression analyses on the separate alleles were similar to the results of the genotype analyses (table 3). Cases more often had the s-allele as compared to the referents. The OR for non-response for the s-allele versus the l-allele was 1.60 (95%-CI 0.66-3.89).

Table 3: Results of logistic regression analysis of serotonin transporter 5-HTTLPR alleles and non-response to SSRI treatment

Genotype	Frequency % (n†)		OR (95% CI)	Adjusted OR *
	Cases†	Referents†		
5-HTTLPR				
l/l	29.2 (14)	36.0 (58)	1 (reference)	1 (reference)
s/s + s/l	70.8 (34)	64.0 (103)	1.37 (0.68-2.76)	1.60 (0.66-3.89)

* adjusted for age, gender, type of SSRI, level of education and marital status
† numbers do not add up to 50 (cases) or 164 (referents) due to missing values in genotype data

There was a suggestive interaction between the allele and gender, tested in a logistic regression model (p = 0.065). Stratified analyses revealed that for men, a statistically non-significant negative association was observed between the 5-HTTLPR s-allele and non-response; OR 0.29 (95%-CI 0.04-2.34). For women, a statistically significant positive association was observed between the s-allele and non-response; OR 3.54 (95%-CI 1.05-11.92).

Table 4: Results of logistic regression analysis of serotonin transporter 5-HTTLPR genotype and non-response to SSRI-treatment for men and women separately

	Genotype	Frequency % (n†)		OR (95% CI)	Adjusted OR *
		Cases	Referents		
Men	5-HTTLPR				
	l/l	43.8 (7)	31.9 (15)	1 (reference)	1 (reference)
	s/s + s/l	56.3 (9)	68.1 (32)	0.60 (0.19-1.93)	0.29 (0.04-2.34)
Women	5-HTTLPR				
	l/l	21.9 (7)	37.7 (43)	1 (reference)	1 (reference)
	s/s + s/l	78.1 (25)	62.3 (71)	2.16 (0.86-5.43)	3.54 (1.05-11.92)

* adjusted for age, gender, type of SSRI, level of education, marital status
† numbers do not add up to 50 (cases) or 164 (referents) due to missing values in genotype data

The interaction between age and allele in the logistic regression was not statistically significant (p = 0.204) but was further explored given low power of test for interaction. Thus, stratification by age (≤ 44 years and > 44) revealed a positive association for the s-allele in patients ≤ 44 years old (OR 9.34, 95%-CI 1.41-61.98) but not for patients > 44 years (OR 1.13, 95%-CI 0.32-3.98).

DISCUSSION

In this study, we analysed the association between two polymorphisms (5-HTTLPR and STin2) in the serotonin transporter gene and SSRI response in patients diagnosed with major depressive disorder. Genotype frequencies are comparable to frequencies reported in previous studies on depression [13]. Genotype frequencies in cases were in Hardy-Weinberg equilibrium in our population, however referent patients were not. Deviations from Hardy-Weinberg would be expected if serotonin transporter genotype was associated with the development of depression. Previously, the association between serotonin transporter genotype and depressive disorder has been studied extensively, however with inconsistent results as reported in two recent meta-analyses [25,26].

Logistic regression results indicate that the overall effect of 5-HTTLPR on SSRI treatment outcome is small. In addition, STin2 appears to have no influence on SSRI treatment outcome. Some previous studies suggested an increased risk of SSRI non-response for individuals with the 10-allele. However, STin2 lies in an intron outside the coding area but in close proximity of the 5-HTTLPR polymorphism of which the s-allele is known to decrease serotonin transporter expression in neurons. It is possible that previous positive results for STin2 influence on SSRI treatment outcome can be attributed to the 5-HTTLPR polymorphism since these polymorphisms are likely to be in linkage disequilibrium.

Although the overall genotype effect appears to be small, there may be a gender-dependent association between serotonin transporter genotype and SSRI non-response. Male patients may have an SSRI treatment response that is not clearly affected by serotonin transporter genotype while results in women with the s-allele show a less favourable response to treatment. Results also suggest an age-dependent effect; patients in the lowest age category had a less favourable SSRI response with the s-allele. Overall, results were in agreement with previous studies among Caucasian patients [3-5,7,9,10,13] which suggests a small, not statistically significant, positive association between the s/s genotype and SSRI non-response. To our knowledge, a gender-dependent influence of serotonin transporter genotype in SSRI treatment outcome has not been reported up till now.

Our findings suggest that patients heterozygous for the s-allele are more at risk for developing SSRI non-response compared to patients homozygous for the s-allele; this was at odds with our expectations that risk for non-response would grow larger as the number of s-allele copies increased. These observations suggest that the influence of the serotonin transporter genotype could be of minor importance in predicting treatment outcome for patients with the s/s genotype. Although speculative, treatment outcome in s/s patients may be more dependent on external factors. Several studies have reported a gene-environment interaction between 5-HTTLPR and stressful life events in major depression [27,28]. Kendler et al recently reported an increased risk in depressive disorder after mild stressful events in patients with the s/s genotype but not in patients with the s/l or l/l genotype [27]. Along this line, one could hypothesize that treatment response in patients with the s/s genotype is more dependent on contextual (e.g. amelioration of social problems) or psychological factors (e.g. problem solving) than on SSRI responsivity.

Previous studies have suggested differences in treatment outcome due to pharmacological differences in men and women. Women are thought to have a better SSRI response whereas men would respond better to tricyclic antidepressants which also influence noradrenergic pathways [29]. Our findings support the idea of pharmacodynamic and pharmacokinetic differences between men and women leading to differences in antidepressant treatment outcome.

Our observations suggest a non-significant age-dependent influence of serotonin transporter genotype; patients under the age of 44 years old appear to have an increased risk of SSRI non-response given the presence of the s-allele. This could imply a different depression etiology in younger patients; possibly serotonergic pathways are more important in younger patients. Another explanation may be that older patients experience more physical complaints which could influence treatment outcome more strongly than a possible genotype effect [30].

Two variables that also could be considered as potential confounders or effect modifiers for the association between SSRI response and genotype are family history of depression and previous antidepressant treatment. In the analyses we did not include these variables since face-to-face interviews with patients are probably not an accurate method to record information on family history and previous treatment. However, if family history and previous treatments were included as confounders, observed ORs were not altered.

A limitation of the present study is sample size. The study was relatively small, causing large confidence intervals; study results should therefore be interpreted with caution. Because of the small sample size, genotype effects that are small could not be detected, even though they could be relevant for psychiatric practice.

In the allele analyses, we assumed a dominant model for the 5-HTTLPR s-allele. This choice was made based on genotype results from the present study that indicated a 5-HTTLPR s/l influence that is similar to the s/s influence. However, even though studies in cell lines and several case-control studies also suggest a dominant s-allele [4,6,13,24], results are not consistent and others have suggested a dominant l-allele instead [5,7,10,31].

Differences in ethnicity are a problem in pharmacogenetic research due to the risk of population stratification [32,33]. In the present study, only Caucasian subjects were included. By excluding different ethnicities, we avoided the risk of population stratification. Another problem frequently occurring, especially in studies with a case-control or case-referent design, is selection bias. To test for selection bias, ABO blood group was assessed in cases and referent patients. Calculated exact 95% confidence intervals around observed ABO blood group frequencies in the study population did not exclude ABO from blood group frequencies in the general population. In addition, ABO blood group frequencies in cases did not differ significantly from the frequencies in the referents. Therefore, it is likely that selection bias did not influence study results.

In summary, the overall effect of 5-HTTLPR on treatment outcome, if any, appears to be small and it is questionable whether this effect would be relevant in clinical psychiatric practice. STin2 appears to have no influence on treatment outcome. In specific subgroups of patients on the other hand, serotonin transporter genotype influences seem to be more prominent. These influences could in fact be important for clinical practice; women and younger patients appear to be at greater risk for SSRI non-response with the 5-HTTLPR s-allele. Nevertheless, these results should be interpreted with caution because of the small sample size. Further research that focuses on the influence of genetic variation in subgroups of patients, is needed in order to replicate these findings before any recommendations can be formulated with respect to genetic testing in psychiatric practice.

Additionally, it would be useful to include the present study in a meta-analysis on serotonin transporter genotype to assess the presence and clinical relevance of small genotype effects that could not be evaluated in the present study due to the limited sample size. Since SSRI non-response is common in patients with major depressive disorder, small genotype effects could still be relevant for clinical psychiatric practice.

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5

Serotonin transporter polymorphisms and the occurrence of adverse events during treatment with selective serotonin reuptake inhibitors

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ABSTRACT

Background
During treatment with selective serotonin reuptake inhibitors (SSRIs) for major depression, some patients experience adverse events whereas others do not. Assessment of predictors for SSRI-induced adverse events would be useful for the identification of patients likely to develop these events. This study evaluates the association between adverse events during SSRI treatment and two polymorphisms in the serotonin transporter (5-HTTLPR and STin2).

Materials and methods
For this study, we included 214 patients meeting the DSM-IV criteria for major depressive disorder and using an SSRI for at least 6 weeks. Blood samples or buccal swabs were taken from all participants in order to determine the 5-HTTLPR an STin2 genotype. Information on adverse events was gathered through interviews and general practitioners' files. The association between the serotonin transporter genotype and the presence of adverse events was assessed by use of logistic regression.

Results
Patients with the 5-HTTLPR s/s or s/l genotype appeared to have an increased risk of adverse events during SSRI treatment. This association was strongest for the general adverse events (dermatologic reactions, weight change and fatigue); Odds Ratio (OR) 1.77 (95%-CI 0.80-3.92) for the s/s genotype and OR 2.37 (95% CI 1.13-4.96) for the s/l genotype. For STin2, results were inconsistent and observed associations were weak and statistically non-significant.

Discussion
Our findings indicate that patients with the 5-HTTLPR s/s or s/l genotype have an increased risk of developing adverse events, especially general adverse events, during SSRI treatment. For the STin2 genotype, no clear relation with occurrence of adverse events seems to exist. At this moment, available evidence is however too limited to incorporate a genetic test on 5-HTTLPR or STin2 in psychiatric practice to identify patients at risk for adverse events during SSRI treatment.

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are increasingly becoming the first-choice medication for depression treatment [1]. It has previously been suggested that SSRI response is partly under genetic control. Two polymorphisms (5-HTTLPR and STin2) in the gene that codes for the serotonin transporter (SLC6A4) have been proposed as possible explanations for observed inter-individual differences in SSRI response [2-13].

Initially, clinicians thought that SSRIs were almost free of adverse events due to their selectivity [14]. However, this opinion has changed; a large part, around 75%, of the patients treated with SSRIs report one or more adverse events during treatment [15]. Since adverse events are the main reason for early discontinuation of SSRI treatment [16], which is associated with higher relapse and recurrence rates and with higher overall healthcare costs [17], it would be useful if clinicians were able to identify patients that are susceptible for developing certain adverse events prior to SSRI treatment. This would allow them to anticipate on these adverse events and perhaps initiate prophylactic treatment [18], prescribe a different antidepressant or plan earlier patient follow-up. .

Previous studies have reported an association between the occurrence of adverse events during SSRI treatment and different serotonin transporter genotypes. For example, the s/s genotype of 5-HTTLPR has previously been associated with insomnia, agitation [18] and disturbances in circadian rhythms and level of alertness as well as with a greater severity of adverse events [19] during SSRI treatment and lack of effect [3]. This genotype has also been suggested in relation to early treatment discontinuations caused by adverse events including gastrointestinal complaints, fatigue, agitation, sweating and dizziness [19].

In the present study, we evaluated the association between specific adverse events and 5-HTTLPR and STin2 genotypes in patients with major depressive disorder who were treated with an SSRI.

METHODS

Patient population

Information for this study was obtained from our previous study on the influence of 5-HTTLPR and STin2 on treatment effect in depression [Smits et al, 2005; submitted]. Patients were recruited in regional mental health services and in one of 16 general practitioners practices affiliated to the Registration Network Family Practices of Maastricht University [20] in the area of South-Limburg in the Netherlands. We made no further distinction between patients recruited in mental health services and patients recruited in general practitioners practices since we were interested in the influence of serotonin transporter genotype on the occurrence of adverse events during SSRI treatment. However, we evaluated whether results would have changed if only general practitioners patients, which were the majority of the patients, were included in the analyses. A total of 214 outpatients were included in the study. Inclusion criteria were: between 18 and 64 years old, Caucasian, diagnosis of major depression according to the DSM-IV, SSRI use for at least 6 weeks and living in the province of Limburg.

Exclusion criteria for the study were: additional diagnoses on axis-I other than anxiety disorder or panic disorder, diagnosis of bipolar depression, pregnancy and inability to complete an interview in Dutch. Written informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of Maastricht University/Academic Hospital Maastricht.

Data collection

Blood samples were taken from using the BD Vacutainer system with EDTA tubes. DNA was extracted from whole blood with the Wizard Genomic DNA Purification Kit (Promega, Leiden, the Netherlands), according to the manufacturers procedure. If it was not possible to take blood samples from a participant, buccal cells were collected by use of Sterile Omniswabs from Whatman.

Information about the most frequently occurring adverse events during SSRI treatment was collected through structured face-to-face interviews by a trained interviewer. Each adverse event was assigned to one of six main classes of adverse events i.e. autonomic (comprising dry mouth and sweating), central/pheripheral nervous system (comprising headache, dizziness and tremor), gastrointestinal (comprising nausea, diarrhea and constipation), psychiatric (comprising somnolence, insomnia, asthenia, anxiety and agitation), urogenital (comprising sexual problems such as ejaculation difficulty) and general (comprising dermatologic reactions, weight gain, weight loss and fatigue). Additional information on treatment, compliance and work-related items was also collected through an interview. Interviews were performed blind to genotype status.

Genotyping

Genomic DNA was isolated either from 8 ml whole blood (EDTA tubes) using the Wizard Genomic DNA purification kit (Promega, Leiden, the Netherlands) or from buccal cells (2 Omniswabs per sample, Whatman) with the QIAamp DNA Mini Kit (Westburg, Leusden, The Netherlands). For both procedures the manufacturers protocols were followed.

Determination of the 5-HTTLPR genotype was performed as previously described [21], with some modifications. A FAM-labeled forward primer: 5'-GGCGTTGCCGCTCT GAATGC-3' was used together with the following reverse primer: 5'-GAGGGACTGAGCTGGACAACCCAC-3'. Polymerase chain reaction (PCR) was carried out in 96-well microtiterplates on a Biometra T1 Thermocycler (Westburg, Leusden, The Netherlands). We used approximately 10 – 100 ng of genomic DNA in a 25 µl reaction mixture containing 1 x PCR buffer (Invitrogen, Breda, The Netherlands), 0.2 mM dNTPs, 0.4 µM of each primer, 0.75 mM $MgCl_2$, and 1U Taq DNA polymerase (Invitrogen, Breda, The Netherlands). Cycling conditions were: initial 3 min. denaturation at 95°C; 5 cycles of denaturation at 94°C for 30 sec., annealing at 65°C (touch down 0.3°C) for 1 min. and extension at 72°C for 1 min.; 35 cycles of denaturation at 94°C for 30 sec., annealing at 63°C for 1 min. and extension at 72°C for 1 min.; and a final extension for 10 min. at 72°C.

Determination of the STin2 genotype was performed as previously described [22], with some modifications. A FAM-labeled forward primer: 5'- GTCAGTATCACAGGCTGCGAG -3' was used together with the following reverse primer: 5'- TGTTCCTAGTCTTACGCCA GTG -3'. Polymerase chain reaction (PCR) was carried out in 96-well microtiterplates on a Biometra T1 Thermocycler (Westburg, Leusden, The Netherlands). We used approximately 10 – 100 ng of genomic DNA in a 25 µl reaction mixture containing 1 x PCR buffer (Invitrogen, Breda, The Netherlands), 0.2 mM dNTPs, 0.4 µM of each primer, 0.75 mM $MgCl_2$, and 1U Taq DNA polymerase (Invitrogen, Breda, The Netherlands). Cycling conditions were: initial 3 min. denaturation at 94°C; 35 cycles of denaturation at 94°C for 30 sec., annealing at 60°C for 45 sec. and extension at 72°C for 45 sec.; and a final extension for 8 min. at 72°C.

For each sample, PCR products were pooled (1 µl each) and subsequently size-resolved on an ABI3100 Genetic Analyzer, using 36 cm capillaries filled with POP6 polymer. The peaks corresponding to the different alleles (STin2.9: 248 bp, STin2.10: 265 bp, STin2.12: 299 bp, 5HHT Short: 478 bp and 5HHT Long: 520 bp) were identified using Genescan Analysis software version 3.7 (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Two researchers independently scored genotypes, and a third expert judged any discordant results before entering final values in the database.

Data analysis

Deviations from Hardy-Weinberg equilibrium were evaluated by use of the chi-square test. The association between adverse events groups and genotype was evaluated by use of logistic regression analysis. For 5-HTTLPR, the l/l genotype group was designated as the reference group as this group was expected to have the least adverse events compared to the other genotypes [18,19]. For STin2, the 12/12 genotype was designated as the reference group. All potential confounding variables that were measured in the study were included as covariates in the logistic regression model. Variables that were considered as potential confounders were age, gender, level of education and marital status. All statistical analyses were performed by use of the SPSS package, version 12.0.

RESULTS

Two-hundred-and-fourteen patients diagnosed with major depressive disorder according to the DSM-IV criteria were included in the study. Eighty-six percent of the participants reported having experienced one or more adverse events during the SSRI treatment. Genotyping was not possible for all patients due to problems with the PCR reaction. For the 5-HTTLPR, we were able to determine the genotype of 209 participants. For STin2, this number was 212.

Table 1 presents baseline characteristics of all participants in the study. More female patients, 69.6%, participated in the study and the most frequently prescribed SSRI was paroxetine. The larger part of the population was born in the Netherlands. Other birth countries that were reported by the participants included Belgium (1), Germany (4), the United Kingdom (1), Poland (1), Romania (1), Greece (1) and Indonesia (1). Most patients had a positive family history of depression and 49.5% had received a tricyclic, SSRI or an other antidepressant during a previous episode of depression.

Table 1: Baseline characteristics of study population

		Overall (n=214)	
		% (N)	Mean (SD)
Age			48.48 (10.05)
Gender	Men	30.4 (65)	
	Women	69.6 (149)	
Type of SSRI	Paroxetine	57.9 (124)	
	Fluoxetine	16.4 (35)	
	Fluvoxamine	8.4 (18)	
	Sertraline	9.3 (20)	
	Citalopram	7.9 (17)	
Country of birth	Netherlands	95.3 (204)	
Family history	Yes	79.0 (169)	
	No	21.0 (45)	
Previous treatments	Yes	49.5 (106)	
	No	50.5 (108)	

The frequencies of adverse events are presented in table 2. Overall, more patients with the s/l genotype and the 12/12 genotype reported adverse events, 90.7 and 87.0% respectively. The most frequently occurring adverse events were psychiatric adverse events such as somnolence, insomnia, asthenia, anxiety and agitation. Also, the autonomic adverse events (dry mouth and sweating) were very common.

Percentages of adverse events were slightly higher for the 5-HTTLPR s/s genotype compared to the 5-HTTLPR l/l genotype, with the exception of the autonomic adverse events. For STin2, frequencies of adverse events were higher in the 12/12 genotype group for all adverse events except gastrointestinal adverse events. STin2 frequencies were in Hardy-Weinberg equilibrium, deviations from Hardy-Weinberg equilibrium were borderline significant for 5-HTTLPR ($p = 0.05$).

Table 2: Adverse events frequencies in the genotype groups

	Adverse events % (N)						
	All	Auto-nomic	Central/ peripheral nervous system	Gastro-intestinal	Psychiatric	Uro-genital	General
Genotype *5-HTTLPR*							
s/s	84.3 (43)	52.9 (27)	41.2 (21)	41.2 (21)	66.7 (34)	45.1 (23)	47.1 (24)
s/l	90.7 (78)	64.0 (55)	47.7 (41)	40.7 (35)	74.4 (64)	52.3 (45)	53.5 (46)
l/l	81.9 (59)	59.7 (43)	37.5 (27)	36.1 (26)	61.1 (44)	36.1 (26)	33.3 (24)
STin2							
10/10	83.3 (30)	58.3 (21)	22.2 (8)	38.9 (14)	58.3 (21)	41.7 (15)	50.0 (18)
10/12	86.9 (73)	57.1 (48)	48.8 (41)	41.7 (35)	69.0 (58)	44.0 (37)	36.9 (31)
12/12	87.0 (80)	64.1 (59)	45.7 (42)	37.0 (34)	70.7 (65)	46.7 (43)	51.1 (47)

Table 3 shows the results of the logistic regression analyses on the association between genotype and adverse events. The results show a small positive association between the s/l genotype and the occurrence of any adverse events, OR 1.42 (95%-CI 0.43-4.74). The risk of developing adverse events with the s/s genotype was highest, however not statistically significant, for general adverse events (dermatologic reactions, weight gain, weight loss and fatigue); OR 1.73 95%-CI 0.78-3.84. This was also seen for the heterozygous patients; patients with the s/l genotype had an increased risk of developing general adverse events; OR 2.37 95%-CI 1.13-4.96.

For the STin2 genotype, results are less clear. Overall, patients with the 10/10 genotype did not have an increased risk of adverse events. Patients with the 10/12 genotype appeared to have a small increased risk of adverse events, OR 1.36 (95%-CI 0.48-3.86). For central/peripheral nervous system adverse events however, patients with the 10/10 genotype had a decreased risk of developing SSRI-induced adverse events, OR 0.32 (95%-CI 0.12-0.84).

Table 3: The association between genotype and adverse events

	Adverse events		
	All	Autonomic	Central/peripheral nervous system
Genotype	Adjusted OR (95% CI) *		
5-HTTLPR			
s/s	0.74 (0.24-2.31)	0.72 (0.32-1.60)	1.28 (0.57-2.86)
s/l	1.42 (0.43-4.74)	1.07 (0.51-2.26)	1.52 (0.74-3.13)
l/l	1 (reference)	1 (reference)	1 (reference)
STin2			
10/10	0.70 (0.20-2.44)	0.83 (0.35-1.97)	0.32 (0.12-0.84)
10/12	1.36 (0.48-3.86)	0.70 (0.36-1.36)	1.23 (0.65-2.33)
12/12	1 (reference)	1 (reference)	1 (reference)

* adjusted for age, gender, type of SSRI, level of education and marital status

If analyses were performed for general practitioners' patients only, observed risks on adverse events were not altered much except for the risk on psychiatric adverse events for patients with the s/l genotype (OR 3.0 95% CI 1.18-7.59).

DISCUSSION

We assessed the association between two polymorphisms in the serotonin transporter gene, 5-HTTLPR and STin2, and the occurrence of adverse events during SSRI treatment. Adverse events were categorized as autonomic, central/peripheral, gastrointestinal, psychiatric, urogenital and general adverse events. In our population, 86% of the patients reported one or more adverse events during SSRI treatment. This percentage is comparable to previously reported percentages [15].

Patients with the s/s or s/l genotype seemed to have a higher risk for developing adverse events, especially for general adverse events. Remarkably, the association between genotype and the development of adverse events was stronger in patients heterozygous for 5-HTTLPR. For the STin2 genotype results were less consistent and, patients with the 10/12 or 10/10 genotype did not seem to have an increased risk on developing adverse events. For central/peripheral nervous system adverse events results suggest a decreased risk for patients with the 10/10 genotype. Excluding mental health services patients did not alter these conclusions, for psychiatric adverse events the observed association was even more strong and statistically significant for patients with the 5-HTTLPR s/l genotype.

STin2 frequencies were in Hardy-Weinberg equilibrium in our population, however 5-HTTLPR frequencies were not. Deviations from Hardy-Weinberg would be expected if serotonin transporter genotype was associated with the development of depression. Previously, the association between serotonin transporter genotype and depressive disorder has been studied extensively, however with inconsistent results as reported in two recent meta-analyses [23,24].

Table 3 continued

Adverse events			
Gastrointestinal	Psychiatric	Urogenital	General
	Adjusted OR (95% CI) *		
1.44 (0.62-3.26)	1.29 (0.57-2.92)	1.44 (0.65-3.18)	1.77 (0.80-3.92)
1.13 (0.54-2.38)	1.74 (0.81-3.76)	1.85 (0.90-3.83)	2.37 (1.13-4.96)
1 (reference)	1 (reference)	1 (reference)	1 (reference)
1.25 (0.52-3.04)	0.56 (0.23-1.36)	0.75 (0.32-1.76)	1.04 (0.44-2.44)
1.26 (0.65-2.45)	1.02 (0.51-2.06)	1.06 (0.56-2.01)	0.57 (0.30-1.10)
1 (reference)	1 (reference)	1 (reference)	1 (reference)

* adjusted for age, gender, type of SSRI, level of education and marital status

These results are in general agreement with those of previous studies of the association between 5-HTTLPR genotype and the occurrence of adverse events during SSRI treatment. The 5-HTTLPR s-allele has previously been associated with complaints such as insomnia, agitation, fatigue, dizziness and gastrointestinal complaints during SSRI treatment [18,19]. To our knowledge, the influence of STin2 genotype on the development of adverse events has not been evaluated up till now. The most frequently reported adverse events in our population were psychiatric adverse events (somnolence, insomnia, asthenia, anxiety and agitation), reported by 67.8% of the participants. Also, autonomic adverse events (dry mouth and sweating) were very common among the study participants, 60.3% of the participants reported one or more autonomic adverse events. This is in agreement with previous studies of adverse events reporting anxiety, agitation and insomnia as the most frequently occurring complaints during SSRI treatment [14]. In our population, gastrointestinal complaints were less frequent (39.3%) than expected from previously reported percentages [14]. Since gastrointestinal disturbances are a common reason to change or discontinue SSRI treatment at an early stage, the low percentage of patients with gastrointestinal complaints in our population might have been caused by our criterion to exclude patients from the study if they had used an SSRI for less than six weeks.

Although the risk of developing adverse events was increased for all classes of adverse events in patients with the 5-HTTLPR s/s or s/l genotype, the increased risk was especially strong for the general adverse events. The class of general adverse events contains complaints such as dermatologic reactions, weight change and fatigue. In general, adverse events during SSRI treatment are attributed to stimulation of different serotonin receptors. For example, the 5HT2 receptors are thought to influence mood, anxiety and temperature, but also sexual function, sleep and eating behavior [25]. In addition, SSRIs are known to cause dermal side effects through elevated serotonin concentrations [26]. It could be hypothesized that the 5-HTTLPR polymorphism may moderate some of the SSRI-induced adverse events caused by elevated serotonin levels and stimulation of serotonin receptors. It is however unclear why observed risks were especially elevated for the group of general adverse events.

Unexpectedly, our findings suggest that patients with the 5-HTTLPR s/l genotype have an higher risk for developing adverse events as compared to patients with the s/s genotype. Previous studies of the influence of serotonin transporter genotype on adverse events only performed allele analyses in which patients with the s-allele were compared to patients with the l/l genotype. For this reason, other studies have not reported any differences between the s/s and s/l genotype for the risk on adverse events. In a previous study on the association between serotonin transporter genotype and response on antidepressant treatment within the same study population, we observed that patients with the s/l genotype also had an higher risk of non-response as compared to patients with the s/s genotype [Smits et al, 2005; submitted]. Possibly, biological mechanisms leading to the development of adverse events during SSRI treatment may differ in patients with the s/l genotype compared to patients with the s/s genotype.

Several limitations of this study should be mentioned. First, we are aware of the limited sample size of the study that caused large confidence intervals and less precise estimates. The results were not corrected for multiple testing since this study was only explorative. Study results should therefore be interpreted with caution. Also small genotype effects that are potentially relevant for clinical practice could not be detected due to the small sample size.

Second, study participants reported a number of different adverse events during SSRI treatment. To facilitate analyses on the relation between serotonin transporter genotype and adverse events, each reported complaint was assigned to one of the main adverse events classes (autonomic, central/peripheral nervous system, gastrointestinal, psychiatric, urogenital and general adverse events). This division is identical to a previous study on SSRI-induced adverse events [14]. However, different results might have been obtained if adverse events had been grouped otherwise.

Third, although we controlled for several potential confounders, we cannot exclude residual confounding by unmeasured variables, incorrectly measured variables or the categorization of continuous confounders such as age [27]. Unknown genetic or environmental factors could be involved in the development of adverse events during SSRI treatment. To our knowledge, no information from previous studies is available of factors predicting adverse events occurrence during antidepressant treatment.

Two variables that also could be considered as potential confounders or effect modifiers for the association between SSRI response and genotype are family history of depression and previous antidepressant treatment. In the analyses we did not include these variables since we gathered information on these variables through face-to-face interviews which are probably not an accurate method to record information on family history and previous treatment. However, if family history and previous treatments were included as confounders, observed ORs were not altered.

Furthermore, differences in ethnicity always are a problem in pharmacogenetic research due to the risk of population stratification [28,29]. In the present study, only Caucasian subjects were included in the analyses. Therefore, ethnicity probably will not have biased the study results. 5-HTTLPR or STin2 genotype effects on adverse events in other ethnic groups have not been evaluated yet.

The principal aim of this study was to evaluate whether polymorphisms in the serotonin transporter are useful as a tool to identify patients at risk for developing adverse events during SSRI treatment. Overall, patients with the 5-HTTLPR s/s or s/l genotype appear to have an higher risk of developing adverse events, especially general adverse events. For the STin2 polymorphism results are more diffuse but overall the STin2 genotype appears to have no clear influence on the occurrence of adverse events except for the central/peripheral nervous system adverse events in which the 10/10 genotype is associated with a decreased risk.

Our results suggest that genetic factors could act as risk factors for the development of adverse events during SSRI treatment. However, at this moment available evidence is too limited to incorporate a genetic test in psychiatric practice in order to distinguish between patients that are at risk for adverse events and patients that are not. Further research is needed to confirm the association between serotonin transporter genotype and adverse events before clinicians can use this information as a guideline in prescribing antidepressant treatments.

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6

Serotonin transporter gene polymorphisms and somatic comorbidity during treatment with selective serotonin reuptake inhibitors in depression

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ABSTRACT

Background
It has been suggested that treatment response to selective serotonin reuptake inhibitors (SSRIs) in major depressive disorder is influenced by polymorphisms in the serotonin transporter gene (such as 5-HTTLPR and STin2). In addition, the presence of concomitant somatic disorders, particularly pain-related, may also increase the risk of SSRI non-response. Since the serotonin transporter is thought to be involved in nociceptive processing, an association between genotype and SSRI non-response could be due in part to mediation of the risk of (pain-related) comorbidity in depression. This study evaluates the association between the presence of comorbidity with or without pain and serotonin transporter genotype in major depressive disorder.

Materials and methods
164 patients meeting the DSM-IV criteria for major depressive disorder who used an SSRI for at least six weeks were included. Blood samples or buccal swabs were taken from all participants to determine the 5-HTTLPR and STin2 genotype. Additional information on comorbidity was gathered through face-to-face interviews and general practitioners' files. Reported comorbidity was divided in comorbidity with pain and without pain. The association between genotype and comorbidity was assessed by use of logistic regression.

Results
More comorbidity was reported by patients with the 5-HTTLPR s/s genotype as compared to patients with the s/l and l/l genotype (65.0% versus 44.4 and 48.3%); Odds Ratio (OR) 2.74 (95%-CI 1.04-7.19) for patients with the s/s genotype. Patients with the s/s genotype also had an increased risk on comorbidity with pain, OR 2.47 (95%-CI 0.93-6.52). For STin2, patients with 10/12 genotype appeared to have a decreased risk of total comorbid somatic disorders (OR 0.40 95%-CI 0.18-0.89) and comorbidity with pain (OR 0.74 95%-CI 0.26-2.16).

Discussion
Our findings indicate that patients with the 5-HTTLPR s/s genotype have an increased risk of comorbid somatic disorders. For STin2, patients with the 10/10 or 10/12 genotype have a decreased risk of comorbid complaints. These results imply that the presence of comorbid complaints may act as a confounder in the association between serotonin transporter genotype and treatment response.

INTRODUCTION

Major depression is among the top 5 leading causes of disease burden worldwide. A large part of depressive patients is treated with an antidepressant. Unfortunately, around 30% of these patients do not exhibit an adequate positive response to initial treatment [1] and the effect of an antidepressant can only be evaluated after a relatively long period (about 6 weeks). For this reason, researchers have focused on finding predictors of therapeutical response to antidepressant treatment. Two polymorphisms (5-HTTLPR and STin2) in the serotonin transporter gene (SLC6A4) have been suggested as possible explanations for observed inter-individual differences in treatment response since the serotonin transporter is the main target for antidepressants. However, available evidence is not conclusive and the question whether this genetic variation influences treatment response cannot be conclusively answered at this moment [2].

A large part, 50-80%, of depressive patients report one or more somatic complaints and the majority of these complaints is pain-related [3-6]. There is evidence that treatment response can also be influenced by somatic complaints, especially pain-related complaints. Somatically ill patients with major depression have an increased risk of antidepressant non-response and incomplete remission from the depressive episode [7-9]. Polymorphisms of the serotonin transporter have previously been associated with disorders such as fibromyalgia and irritable bowel syndrome, disorders that are common in patients with major depression [5,10-12]. This raises the question whether antidepressant non-response can either be explained by the presence of a certain genotype or by the presence of somatic comorbidity. It is therefore possible that any association between polymorphisms of the serotonin transporter and antidepressant non-response is attributable to the presence of comorbidity related to these polymorphisms i.e. comorbidity could act as a potential confounder in the association between serotonin transporter genotype and treatment response. In the present study, we evaluated the association between serotonin transporter genotype and the presence of somatic comorbidity among 164 patients with major depressive disorder who were treated with SSRIs.

METHODS

Study population

Data for this study were obtained from a previous study of the influence of 5-HTTLPR and STin2 on treatment effect in depression [Smits et al, 2005; submitted]. Since standardized information on comorbid somatic disorders was only available for patients who were treated by their general practitioner, the analyses for the present study were restricted to these patients (n=164). All patients were recruited from 16 general practice centres of the Registration Network Family Practices of Maastricht University [13] and met the DSM-IV criteria for major depressive disorder as diagnosed by their general practitioner. Other inclusion criteria were: between 18 and 64 years old, Caucasian and SSRI use for at least 6 weeks. Exclusion criteria for the study were: additional diagnoses on DSM-axis-I other than an anxiety disorder, diagnosis of bipolar disorder, pregnancy or inability to complete an interview in Dutch.

Informed consent was obtained from all participants. The study was approved by the Medical Ethics Committee of Maastricht University/University Hospital Maastricht.

Data collection
Blood samples were taken from all participants using the BD Vacutainer system with EDTA tubes. If it was not possible to take blood samples from a participant, buccal cells were collected by use of Sterile Omniswabs from Whatman. Information on comorbidity during SSRI treatment was collected through structured face-to-face interviews by a trained interviewer and from the patients' general practitioners' files. During the interviews, patients were asked if they suffered from coronary heart diseases, hypertension, lung problems, kidney problems, diabetes, arthritis or any other (chronic) medical problem. Interviews were performed blinded for genotype.

Pain status
Known comorbidity was divided into two groups: conditions with or without painful symptoms associated with the disorder. This classification was done by two clinicians (MP and JS) independent of each other without any other knowledge of the patient, including their genotype. Each comorbid somatic disorder, either self-reported or from the general practitioner's file, was assigned to one of the two groups as shown in table 1. If patients reported both painful comorbidity and comorbidity without pain, they were assigned to the "pain" group.

Genotyping
Genomic DNA was isolated either from 8 ml whole blood (EDTA tubes) using the Wizard Genomic DNA purification kit (Promega, Leiden, the Netherlands) or from buccal cells (2 Omniswabs per sample, Whatman) with the QIAamp DNA Mini Kit (Westburg, Leusden, The Netherlands). For both procedures the manufacturers' protocols were followed.

Determination of the 5-HTTLPR genotype was performed as previously described [14], with some modifications. A FAM-labeled forward primer: 5'-GGCGTTGCCGCTCT GAATGC-3' was used together with the following reverse primer: 5'-GAGGGACTGAGCTGGACAACCCAC-3'. Polymerase chain reaction (PCR) was carried out in 96-well microtiterplates on a Biometra T1 Thermocycler (Westburg, Leusden, The Netherlands). We used approximately 10 – 100 ng of genomic DNA in a 25 µl reaction mixture containing 1 x PCR buffer (Invitrogen, Breda, The Netherlands), 0.2 mM dNTPs, 0.4 µM of each primer, 0.75 mM $MgCl_2$, and 1U Taq DNA polymerase (Invitrogen, Breda, The Netherlands). Cycling conditions were: initial 3 min. denaturation at 95°C; 5 cycles of denaturation at 94°C for 30 sec., annealing at 65°C (touch down 0.3°C) for 1 min. and extension at 72°C for 1 min.; 35 cycles of denaturation at 94°C for 30 sec., annealing at 63°C for 1 min. and extension at 72°C for 1 min.; and a final extension for 10 min. at 72°C. Determination of the STin2 genotype was performed as previously described [15], with some modifications. A FAM-labeled forward primer: 5'- GTCAGTATCACAGGCTGCGAG -3' was used together with the following reverse primer: 5'- TGTTCCTAGTCTTAC GCCAGTG -3'.

Table 1*:* Classification of somatic comorbidity according to the presence of pain

No pain	Pain
Allergies	Abdominal/stomach pain
Asthma	Angina pectoris
Atherosclerosis	Appendicitis
Benign neoplasm	Arthritis
Cataract	Carpal tunnel syndrome
Cirrhosis	Cervical spinal complaints
COPD	Cholelithiasis
Dermatological complaints	Chondropathy
Diabetes	Conjunctivitis
Graves' disease	Coronary disease
Hepatitis	Diafragmatic hernia
Hypertension	Diplegia
Hyperthyroidism	Diverticulosis/diverticulitis
Hyperventilation	Facial neuralgia
Hypertension	Fibromyalgia
Hypothyroidism	Fissura ani
Inguinal hernia	Fractures
Lung diseases	General pain complaints
Kidney diseases	Head ache
Polyposis colitis	Hip complaints
Presbyacusis	Irritable bowel syndrome
Psoriasis	Joint problems
Strabismus	Knee compliants
Stroke	Low back pain
Tachycardia	Lung embolism
	Migraine
	Myocardial infarction
	Oesophagitis
	Osteoarthritis
	Peptic ulcer
	Repetitive strain injury (RSI)
	Rheumatoid arthritis
	Sciatica
	Shoulder complaints
	Sinusitis
	Stomach problems
	Urolithiasis
	Varices
	Whiplash syndrome

Polymerase chain reaction (PCR) was carried out as described above. Cycling conditions were: initial 3 min. denaturation at 94°C; 35 cycles of denaturation at 94°C for 30 sec., annealing at 60°C for 45 sec. and extension at 72°C for 45 sec.; and a final extension for 8 min. at 72°C.

For each sample, PCR products were pooled (1 µl each) and subsequently size-resolved on an ABI3100 Genetic Analyzer, using 36 cm capillaries filled with POP6 polymer. The peaks corresponding to the different alleles (STin2.9: 248 bp, STin2.10: 265 bp, STin2.12: 299 bp, 5HHT Short: 478 bp and 5HHT Long: 520 bp) were identified using Genescan Analysis software version 3.7 (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Two researchers independently scored genotypes, and a third expert judged discordant results before entering final values in the database.

Data analysis

Deviations from Hardy-Weinberg equilibrium were analysed by use of the chi-square test. The risk on comorbid somatic disorders in the presence of a certain serotonin transporter genotype was analysed by use of logistic regression analysis. For 5-HTTLPR, the l/l genotype group was designated as the reference group as this group was expected to have lower comorbidity rates as compared to the other genotypes [2]. For STin2, the 12/12 genotype was designated as the reference group [2]. Variables that were evaluated as potential confounders were age, gender, level of education and marital status; these variables were included as covariates in the logistic regression model. All statistical analyses were performed using the SPSS package, version 12.0.

RESULTS

For the 5-HTTLPR polymorphism, we were able to determine the genotype in 161 patients, for the STin2 polymorphisms this number was 162. In table 2 the baseline characteristics for these patients are presented. More female patients (71.3%) than male participated in the study and the most frequently prescribed antidepressant was paroxetine.

The mean age in our population was 50.1 years (SD 9.5); range 23-64. Almost all patients were born in the Netherlands, birth countries that were also reported in our population were Germany (4), Romania (1), United Kingdom (1), Poland (1) and Indonesia (1). Most patients reported a positive family history of depressive complaints and 48.2% had already received an antidepressant during a previous episode of depression. Genotype frequencies were in Hardy-Weinberg equilibrium for STin2. For 5-HTTLPR, however, frequencies were not in Hardy-Weinberg equilibrium ($p = 0.03$).

Table 2: Characteristics of the study population

		Overall
		% (N)
Gender	Women	71.3 (117)
SSRI	Paroxetine	61.6 (101)
	Fluoxetine	17.1 (28)
	Fluvoxamine	8.5 (14)
	Sertraline	7.9 (13)
	Citalopram	4.9 (8)
Country of birth	Netherlands	95.1 (156)
Family history of depression	Yes	81.7 (134)
Previous treatments for depression	Yes	48.2 (79)
5-HTTLPR genotype*	s/s	24.8 (40)
	s/l	39.1 (63)
	l/l	36.0 (58)
STin2 genotype*	10/10	15.4 (25)
	10/12	41.4 (67)
	12/12	43.2 (70)
Somatic comorbidity	Yes	51.2 (84)
Painful somatic comorbidity	Yes	49.4 (81)

* numbers do not add up to 164 due to missings

Frequencies of all comorbid complaints are shown in table 3. Overall, 51% of the participants reported one or more somatic disorders (N= 84). Almost all (N= 81) of these patients suffered from one or more painful complaints. For 5-HTTLPR, patients with the s/s genotype reported more comorbidity as compared to patients with the s/l and l/l genotype 65.0% versus 44.4 and 48.3% respectively.

Table 3: Percentages of comorbidity according to presence of pain in different genotypes

	Comorbidity		
	No somatic comorbidity % (N)	Somatic comorbidity without pain % (N)	Somatic comorbidity with pain % (N)
5-HTTLPR			
s/s	35.0 (14)	5.0 (2)	60.0 (24)
s/l	55.6 (35)	1.6 (1)	42.8 (27)
l/l	51.7 (30)	-	48.3 (28)
STin2			
10/10	44.0 (11)	-	56.0 (14)
10/12	55.2 (37)	-	44.8 (30)
12/12	44.3 (31)	2.8 (2)	52.9 (37)

For STin2, patients with the 10/10 and 12/12 genotype reported similar numbers of somatic problems (56.0% versus 55.7%). Patients with the 10/12 genotype reported less somatic comorbidity; 44.8%.

Table 4 shows crude and adjusted odds ratios for 5-HTTLPR and STin2 genotypes in relation to the presence of somatic comorbidity. For total somatic comorbidity (with or without pain), patients with the s/s genotype were more likely to report one or more somatic complaint; OR 2.74 (95% CI 1.04-7.19). A statistically non-significant risk decrease of comorbid complaints was found for patients heterozygous for 5-HTTLPR genotype. The OR for s/s versus l/l genotype did not change much after exclusion of patients with non-painful comorbidity from the comorbidity group(OR=2.47, 95%-CI: 0.93-6.52). For comorbidity without pain, numbers were too small for the calculation of ORs.

Patients with the STin2 10/10 and 10/12 genotypes were less likely to suffer from somatic comorbidity: OR=0.40 (95%-CI: 0.18-0.89) for patients with the 10/12 genotype and OR=0.68 (95%-CI: 0.24-1.95) for patients with the 10/10 genotype. Again, these figures did not change much after exclusion of patients with non-painful comorbidity: OR 0.43 95% CI 0.19-0.96 and OR 0.74 95% CI 0.26-2.16 for patients with the 10/12 and 10/10 genotype, respectively.

Table 4: Associations between 5-HTTLPR and STin2 genotypes and comorbidity

	Unadjusted OR		**Adjusted OR** *	
	Total somatic comorbidity	Somatic comorbidity with pain	Total somatic comorbidity	Somatic comorbidity with pain
5-HTTLPR				
s/s	1.99 (0.87-4.55)	1.84 (0.80-4.24)	2.74 (1.04-7.19)	2.47 (0.93-6.52)
s/l	0.86 (0.42-1.75)	0.83 (0.40-1.69)	0.73 (0.31-1.76)	0.69 (0.28-1.66)
l/l	1 (reference)	1 (reference)	1 (reference)	1 (reference)
STin2				
10/10	1.01 (0.40-2.54)	1.07 (0.42-2.68)	0.68 (0.24-1.95)	0.74 (0.26-2.16)
10/12	0.64 (0.33-1.27)	0.68 (0.35-1.34)	0.40 (0.18-0.89)	0.43 (0.19-0.96)
12/12	1 (reference)	1 (reference)	1 (reference)	1 (reference)

* adjusted for age, gender, type of SSRI, level of education and marital status

DISCUSSION

In this study we analysed the association between two polymorphisms (5-HTTLPR and STin2) in the serotonin transporter gene and somatic comorbidity in depression. In addition, we evaluated whether painful somatic disorders were associated with 5-HTTLPR or STin2. Previous studies have reported that 50% - 80% of the persons suffering from major depression report somatic disorders [3-5].

This is in accordance with results from the present study in which 51% of all patients suffer from at least concomitant somatic disorder. Logistic regression results indicated that patients with the 5-HTTLPR s/s genotype were more likely to to have an increased risk of somatic comorbidity. For the s/l genotype, no clear association with the presence of comorbidity was observed. For the STin2 genotype, patients with the 10/10 and 10/12 genotype had a lower risk of a concomitant somatic disorder.

Our results are in agreement with previous studies on the association between 5-HTTLPR genotype and somatic comorbidity like fibromyalgia, irritable bowel syndrome and migraine [10-12]. Little evidence is available on the association between STin2 and comorbid somatic disorders, but the 10/10 genotype has previously been associated with migraine [16]. Our findings however indicate a decreased risk of comorbid somatic disorders for patients with the 10/10 or 10/12 genotype.

Some limitations of this study should be mentioned. First, we are aware of the limited sample size of the study which gave rise to large confidence intervals. Alphas were not corrected for multiple testing since this study was only exploratory.

Second, comorbidity was assessed through face-to-face interviews and through individual general practitioners' files. Using this method, we aimed to gather information on comorbid somatic disorders that patients experienced during the last few years. However, gaps in general practitioners' files and incorrect patient information might have led to misclassification of some complaints. We expect this misclassification to be non-differential, i.e. independent of genotype. Since non-differential misclassification usually leads to the convergence of effect estimates, this could have led to a dilution of the true association between genotype and comorbidity [17]. In that case, the true association between genotype and comorbidity could be stronger than the association observed in our population.

Third, differences in ethnicity can be a problem in pharmacogenetic research due to the risk of population stratification [18,19]. In the present study, only Caucasian subjects were included in the analyses. Therefore, ethnicity probably will not have biased the study results.

In a systematic review we found evidence for a less favorable response to SSRIs in depressive patients with the 5-HTTLPR s/s genotype [2]. The principal aim of this study was to assess the association between serotonin transporter genotype and somatic comorbidity in depression, in order to evaluate whether the presence of somatic comorbidity could be an explanation for observed non-response during treatment with antidepressants.

Patients with the 5-HTTLPR s/s genotype also appear to have an increased risk of somatic comorbidity. This suggest that the association between the 5-HTTLPR s/s genotype and treatment outcome could have been confounded by the presence of somatic comorbidity. For the STin2 polymorphism, the evidence points to a decreased risk on somatic comorbidity in patients with the 10/10 and the 10/12 genotype. However, due to the small sample size, results should be interpreted with caution.

Based on our results, it is possible that concomitant disorders act as confounding variables in studies on the association between serotonin transporter genotype and SSRI treatment outcome in depressive patients. Further research is needed to confirm the association between serotonin transporter genotype and somatic disorders. In the meantime, researchers evaluating the influence of serotonin transporter genotype on treatment effect, should keep in mind that observed genotype effects on treatment outcome might be biased by the presence of somatic comorbidity.

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7

Does pre-treatment testing on serotonin transporter polymorphisms lead to earlier effects of drug treatment in major depression? A decision analytical model

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ABSTRACT

Introduction
Since approximately 40% of depressive patients do not sufficiently respond to treatment with selective serotonin reuptake inhibitors (SSRI) and the period that the adequacy of the treatment can be assessed is relatively long, a test to identify potential non-responders could be useful in the treatment of depression. Serotonin transporter gene (SLC6A4) variations have been reported to explain inter-individual differences in SSRI effects. In this study we assess, by use of a decision analytical model, whether pre-treatment genetic testing for 5-HTTLPR genotype could be an efficient tool in the treatment of depression.

Methods
We constructed a clinical decision model to compare the current treatment strategy in which all patients start receiving SSRI treatment to an alternative strategy in which a genetic test is used to determine the initial class of antidepressant. SSRI remission rates after 6 weeks of treatment were varied in a sensitivity analysis to test the robustness of the model.

Results
If genetic testing was performed prior to antidepressant prescription, 64.6% of the patients were in remission after 6 weeks of treatment as compared to 60.0% in the current (no-testing) treatment strategy. After 12 weeks, the numbers were 79.5% if an SNRI was used as the first-choice alternative, and 83.2% if a TCA was used as the first-choice alternative as compared to 76.7% in the current strategy. Sensitivity analyses showed that the model was robust to variation of probability estimates within their plausible ranges.

Discussion
Our findings indicate that the use of a genetic test prior to antidepressant treatment would increase the number of patients in remission early in treatment. Since this model is theoretical, it would be necessary to evaluate the use of a genetic test in a randomized clinical trial before the introduction of this strategy in psychiatric practice can be recommended.

INTRODUCTION

Clinical pharmacogenetics is a rapidly expanding domain of research that attempts to describe the influence of genetic variation on treatment response. For a broad range of diseases, it promises drug therapy targeted to the individual patient. However, responses to drugs are complex and are influenced by both environmental and genetic factors. To evaluate the importance of genetic factors in drug reactions, it would be useful to evaluate what would be the minimum difference in treatment effect between genotypes to influence clinical practice for a certain disease.

One of the diseases in which pharmacogenetics could be advantageous to identify patients at risk for treatment non-response, is major depressive disorder. Approximately 30-40% of all depressive patients do not have an adequate therapeutic response on selective serotonin reuptake inhibitors (SSRIs), the most frequently prescribed antidepressants and the time that is needed to evaluate treatment outcome is relatively long, about 6 weeks. Moreover, patients who do not respond to treatment experience a continuous burden of the depression. It has been previously suggested that SSRI response is, at least partly, under genetic control. A polymorphism in the serotonin transporter gene, 5-HTTLPR, has been found to explain part of the observed differences in clinical response [1-13]. If polymorphisms indeed are (partly) involved in SSRI non-response, genetic testing prior to antidepressant prescription and subsequently choosing the antidepressant that has the best expected therapeutic effect, might help caregivers in achieving faster treatment effects. In this study we compared two antidepressant treatment strategies (genetic testing with modification of treatment according to genotype/no genetic testing) in a decision analytical model.

METHODS

Decision analytical model

Decision analysis is a modelling instrument that facilitates a clinician's choice in a specific situation by comparing alternatives and indicating the optimal strategy. We constructed a decision analytic model that distinguishes two situations: the present situation in which depressive patients are treated according to general clinical guidelines and the alternative situation in which genetic testing for 5-HTTLPR is performed and antidepressant prescription is based on these test results. In the current situation we assumed that all patients with a first episode of major depression received a SSRI since this is the medication of first choice in the Netherlands. After 6 weeks the situation was evaluated and patients could be in remission or not. If not in remission, patients either had experienced a non-response (no therapeutically response to the SSRI) or had stopped the treatment due to severe adverse events. In the case of non-response the dose of the SSRI was adjusted, after severe adverse events another antidepressant was prescribed. In the alternative situation the choice of an antidepressant was based on the genetic test results. Patients with a genotype associated with a diminished chance of a clinical response to SSRIs (the s/s and s/l 5-HTTLPR genotype) were given a TCA or a SNRI in a high dosage. The decision analytic model which, due to the large number of nodes, is only partly shown in figure 1, was based on expert opinions and clinical guidelines used in the Netherlands for treating depression.

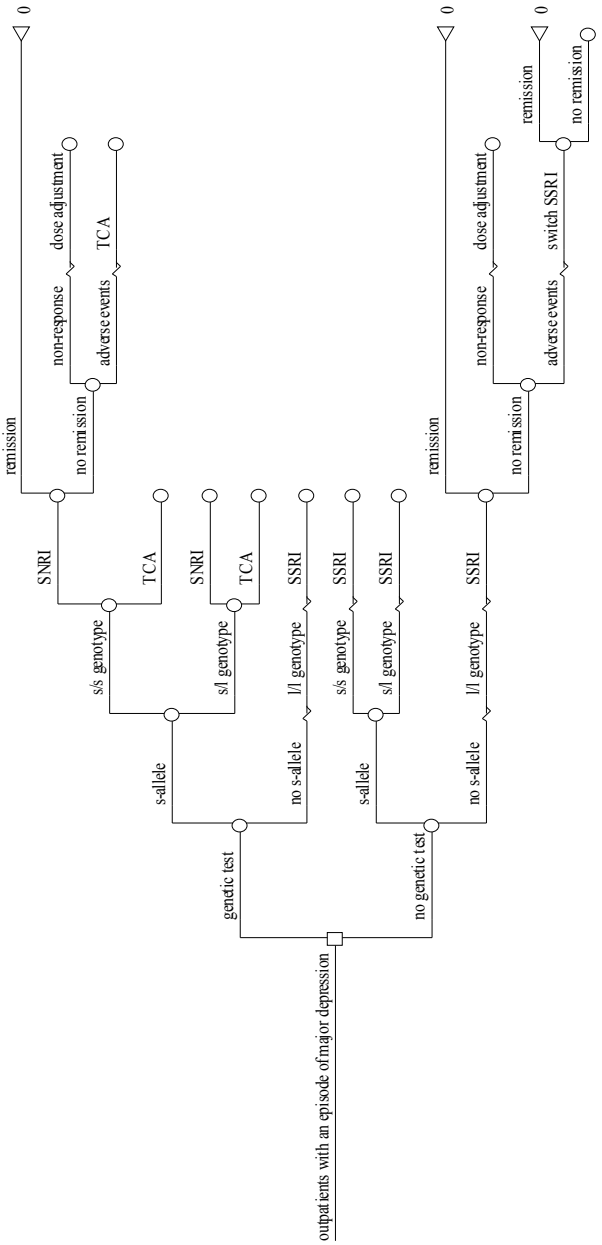

Figure 1: Decision analytical model

The overall duration of the model was set at 12 weeks. We expected that the effect of genetic testing would be most obvious in this period because patients would receive a suitable antidepressant sooner and the number of patients in remission after this period would increase.

Patients with major depression that were not genetically tested all started with SSRI treatment. In case of non-response to the SSRI within 6 weeks after initiating treatment, the dose of the SSRI was adjusted. If patients experienced serious adverse events within 6 weeks after initial treatment with an SSRI, they switched to another SSRI. After 12 weeks (not shown in the figure), treatment effect was again evaluated, in case of non-response after dose adjustment of the first SSRI, patients were switched to another SSRI, in case of adverse events patients received an SNRI. In case of non-response after the second SSRI, patients received an SNRI.

Patients with major depression that were genetically tested for 5-HTTLPR received an antidepressant in accordance with their test results. Patients with a l/l genotype received an SSRI and were treated identically to patients that were not genetically tested. Patients with an s/s or s/l genotype received an SNRI or TCA and were not treated with an SSRI. For patients with an s/s or s/l genotype, treatment with an SNRI or TCA was identical to treatment with SNRI or TCA for patients who were not genetically tested. At 6 weeks after initial treatment, treatment effect was evaluated and in case of non-response patients received a dose adjustment. If patients experienced adverse events, they received another TCA. At 12 weeks (not shown in the figure) patients with non-response were changed to another TCA instead, patients with adverse events received a second TCA or lithium augmentation (not shown in the figure).

For the construction of the decision analytic model the following assumptions were made: 1) antidepressants are prescribed according to the present guidelines for the treatment of depression in the Netherlands indicating that after the second SSRI without sufficient therapeutical response, treatment should be continued with an antidepressant from a different class, 2) after administration of an TCA or SNRI, an SSRI would not be prescribed, 3) after an TCA, an SNRI would not be prescribed, 4) percentages of patients relapsing during the period of 12 weeks were equal for all treatments, 5) if in remission, patients were treated until the end of the 12 week period.

Clinical outcomes

The probabilities used in the model are presented in table 1. The frequencies of the different genotypes were calculated from two previous studies [2] [Smits et al, 2005; submitted]. We estimated that the l/l genotype would be present in 33.2% of the patients. In the remaining patients (66.8%), we assumed that 65.7% would have an s/l genotype and 34.3% would have an s/s genotype. Remission rates, adverse event rates and non-response rates in the current situation were based on the literature or, if no information could be retrieved, on expert opinion. Remission rates for the first 6 week-period were calculated based on data from previous studies [2] [Smits et al, 2005; submitted] on the influence of serotonin transporter polymorphisms on the effect of SSRIs. Since not all information for the model could be extracted from the literature, a team of experts was asked to participate in the construction of the model and the estimation of remission rates and non-response rates and their corresponding sensitivity range. Experts contacted for this purpose were seven psychiatrists with a large experience in the treatment of mood disorders in the Netherlands. All experts were asked to estimate rates and sensitivity ranges that could not be retrieved from literature.

Table 1: Probabilities for decision analytical model

Probability			Value (%)	Sensitivity range (%)	Source
	Genotype				
Frequency	"s-allele"	s/s	34.3	0-60	Calculated [1,2]
		s/l	65.7	20-80	Calculated [1,2]
	"l-allele"	l/l	33.2	20-80	Calculated [1,2]
SSRI					
Remission	1st SSRI	s/s	59.9	0-80	Calculated [1,2]
		s/l	49.5	20-100	Calculated [1,2]
		l/l	73.8	40-100	Calculated [1,2]
	Titration 1st SSRI	All	40.0	10-60	Expert opinion
	2nd SSRI*	s/s	40.0	10-80	Expert opinion
		s/l	40.0	10-80	Expert opinion
		l/l	60.0	10-80	Expert opinion
No remission (non-response rates)	1st SSRI	All	60.0	55-100	Expert opinion [19-22]
SNRI					
Remission	1st SNRI	All	60.0	30-80	Expert opinion
	Titration 1st SNRI	All	40.0	10-60	Expert opinion
No remission (non-response rates)	1st SNRI	All	60.0	60-90	Expert opinion
TCA					
Remission	1st TCA	All	60.0	20-80	Expert opinion
	Titration 1st TCA	All	40.0	20-60	Expert opinion
	2nd TCA*	All	60.0	15-80	Expert opinion
No remission (non-response rates)	1st TCA	All	30.0	20-80	Expert opinion
Other					
Remission	1st TCA after SNRI	All	40.0	15-80	Expert opinion

* after immediate adverse events on 1st antidepressant

To obtain the best estimate, all individual rates were averaged. We assumed an SSRI remission rate of 59.9% for patients with an s/s genotype, for patients with the s/l genotype an SSRI remission rate of 49.5% and for the l/l genotype an SSRI remission rate of 73.8%. For the second SSRI remission rates were assumed to be 40.0% for the s/s and s/l genotypes and 60.0% for the l/l genotype. SNRI remission rates, TCA remission rates, non-response rates and adverse events rates were assumed to be identical for all genotypes. Because not all rates could be extracted from previous studies, assumptions on the probabilities for the different genotypes had to be made.

"No remission" rates presented in table 1 are the rates for patients that experienced a non-response after medication. Adverse events rates were defined in the model as all (100%) patients without remission minus the non-responders. Sensitivity ranges around values were estimated based on expert opinions. The principal outcome measure was the percentage of patients in remission after 6 weeks and 12 weeks.

Analyses

To test the robustness of the decision analysis results concerning the necessary assumptions, univariate sensitivity analyses were performed by using predetermined value ranges for a specific variable. This is the most common form of sensitivity analysis in which one parameter is varied across a specified range. The key parameter in the sensitivity analyses was SSRI remission rate after 6 weeks. In addition, a threshold analysis was used to determine the threshold value; this is the value of a variable at which the conclusion on which strategy is to be preferred would change. Analyses were performed in TreeAge version 3.5.

RESULTS

Main outcome measures of the decision analytical model were percentages of patients in remission for the different genotypes. These are shown in table 2. After 6 weeks, 60.0% of the patients were in remission if no genetic test was performed and all patients would have used an SSRI, after 12 weeks this was 76.7%. If genetic testing was used to guide decisions on antidepressant prescription and patients with the s/s and s/l genotype received an SNRI, 64.6% of the patients were in remission after 6 weeks. After 12 weeks, this was 79.5%. If patients with the s/s and s/l genotype received an TCA after genetic testing, 64.6% of the patients were in remission after 6 weeks and 83.2% after 12 weeks.

Table 2: Percentages patients in remission after 6 and 12 weeks after start of initial therapy

Treatment strategy			Patients in remission after 6 weeks (%)	Patients in remission after 12 weeks (%)
Genetic testing	s/s	SNRI	13.7	17.4
	s/l	SNRI	26.3	33.3
	l/l	SSRI	24.6	28.8
	Total		**64.6**	**79.5**
	s/s	TCA	13.7	18.6
	s/l	TCA	26.3	35.8
	l/l	SSRI	24.6	28.8
			64.6	**83.2**
No genetic testing (reference strategy)	s/s	SSRI	13.7	17.4
	s/l	SSRI	21.7	30.5
	l/l	SSRI	24.6	28.8
	Total		**60.0**	**76.7**

Table 3: Sensitivity analysis

Current Strategy			Alternative strategy		
Remission rates	**Patients in remission (%)**		**Remission rates**	**Patients in remission (%)**	
	6 weeks	12 weeks		6 weeks	12 weeks
s/s SSRI 0.60	13.7	17.4	s/s SNRI 0.60	13.7	17.4
s/l SSRI 0.60	26.3	33.3	s/l SNRI 0.60	26.3	33.3
l/l SSRI 0.60	20.0	26.4	l/l SSRI 0.60	20.0	26.4
Total	*60.0*	*77.1*	*Total*	*60.0*	*77.1*
s/s SSRI 0.40	9.2	14.7	s/s TCA 0.60	13.7	18.6
s/l SSRI 0.64	27.5	34.3	s/l TCA 0.60	26.3	35.8
l/l SSRI 0.70	23.3	28.1	l/l SSRI 0.60	20.0	26.4
Total	*60.0*	*77.1*	*Total*	*60.0*	*80.8*
s/s SSRI 0.45	10.4	15.3	s/s SNRI 0.60	13.7	17.4
s/l SSRI 0.60	26.3	33.3	s/l SNRI 0.60	26.3	33.3
l/l SSRI 0.70	23.3	28.1	l/l SSRI 0.70	23.3	28.1
Total	*60.0*	*76.7*	*Total*	*63.3*	*78.8*
s/s SSRI 0.50	11.5	15.9	s/s TCA 0.60	13.7	18.6
s/l SSRI 0.57	25.2	32.5	s/l TCA 0.60	26.3	35.8
l/l SSRI 0.70	23.3	28.1	l/l SSRI 0.70	23.3	28.1
Total	*60.0*	*76.5*	*Total*	*63.3*	*82.5*
s/s SSRI 0.60	13.7	17.4			
s/l SSRI 0.53	23.0	31.4			
l/l SSRI 0.70	23.3	28.1			
Total	*60.0*	*76.9*			

Table 3 continued

Current Strategy			Alternative strategy		
Remission rates	**Patients in remission (%)**		**Remission rates**	**Patients in remission (%)**	
s/s SSRI 0.65	14.8	18.1			
s/l SSRI 0.50	21.9	30.7			
l/l SSRI 0.70	23.3	28.1			
Total	*60.0*	*76.9*			
s/s SSRI 0.40	9.2	14.7	s/s SNRI 0.60	13.7	17.4
s/l SSRI 0.60	26.3	33.3	s/l SNRI 0.60	26.3	33.3
l/l SSRI 0.75	24.5	29.0	l/l SSRI 0.75	24.5	29.0
Total	*60.0*	*77.0*	*Total*	*64.5*	*79.7*
			s/s TCA 0.60	13.7	13.7
			s/l TCA 0.60	26.3	35.8
			l/l SSRI 0.75	24.5	29.0
			Total	*64.5*	*83.4*
s/s SSRI 0.40	9.2	14.7	s/s SNRI 0.60	13.7	17.4
s/l SSRI 0.55	24.2	32.0	s/l SNRI 0.60	26.3	33.3
l/l SSRI 0.80	26.6	29.8	l/l SSRI 0.80	26.6	29.8
Total	*60.0*	*76.5*	*Total*	*66.6*	*80.5*
s/s SSRI 0.50	11.5	15.9	s/s TCA 0.60	13.7	18.6
s/l SSRI 0.50	21.9	30.7	s/l TCA 0.60	26.3	35.8
l/l SSRI 0.80	26.6	29.8	l/l SSRI 0.80	26.6	29.8
Total	*60.0*	*76.4*	*Total*	*66.6*	*84.2*
s/s SSRI 0.70	16.0	18.7			
s/l SSRI 0.40	17.4	28.0			
l/l SSRI 0.80	26.6	29.8			
Total	*60.0*	*76.5*			

Sensitivity analyses were performed in order to test the robustness of the model. The outcomes of the sensitivity analyses are shown in table 3. Since experts agree that approximately 60% of the patients respond on an initial treatment with SSRIs after 6 weeks, remission rates in the current treatment strategy always add up to 60% at 6 weeks.

The genetic testing strategy had an advantage over the current treatment strategy at 6 and 12 weeks of treatment regardless of alterations in baseline values within the specified sensitivity ranges.

Threshold values for the variables in the decision analytical model were calculated. In our model the preferred strategy is the genetic testing strategy. The threshold value therefore represents the baseline value that causes a change to an advantage of the current testing strategy at 12 weeks. However, in our model for all values within the sensitivity range, the preferred strategy at 12 weeks is the genetic testing strategy.

DISCUSSION

In this decision analytical study, we examined the utility of a genetic test and consequently antidepressant prescription according to the results of this test. We assessed whether this test could be used as a tool to achieve pharmacotherapeutic treatment effects in depression sooner. The introduction of such a genetic test as a tool in the choice of type of antidepressant could be advantageous if more patients would experience remission or if patients experienced earlier remission compared to the current situation in which the choice of an antidepressant is often based on experience and preference of the treating physician.

As expected, we observed an effect of genetic testing at 6 weeks after initial treatment; a higher percentage of patients were in remission in the genetic testing strategy compared to the strategy in which all patients received an SSRI. For patients with the s/s or s/l genotype that received an SNRI after genetic testing, the response rates converge at 12 weeks after start of treatment. For patients receiving TCA treatment however, the observed differences in the two strategies at 12 weeks were even larger than observed at 6 weeks. The absolute risk reduction (ARR), the difference between the two strategies, at 6 weeks is 4.6%. The inverse of the ARR (1/ARR) represents the number of patients that we need to treat according to the genetic testing strategy in order to cause one additional patient in remission. Our results suggest that 21 patients would have to be tested to improve the 6 week results for one patient if an antidepressant was prescribed in accordance with the genetic test result. To improve the 12 weeks results for one patient, 35 patients would have to be tested in case of an SNRI as first treatment choice for the s/s and s/l genotype and 15 patients would have to be tested in case of an TCA as first treatment choice for the s/s and s/l genotype.

Premature antidepressant discontinuation is common in depression. Since early discontinuation is associated with an increased risk of relapse and recurrence [14-16], it is important for depressive patients to adhere to the prescribed antidepressant. In addition overall medical costs are highest in patients that discontinue or switch antidepressants early in treatment [15]. Achieving a treatment response sooner would therefore be valuable for patients as well as caregivers.

Our results indicate that genetic testing in the psychiatric practice would lead to a higher percentage of patients in remission at 6 - 12 weeks after the initial start of the SSRI treatment. Moreover, in a recent study on SSRIs in depression, we observed that patients with the 5-HTTLPR s/s or s/l genotype experienced more adverse events during SSRI treatment [Smits et al submitted]. Since adverse events are the most important reason for early treatment discontinuation [17], genetic testing in depression would not only lead to a higher percentage of remitted patients early in treatment but also to a decrease of patients with SSRI-induced adverse events by prescribing SNRIs or TCAs for patients with s/s or s/l genotypes.

Several limitations of this study should be mentioned. First, in common with other modeling studies, this study is a restricted representation of reality. The course of a depressive episode and the effect of a psychopharmaceutical treatment varies among patients. Patients can experience different treatment effects, for example partial remission, which are not incorporated in the model. However, by modeling treatment options for the theoretical 'average' depressed patient, we were able to use available evidence from literature on general treatment effects.

Second, since literature provides limited information on remission, non-response and adverse events rates, especially after the first 6 weeks of antidepressant treatment, several rates that were used in the model are based on expert opinion. Although we aimed for the best estimate by consulting several experts and averaging their estimates, it is possible that the rates used in the model are not optimal. However, we used broad sensitivity ranges to assess the influence of changes in remission, non-response or adverse events rates. Our results appeared to be robust showing an advantage for genetic testing early in treatment for all possible SSRI remission rates.

Third, our decision analytical model reflects current guidelines for antidepressant prescription in the Netherlands. However, based on individual patient characteristics and patient history, caregivers could (and do) deviate from the guidelines. In our decision analytical model, we did not take these deviations into account since we assumed that these deviations from the current guidelines were not common. Moreover, the options for a caregiver in the treatment of a depressive patients are diverse; it is not possible to include all possible deviations for the guidelines in a decision analytical model.

In our decision analytical model we focused on treatment response and we did not incorporate any costs at all. Obviously, costs related to depressive disorder or its treatment, are costs of the drug, costs of the caregiver and indirect costs, such as missed days at work.

For the larger part, overall costs from a societal perspective in the treatment of depression are largely determined by the costs of failure of treatment. Hence, reducing overall costs is possible through reducing the number of treatment failures, and thus the number of clinical consultations and referrals. The actual cost of the drug is of minor influence on overall costs in depression treatment [18].

Using a genetic test prior to antidepressant prescription increased the number of patients in remission early in treatment, thereby reducing the number of consultations needed by individual patients and resulting costs. In addition, achieving faster treatment effects will also result in a decrease in indirect costs, such as missed days at work, since patients will be able to return to normal daily activities sooner. We hypothesize that introducing a genetic test in psychiatric practice can make savings in overall treatment costs which will make up for the costs of the genetic test. However, this hypothesis should be tested in a cost-effectiveness analysis.

The principal aim of this study was to evaluate whether using a genetic test would be useful to achieve faster treatment effects in depression. Overall, the decision analytical model implies that the implementation of a genetic test prior to antidepressant prescription would lead to a higher number of patient in remission early in treatment. Early discontinuation is associated with an increased risk of relapse or recurrence and with higher overall medical costs. In addition, switching antidepressants is also associated with higher overall medical costs. Achieving faster treatment effects and reducing the number of patients discontinuing or switching antidepressant treatment, could therefore lead to a decrease in the number of recurrences or relapses and lower overall costs. However, since this decision analytical model is only theoretical, it would be necessary to evaluate the use of a genetic test in a randomized clinical trial. In addition, costs should be incorporated in the model to assess the cost-effectiveness of the genetic test before this method could be implemented in every-day psychiatric practice.

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8

General discussion

INTRODUCTION

Pharmacogenetics is an emerging discipline that holds great potential in psychiatry [1,2]. The definition of a pharmacogenetic profile that assists the general practitioner or the psychiatrist in predicting the outcome of an SSRI treatment or the risk on the development of adverse events and selecting patients that are likely to have a beneficial effect of the antidepressant, would be a step towards more efficient antidepressant treatment. In this thesis, we report on six studies relevant to the subject of pharmacogenetics in depression in order to evaluate the usefulness of pharmacogenetics as a tool in the treatment of depression. The goal of the General Discussion is to review the individual study results and to put them into a broader perspective. We will summarize the main results of each of the six studies described in this thesis and additionally discuss the main conclusions for the serotonin transporter genotypes. Strengths and limitations have been discussed in detail in the individual studies, in the General Discussion, we will go into the overall methodological issues. Finally, we will discuss the implications of our findings and make some suggestions for future research on pharmacogenetics in depression.

METHODOLOGICAL ISSUES IN PHARMACOGENETIC RESEARCH

In a newly emerging discipline, such as pharmacogenetics, it is useful to discuss methodological issues in the designing and reporting of studies in order to ensure high study quality. In the second chapter we addressed these issues and presented a list of points of interest for future pharmacogenetic studies. Pharmacogenetic studies evaluate differences in treatment effect based on a patient characteristic (genotype). In epidemiology, this evaluation is called effect modification which is dependent of the choice of the effect measure [3]. Researchers evaluating pharmacogenetic questions often encounter specific methodologic problems. The use of a non-experimental cohort design, for example, could lead to a biased estimate of the genotype effect if the likelihood of treatment is associated with the genotype of interest. Moreover, if researchers use a design in which a control group is lacking, it is impossible to distinguish between the genotype as a prognostic factor or as an effect modifier. Therefore, for the evaluation of effect modification, the randomized clinical trial (RCT) is the most appropriate design. However, it should be emphasized that if data from a completed RCT are used, the division according to genotype is not random and confounding can be present. The distribution of potential confounders in relation with genotype and treatment group should therefore be reported and analyses should be adjusted for these variables. Similarly, the selection of subjects could be biased if the likelihood of selection is associated with the genotype. Data analyses in studies that evaluate treatment differences based on patient characteristics also encounter specific problems, such as the choice of the reference groups. Researchers should consider which reference group is most suitable for their study to prevent that observed treatment differences in genotypes could also be explained by differences in prognosis for certain genotypes.

5-HTTLPR AND STIN2 AND SSRI TREATMENT IN DEPRESSIVE PATIENTS

The central theme of this thesis was the influence of two polymorphisms in the serotonin transporter gene on several aspects of antidepressive treatment. We first summarized available evidence on the influence of 5-HTTLPR and STin2 (Chapter 3) in a systematic review. Second, we conducted a case-referent study on the influence of 5-HTTLPR and STin2 on clinical non-response during treatment with selective serotonin reuptake inhibitors (Chapter 4). In addition, we assessed the influence of these polymorphisms on adverse events and comorbid physical complaints (Chapter 5 and 6).

Serotonin transporter polymorphisms and SSRI non-response

The third and fourth chapter of this thesis focus on the influence of 5-HTTLPR and STin2 on the effect of treatment with selective serotonin reuptake inhibitors (SSRIs) in depression. In the third chapter we summarized all available evidence in a systematic review. In this review, we concluded that although evidence points towards a small increase in the risk of SSRI non-response for patients with the 5-HTTLPR s/s genotype or the STin2 10/12 genotype, due to the limited numbers of studies and the large heterogeneity of the different studies, a definite conclusion on the influence of genotype on treatment effect was not feasible.

In order to expand knowledge on genotype influence on SSRI treatment outcome, we conducted a study in which we assessed the association between 5-HTTLPR and STin2 genotype and SSRI non-response in 214 depressive patients (Chapter 4). Results of this study support the hypothesis that genetic factors are involved in antidepressant response. Patients with the 5-HTTLPR s/s or s/l genotype had a statistically non-significant increased risk on SSRI non-response. Results are in agreement with previous studies among Caucasian patients [4-10] which suggests a not statistically significant, positive association between the s/s genotype and SSRI non-response. The increased risk of SSRI non-response in heterozygous patients has also been observed previously [11].

In our study, genotype effects were more prominent in several subgroups of patients. Especially female patients and younger patients appeared to be susceptible for serotonin transporter genotype effects. Women and patients under 44 years old with the 5-HTTLPR s-allele showed a less favorable response to SSRI treatment. This gender- and age-dependent influence was only observed for the 5-HTTLPR genotype, no clear effect was observed for the STin2 genotype. To our knowledge, an increased risk in female patients and patients under 44 years old with the s-allele has not been reported before. However, since our sample size was small, these results should be replicated in a larger population to exclude the possibility of a chance finding.

Serotonin transporter polymorphisms and adverse events during SSRI treatment

Adverse events are frequent during treatment with antidepressants and are associated with incomplete recovery and lower response rates on antidepressants [12-14]. Previously, the serotonin transporter genotype has been associated with adverse events during SSRI treatment [15,16]. However, the available evidence on this association is limited.

We assessed the influence of 5-HTTLPR and STin2 genotype on the occurrence of adverse events during SSRI treatment in 214 depressive patients (Chapter 5). Our results indicated that patients with the 5-HTTLPR s-allele had an increased risk on adverse events during treatment with SSRIs. This risk was especially increased for complaints that were classified as 'general adverse events' [17]. This group contained adverse events such as dermatologic reactions, weight change and fatigue. STin2 genotype had no clear influence on the occurrence of adverse events during SSRI treatment. These results are in general agreement with those of previous studies of the association between 5-HTTLPR genotype and the occurrence of adverse events during SSRI treatment. The 5-HTTLPR s-allele has previously been associated with complaints such as insomnia, agitation, fatigue, dizziness and gastrointestinal complaints during SSRI treatment [15,18]. To our knowledge, the influence of STin2 genotype on the development of adverse events has not been evaluated up till now.

Serotonin transporter polymorphisms and comorbidity in depression

A large number of the depressive patients also experience physical complaints, especially complaints that are pain-related [19-22]. Somatically ill depressive patients have an increased risk of SSRI non-response and less complete recovery from the depressive episode [23-25]. In chapter 6, we describe a study in which we assessed the association between 5-HTTLPR and STin2 genotype and comorbidity in depression. In addition, we evaluated whether serotonin transporter genotype is associated with the presence of pain related to somatic comorbidity. Results from this study indicate that the 5-HTTLPR s/s genotype is associated with an increased risk on comorbid physical complaints. However, there was no evidence of a clear relation with pain. For STin2 genotype, our results suggest that patients with the 10/10 or 10/12 genotype have a decreased risk of comorbidity. Our results imply that comorbid physical complaints could act as a confounder in the relation between serotonin transporter genotype and SSRI non-response. Our results are in agreement with previous studies on the association between 5-HTTLPR genotype and somatic comorbidity like fibromyalgia, irritable bowel syndrome and migraine [26-28]. Little evidence is available on the association between STin2 and comorbid somatic disorders, but the 10/10 genotype has previously been associated with migraine [29].

5-HTTLPR and STin2 genotypes: the consequences

In the studies described in this thesis we focused on two polymorphisms in the serotonin transporter gene, 5-HTTLPR and STin2 [30-35]. Several other genetic factors have been suggested to influence treatment in depression as well, such as the tryptophan hydroxylase (TPH) gene [36-38]. Also, combined effects of different genetic factors have been proposed to influence treatment effect [38]. Conclusions on other genetic factors were not possible based on our studies. We can draw the following conclusions with regard to the different genotypes of the serotonin transporter.

<u>5-HTTLPR s/s genotype</u>

Based on our study results and results from previous studies among Caucasian patients, patients with the 5-HTTLPR s/s genotype seem to have an increased risk of a less favorable response on SSRI treatment in depression as compared to patients with the l/l genotype [4-11].

Besides, they seem to have an increased risk of adverse events during SSRI treatment and an increased risk of physical complaints during depression. This has also been suggested in previous studies that observed an association between 5-HTTLPR s/s genotype and the occurrence of certain adverse events [15,18] and between the s/s genotype and specific somatic comorbidity [21,26-28].

5-HTTLPR s/l genotype

Patients with the s/l genotype also seem to have an increased risk on SSRI non-response in our population. This risk is more increased as compared to the risk for patients with the s/s genotype. Although this has also been observed in previous studies [6,11], results from previous studies are not consistent and some researchers have concluded that the s/l genotype is not associated with a less favourable result [4,7,8]. In our study, patients with the s/l genotype also appear to have an increased risk on adverse events during treatment as compared to patients with the l/l genotype. Since most studies only compared the s-allele to the l-allele and did not evaluate the different genotypes, the s/l genotype influence has not been described in previous studies. One study that did evaluate the influence of the different genotypes on the occurrence of insomnia and agitation concluded that patients with the s/l genotype did not have an increased risk on adverse events [15]. Patients with the s/l genotype do not seem to have an increased risk on physical complaints during depression based on our study results. Also, in previous studies, no increased risk on comorbidity for patients with the s/l genotype is observed [21,26-28].

STin2 10/10 genotype

In our population, patients with the 10/10 genotype do not seem to have an increased risk of SSRI non-response. Previous studies have suggested an increased risk of non-response for Asian patients with the 10/10 genotype but the number of subjects in these studies was often small [39,40]. Patients with the 10/10 genotype do not appear to have an increased risk of more adverse events during treatment compared to patients with the 12/12 genotype. To our knowledge, the influence of STin2 genotype on the occurrence of adverse events has not been evaluated in previous studies. On the other hand, patients with a 10/10 genotype in our population seem to have a decreased risk on somatic complaints during depression. Available evidence on the association between STin2 and comorbidity is limited, the 10/10 genotype has previously been associated with migraine [29].

STin2 10/12 genotype

Patients with the 10/12 genotype did not appear to have an increased risk on SSRI non-response as compared to patients with the 12/12 genotype in our population. Previous studies in Asian patients did observe a less favourable response with the 10/12 genotype [39,40]. It also appears that the 10/12 genotype did not increase the risk of adverse events during SSRI treatment in the patients in our study. The 10/12 genotype appeared to be associated with a decrease of the risk on physical complaints during depression in our population. Information from previous studies on the influence of STin2 on the occurrence of adverse event or on comorbidity was not available.

Patients subgroups

Based on the studies in this thesis we also aimed to formulate conclusions, although cautiously, on specific subgroups of patients. Our results suggested a gender-dependent influence of 5-HTTLPR. Female patients with the s/s or the s/l genotype appeared to have an increased risk on SSRI non-response. For male patients, this risk seemed not to be increased. Although previous studies have mentioned a gender-dependent genotype influence in, for example, suicide attempts or certain personality traits [41,42], the influence of 5-HTTLPR on treatment outcome has not been evaluated up till now in men and women separately.

The gender-dependent influence of 5-HTTLPR could possibly be explained through differences in serotonin synthesis, differences in neurotransmission [43,44] or differences in serotonin transporter expression due to estrogen and progesterone [44,45] between men and women that were reported by previous studies [43,45]. Our results also suggested an age-dependent influence of 5-HTTLPR. Patients under 44 years old with the s/s or s/l genotype seem to have an increased risk on SSRI non-response whereas this risk appeared not to be increased in patients over 44 years old. Although, age has been proposed as moderator for antidepressant response in a previous study [46], the influence of 5-HTTLPR on treatment outcome has not been evaluated in different age groups up till now. The observed age-dependent influence of 5-HTTLPR might be explained by age modification of serotonin transporter affinity [47]. However, it should be mentioned that the subgroups of patients in which these effects, the gender-dependent as well as the age-dependent effects, were evaluated were small and these conclusions should be confirmed in a larger sample.

Strengths and limitations

We evaluated our research questions in a case-referent design which enables unbiased estimation of relative risks without the need of a rare-disease assumption [48]. However, other sources of bias might influenced our study results. These sources will be discussed here.

Information bias

Depression was diagnosed by a psychiatrist or a general practitioner according to the DSM-IV criteria. Although the recognition of depression by general practitioners has been questioned, participating general practitioners were instructed to diagnose according to DSM-IV and, in case of doubt, diagnose patients as having 'depressive complaints'; these patients were not considered for inclusion. SSRI non-response was defined as having used an SSRI for at least 6 weeks in an appropriate dosage without any therapeutical response as diagnosed by the psychiatrist. Although misdiagnosis cannot completely be excluded, it is not likely that patients participating in our study were erroneously diagnosed as 'depressive' or as 'non-responder'.

The occurrence of adverse events during SSRI treatment was assessed through a structured face-to-face interview. Due to the use of this method, bias possibly occurred since patients often have problems recalling certain events. Previous studies have shown that this often leads to the underestimation of, for example, the prevalence of depressive symptoms [49].

However, any incomplete recall in our study is likely to be non-differential; i.e. not associated with serotonin transporter genotype. Therefore, it is less likely that this type of bias has influenced our conclusions. Moreover, since in our population adverse events were not recorded if participants doubted if the events occurred, bias due to incomplete recall has probably caused an underestimation of the number of adverse events during treatment. As this underestimation is not likely to be associated with genotype, this would probably not have changed our conclusions.

Recall bias is not likely in the comorbidity analyses since we have used general practitioners' files to assess information on these complaints. However, gaps in general practitioners' files could have caused non-differential misclassification that could have led to a dilution of the association between genotype and comorbidity [3]. In that case, the true association would be even stronger than observed in our population.

Genotyping was performed blinded to patients status (case or referent) according to a strict protocol. Two researchers independently scored genotypes, discordant results were judged by a third expert before final results were entered in the database. Using this procedure, the risk on bias through measurement errors was minimized.

Selection bias

As discussed previously (chapter 2), the selection of patients in pharmacogenetic studies could yield biased results if the likelihood of selection is associated with the genotype. It is unlikely that this has occurred in our population since we approached all patients in the general practitioners' practices and mental health services that met the inclusion criteria. Another frequent problem in pharmacogenetic studies is population stratification [31,50]. However, since we only included Caucasian patients in our population, this will probably not have biased our results.

Confounding bias

Several factors possibly influence the association between genotype and treatment outcome, adverse events and comorbidity. Although we included several potential confounders in our analyses we cannot exclude the possibility that other factors have influenced the observed associations. Unknown or unmeasured genetic or environmental factors could be involved in the association between genotype and treatment outcome, adverse events or comorbidity. Especially factors such as stressful life events and personality traits such as neuroticism are possibly associated with serotonin transporter genotype and treatment outcome [51-53].

The occurrence of adverse events during treatment and the presence of somatic comorbidity was associated with serotonin transporter genotype in our population. This raises the question whether the observed association between SSRI non-response and serotonin transporter genotype can be explained by the presence of polymorphisms or by the presence of adverse events or somatic disorders that are associated with a less favourable treatment effect. Possibly, patients with the s/s or s/l genotype have a less favourable response to SSRIs because they experience more adverse events and are likely to discontinue treatment due to these events.

Moreover, these patients also have an increased risk on somatic comorbidity which is also related to a less favourable treatment effect. We can therefore not exclude the possibility that the increased risk on SSRI non-response in patients with the s/s or s/l genotype as observed in chapter 4, could be explained by more adverse events or somatic comorbidity in patients with these genotypes.

As described in chapter 2, it is not possible to distinguish between the genotype as a prognostic factor or as an effect modifier using a design without a control group. However, the main question in our studies was whether serotonin transporter genotype could be used to predict treatment outcome after a certain treatment period. Since in this case the focus is on prediction rather than causality, there is no need to make this distinction.

USING GENETIC TESTS IN PSYCHIATRIC PRACTICE

In chapter 7 of this thesis we concentrate on the question whether the implementation of a genetic test in psychiatric practice would be a useful tool to achieve faster pharmacotherapeutic treatment effects. We evaluated this question in a theoretical decision analytical model. Results from this study indicate that genetic testing prior to antidepressant prescription yields a higher percentage of patients in remission early in treatment: after 6 or 12 weeks. Patients discontinuing treatment within this period due to adverse events of non-response have a increased risks of relapse or recurrence [12] and have higher 12-month medical costs compared to patients who remain on therapy for at least 90 days [14]. Achieving a faster treatment effect could result in a higher number of patients adhering to treatment. Since serotonin transporter genotype has also been associated with the occurrence of adverse events (chapter 5), using a genetic test to identify patients at risk for non-response could also be used to identify patients at higher risk for adverse events. The decision analytical model was tested in a sensitivity analysis to evaluate the robustness of the model if baseline values such as remission rates, non-response rates, adverse events rates and genotype frequencies were altered and our model turned out to be robust for alterations at baseline. Even if baseline values were changed according to the sensitivity range, results showed an advantage for the genetic testing strategy over the strategy that is currently used in psychiatry. These results suggest that genetic testing could be an appropriate tool to achieve faster treatment effects in depressive patients.

In the current decision analytical model, the occurrence of adverse events was not taken into account. Since adverse events are possibly associated with the 5-HTTLPR genotype, incorporating the occurrence of adverse events during treatment in the model could alter the outcome of the analyses. Adverse events are associated with early treatment discontinuation and higher overall medical costs [12,13]. It is therefore likely that incorporating these advents in the decision analytical model would cause an even larger difference between the two strategies in favor of the genetic testing strategy.

We did not include any costs of treatment in the analyses. However, we assume that the implementation of a genetic test to identify patients at risk for SSRI non-response is likely to be cost-effective.

The 5-HTTLPR s/s and s/l genotype is prevalent in the population, the genetic test is relatively sensitive and specific, no alternative test is available to individualize antidepressive treatment, untreated depression is associated with poor prognosis and the implementation of a test could reduce healthcare costs by increasing the number of patients in remission early in treatment and reducing the number of clinical consultations and referrals [54].

FINAL CONCLUSIONS

In the introduction of this thesis we described our general aim as the evaluation of the usefulness of pharmacogenetics in the treatment of depression, since current evidence on the influence of genotype in the antidepressive treatment was not conclusive. Moreover, we aimed at providing additional evidence on subjects that are important in the evaluation of the usefulness of pharmacogenetics in the treatment of depression.

In this thesis, we found support for the relation between serotonin transporter genotype and several factors involved in the treatment of depression (SSRI response, the occurrence of adverse events and comorbid physical complaints during treatment). Furthermore, a decision analytical model indicated that the use of a genetic test prior to antidepressant prescription may cause an increased number of patients in remission early in treatment. Our studies suggest that pharmacogenetics could fulfill an important role in the treatment of depression by identifying patients that are likely to benefit more from an antidepressant other than an SSRI. However, it should be realized that these conclusions were based on a small study population and many questions, such as on the influence of other genes and the possibility of gene-environment interactions, remain unanswered.

PUBLIC HEALTH IMPLICATIONS

Mood disorders such as depression are common in the Netherlands with a one-year prevalence rate of approximately 7% for mood disorders and 6% for major depression in the adult population between 18 and 64 years old [55,56]. Using the rate of 6% for major depression we estimate that over 700,000 persons between 18 and 64 years old would have suffered from major depression in 2005. First incidence rates for depression in the population between 18 – 64 years old is 22.1 per 1000 persons per year; 17.3 per 1000 men per year and 38.8 per 100 women per year [57]. Given these incidence rates and estimating around 12 million persons between 18 -64 years old in the Netherlands in 2005 (Central Bureau of Statistics, www.cbs.nl) over 265,000 persons would have suffered from a first episode of depression in 2005 in the Netherlands. Half of the depressive population receives professional treatment, and around 40% of the patients that are treated receive an antidepressant [58]. If we assume that 85% of the depressive patients receive an SSRI and 30-40% of these patients do not experience a sufficient response to initial SSRI treatment [30,59,60] 13,525 – 18,020 patients were initially treated with SSRIs without a satisfactory outcome in 2005 in the Netherlands. Since patients are usually treated for 6 weeks before treatment outcome is established, these patients were each treated with SSRIs for at least 42 days without sufficient result. This causes not only large healthcare costs but also a large number of days with depressive complaints for the individual patient.

The ability to predict treatment outcome prior to antidepressant prescription, for example through a genetic test, could reduce the number of days patients are treated without sufficient response and would be beneficial for the caregiver as well as for the patient. It should however be questioned whether all patients are willing to undergo a genetic test. These tests would only give an indication whether an SSRI would be a suitable antidepressant. A successful treatment outcome would never be guaranteed since multiple factors influence treatment.

RECOMMENDATIONS FOR FUTURE RESEARCH

Although the studies in this thesis provide additional information on the use of pharmacogenetics in the treatment of depression, several questions on the prediction of treatment outcome using genetic information remain unanswered. It is unlikely that polymorphisms in the serotonin transporter gene are the only genetic factors influencing antidepressant response in depressive patients. There are indications that other gene variations also influence treatment outcome in depression [36-38,59,61,62].

Future studies should pursuit the combination of multiple candidate gene polymorphisms since previous studies have suggested an additive effect of polymorphisms [59,63]. In addition, the use of haplotypes might results in a better predictive model for SSRI response [59]. Eventually, the construction of a genetic model including multiple genes influencing treatment outcome in depression, could lead to the identification of subgroups of patients that respond more favorably to one treatment or another.

However, treatment outcome depends on numerous factors, some are biological (such as genotype) [5,10] and some are contextual (such as amelioration of social problems) or psychological (such as problem solving) [51,52]. At this moment it is unclear how these different factors interact to eventually cause treatment response. Future research should work towards a better understanding of the interaction between genetic and environmental factors that influence treatment outcome in depression. Previous studies have reported gene-environment interactions between serotonin transporter genotype and environmental factors such as stressful life events [51,52,64,65], although not consistent [66].

To obtain the optimal antidepressant treatment outcome for individual patients, one should therefore not only focus on biological factors (using a genetic test to determine the appropriate antidepressant) but also take into account environmental factors if patients have a certain genetic constitution. Since we observed an age- and gender-dependent influence of the serotonin transporter genotype on treatment outcome, it is possible that factors influencing treatment are different for subgroups of depressive patients.

The studies in this thesis provide evidence for an association, although not very strong, between genotype and adverse events and physical complaints in depressive patients. Partially, these associations have also been suggested in previous studies, such as the association between 5-HTTLPR genotype and some adverse events [15,18] and between 5-HTTLPR and somatic comorbidity [21,26-28].

The occurrence of adverse events and the presence of physical complaints are important factors that influence treatment outcome. Since the underlying mechanisms for these associations are not completely clear, this relation should be further elucidated before this information can be useful in the treatment of depression. It would also be worthwhile to include other polymorphisms, for example in serotonin receptors, in studies of genotype and adverse events.

Until a more complete model including genetic and environmental factors influencing treatment outcome in depression is available, current genetic knowledge could be used for a more individualized antidepressant treatment. The implementation of genetic testing prior to antidepressant prescription to achieve a faster treatment effect, is only recommended if the test is indeed a cost-effective tool in antidepressant treatment. In this thesis, we hypothesized by use of a decision analytical model that such a test would be useful as well as cost-effective since depression as well as the 5-HTTLPR s/s and s/l genotype are common and no other test is currently available to identify patients at risk for antidepressant non-response. However, our decision analytical model is only theoretical. It would be necessary to evaluate the use of a genetic test in a clinical trial in which patients are randomized in groups with different treatment strategies (genetic testing versus current treatment strategy) and consequently receive an antidepressant according to their genotype or according to the current strategy, before genetic testing on serotonin transporter genotype can be used in the treatment of depression.

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SUMMARY

Depression is a common illness affecting millions of people over the world. A large part of the depressive population receives pharmacological treatment and the most frequently prescribed antidepressants are selective serotonin reuptake inhibitors (SSRIs). However, the period until the effect of an SSRI is established can amount to 6 weeks. During this period, adverse effects can already occur. Moreover, in a large part of the depressive patients, 30 – 40%, a sufficient therapeutical effect remains absent. Therefore it would be useful to be able, before treatment, to identify patients at increased risk of non-response to SSRIs. These patients could be given alternative treatment.

Over the last few years, evidence has accumulated indicating that antidepressant response is under genetic control. Genetic variants involved in the serotonergic system have been proposed as possible explanations for the observed differences in treatment effect among depressive patients. Previous studies have mainly focused on two polymorphisms in the serotonin transporter gene, 5-HTTLPR and STin2. However, available evidence is limited and results from previous studies are inconsistent, possibly due to variations in methodology. There is a need for methodological guidelines for studies on drug-gene interactions to assure high quality studies and facilitate the use of individual study results in meta-analyses. In addition, there is a lack of evidence on several topics that are also important in the evaluation of the usefulness of pharmacogenetics in depression, such as the effectiveness of a genetic test in psychiatric practice and the influence of comorbidity on SSRI non-response. The general aim of the studies in this thesis was to evaluate the usefulness of pharmacogenetics in the treatment of depression by adducing additional evidence on the influence of the serotonin transporter genotype on SSRI response, adverse events and somatic comorbidity. In addition, the effectiveness of a genetic test in psychiatric practice was evaluated in a clinical decision analytic model.

In the introduction (chapter 1), the rationale of the studies and a description of available evidence on the influence of 5-HTTLPR and STin2 on treatment response, adverse events and somatic comorbidity is given.

Chapter 2 describes methodological issues that are important in the design of pharmacogenetic studies. Available studies on drug-gene interactions appear to be heterogeneous and relevant methodological issues for pharmacogenetic studies have received little attention. We discuss several topics that should be addressed in pharmacogenetic studies, such as the choice to express treatment effect in relative or absolute terms. The interpretation of drug-gene interaction, which in epidemiology could be called effect modification, could depend on the choice of an effect measure. Other issues that are important especially in pharmacogenetic studies are the possibility of bias if analyses are not adjusted for differences in the distribution of possible confounders in the genotype groups, and the risk of selection bias if the likelihood to be included in the study is associated with the genotype. In addition we presented several guidelines that could serve as a basis for further discussion on methodology in pharmacogenetic studies.

Available evidence on the relation between 5-HTTLPR and STin2 and SSRI response was summarized in a systematic review (chapter 3). Nine studies were identified that addressed the association between serotonin transporter polymorphisms and SSRI response in Caucasian or Asian patients with major depression. SSRI response was expressed as either the decrease of depression scores on the Hamilton Rating Scale for Depression (HAM-D) or the Montgomery and Ashberg Depression Rating Scale (MADRS) or as the percentage of antidepressant responders. Although results were heterogeneous, a pooled analysis showed that Caucasian patients with the 5-HTTLPR s/s genotype appeared to have a lower decrease of depression scores on the HAM-D at both 4 weeks weighted mean decrease in HAM-D score: 26.2 % (s/s) vs. 51.5% (s/l) and 50.2% (l/l), and 6 weeks, 40.7% (s/s) versus 56.0% (s/l) and 52.4% (l/l). The one study on Caucasian patients that reported percentages of antidepressant responders also showed poorer response rates at 6 weeks of medication for patients with the s/s (70.4%) and s/l (75.5%) genotype as compared to patients with the l/l genotype (87.5%) ($p = 0.029$). However, even though studies were only included if they met our inclusion criteria, there was considerable heterogeneity between individual studies with respect to population characteristics, type of intervention, outcome measurement and validity. The diversity of designs is likely to have contributed to the observed heterogeneity of individual study results. Due to this heterogeneity, the question whether serotonin transporter genotype could be used as a tool to identify patients at risk for SSRI non-response could not be answered conclusively based on the available evidence.

Several factors important in the treatment of depression were analyzed in relation to serotonin transporter genotype (chapter 4, 5 and 6) to evaluate if pharmacogenetics could be useful in the treatment of depression. In chapter 4, we assessed the influence of 5-HTTLPR and STin2 on SSRI treatment effect in 214 Caucasian depressive patients. Patients with the 5-HTTLPR s/s or s/l genotype appeared to have an increased risk on SSRI non-response, although not statistically significantly so; Odds Ratio (OR) 1.27 (95%-CI 0.44-3.72) for the s/s genotype and OR 1.86 (95%-CI 0.71-4.88) for patients with the s/l genotype. For STin2, results showed no clear association between SSRI non-response and the 10/10 genotype or the 12/10 genotype; OR 1.07 (95%-CI 0.40-2.87) and OR 0.73 (95%-CI 0.32-1.68) respectively. There was a suggestion for interaction between allele and gender, $p = 0.065$. Analyses stratified for gender indicated that female patients with the 5-HTTLPR s-allele had an increased risk on SSRI non-response, OR 3.54 (95%-CI 1.05-11.92), whereas no such increased risk was observed in male patients with the s-allele, OR 0.29 (95%-CI 0.04-2.34). Stratification by age ($\leq$ 44 years and $>$ 44) showed a positive association for the s-allele in patients $\leq$ 44 years old (OR 9.34, 95%-CI 1.41-61.98) but not for patients $>$ 44 years (OR 1.13, 95%-CI 0.32-3.98). We concluded that, although the overall influence of serotonin transporter genotype appears to be small, the influence in specific subgroups of patients could be important for clinical practice.

Chapter 5 describes a study on the association between serotonin transporter genotype and the occurrence of adverse events during treatment. Results indicate that patients with the 5-HTTLPR s/l genotype have an increased risk of adverse events of any kind, OR 1.42 (95%-CI 0.43-4.74). Patients with the s/s genotype appeared to have an increased risk of some kinds of adverse events, but not all.

The risk on adverse events with the s/s or s/l genotype was highest for general adverse events (dermatologic reactions, weight gain, weight loss and fatigue); OR 1.73 95%-CI 0.78-3.84 for the s/s genotype and OR 2.37 95%-CI 1.13-4.96 for the s/l genotype. The STin2 genotype did not appear to have an influence on the occurrence of adverse events except for the central/peripheral nervous system adverse events in which the 10/10 genotype was associated with a decreased risk. Our results suggest that the serotonin transporter genotype is involved in the development of adverse events during SSRI treatment, however, these results should be replicated.

Patients with depression often report somatic comorbidity, comorbidity could also influence antidepressant treatment effect. In chapter 6 we assessed the association between serotonin transporter genotype and somatic comorbidity, in particular painful comorbidity, in depression. Fifty-one percent of the patients in our study reported one or more somatic disorders and almost all reported disorders could be categorized as painful comorbidity. Patients with the s/s genotype reported more comorbidity as compared to patients with the s/l or l/l genotype. For STin2, patients with the 10/12 genotype reported less comorbidity as compared to patients with the 10/10 or 12/12 genotype. An increased risk of somatic comorbidity was observed for patients with the s/s genotype, 2.74 (95% CI 1.04-7.19). The OR did not change much after exclusion of patients with non-painful comorbidity (OR 2.47, 95%-CI: 0.93-6.52). For comorbidity without pain, numbers were too small for the calculation of ORs. Patients with the STin2 10/10 and 10/12 genotypes were less likely to suffer from somatic comorbidity; OR 0.40 (95%-CI: 0.18-0.89) for 10/12 genotype and OR 0.68 (95%-CI: 0.24-1.95) for the 10/10 genotype. Again, these figures did not change much after exclusion of patients with non-painful comorbidity; OR 0.43 95% CI 0.19-0.96 and OR 0.74 95% CI 0.26-2.16 for patients with the 10/12 and 10/10 genotype, respectively. We concluded that the presence of somatic disorders in depression might be related to serotonin transporter genotype. Since somatic comorbidity has been found to negatively influence treatment effect in depression, it is possible that the previously observed association between serotonin transporter genotype and SSRI treatment response has been biased by the presence of somatic comorbidity.

In chapter 7, we present a decision analytical model that compares the current treatment strategy in which all patients initially receive an SSRI with an alternative treatment strategy in which antidepressants are prescribed according to genetic test results. The results suggest that incorporating a genetic test on 5-HTTLPR in the treatment of depression could yield higher percentages of patients in remission after 6 or 12 weeks of treatment. After 6 weeks of treatment, 60.0% of the patients were calculated to be in remission if no genetic test was performed and all patients would have used an SSRI; after 12 weeks this percentage was 76.7%. If pre-treatment genetic testing was used to guide decisions on antidepressant prescription and patients with the s/s and s/l genotype received an SNRI, 64.6% of the patients were in remission after 6 weeks. After 12 weeks, this was 79.5%. If patients with the s/s and s/l genotype received an TCA after genetic testing, 64.6% of the patients were in remission after 6 weeks and 83.2% after 12 weeks. The robustness of the decision analytical model was evaluated in sensitivity analyses and the conclusion remained in favour of the alternative strategy regardless of any alterations in baseline values.

We concluded that implementation of a genetic test on 5-HTTLPR could increase the number of patients in remission and decrease the number of early discontinuations, thereby reducing overall healthcare costs.

In chapter 8 the main findings of the studies described in this thesis are summarised and discussed. In this thesis, we found support for the hypothesis that serotonin transporter genotype is involved in several aspect in the treatment of depression (response, adverse events and comorbidity). We observed an association between serotonin transporter genotype and SSRI response, the occurrence of adverse events and the presence of somatic comorbidity in depressive patients. In addition, we showed in a decision analytical model that incorporating a genetic test in psychiatric practice could yield higher percentages of patients in remission after antidepressive treatment. In conclusion, we can state that pharmacogenetics could be an important and relevant addition to improve the treatment of major depressive disorder.

SAMENVATTING

Depressie is een veel voorkomende ziekte die wereldwijd miljoenen mensen treft. Een groot deel van deze mensen krijgt een farmacologische behandeling tegen de depressie. De meest voorgeschreven antidepressiva zijn de selectieve serotonine heropname remmers (SSRI). De periode die echter nodig is voordat vastgesteld kan worden of een SSRI effectief is, kan oplopen tot 6 weken. Daarnaast blijkt dat in een groot deel van de patiënten, 30-40%, een voldoende therapeutisch effect achterwege blijft. De mogelijkheid om, voorafgaand aan behandeling, patiënten die geen baat zullen hebben van een SSRI te identificeren zou clinici in staat stellen hierop te anticiperen en een ander antidepressivum voor te schrijven.

De laatste jaren zijn er steeds aanwijzingen gevonden die erop duiden dat de respons op antidepressiva onder invloed staat van genetische factoren. Genetische varianten die betrokken zijn bij het serotonerge systeem zijn genoemd als mogelijke verklaring voor de geobserveerde verschillen in behandelingseffect bij depressieve patiënten. In eerdere studies ging de aandacht voornamelijk uit naar twee polymorfismen in de serotonine transporter, 5-HTTLPR en STin2. Het aanwezige bewijs is echter beperkt en de resultaten uit eerdere studies zijn inconsistent. Mogelijk is diversiteit in methodologie een oorzaak voor de inconsistente resultaten uit eerder onderzoek waardoor het moeilijk is om een eenduidige conclusie te formuleren over de invloed van 5-HTTLPR en STin2 op behandelingseffect. De formulering van methodologische richtlijnen is noodzakelijk om een hoge kwaliteit van toekomstig onderzoek te garanderen en om de inclusie van de resultaten in een meta-analyse te vergemakkelijken. Tevens is het uitbreiden van de kennis over verschillende factoren die belangrijk zijn in de evaluatie van de bruikbaarheid van farmacogenetica bij depressie, zoals de voorspellende waarde van het genotype voor de kans op bijwerkingen of comorbiditeit, essentieel voor het totaalbeeld. In dit proefschrift wordt de bruikbaarheid van farmacogenetica in de behandeling van depressie geëvalueerd door nieuw bewijs aan te dragen over de invloed van het serotonine transporter genotype op SSRI respons, bijwerkingen en somatische comorbiditeit. Daarnaast wordt de mogelijke effectiviteit van een genetische test in de psychiatrische praktijk geëvalueerd in een besliskundig model.

In de inleiding van dit proefschrift (hoofdstuk 1) worden de beweegredenen voor het opzetten van de studie en het beschikbare bewijs over de invloed van 5-HTTLPR en STin2 op behandelingsrespons, bijwerkingen en somatische aandoeningen beschreven.

Hoofdstuk 2 beschrijft een studie naar methodologische onderwerpen die belangrijk zijn in het design van farmacogenetische studies. Beschikbare studies zijn heterogeen en relevante methodologische factoren kregen tot nu toe weinig aandacht in farmacogenetische studies. Wij bespreken verschillende onderwerpen die aan de orde zouden moeten komen in studies naar gen-medicatie interacties, zoals de keuze om het behandelingseffect uit te drukken in relatieve dan wel absolute waardes. De interpretatie van gen-medicatie interactie wordt in de epidemiologie beschreven als effectmodificatie. De conclusie dat effectmodificatie aanwezig is, is afhankelijk van de keuze van een effectmaat.

Andere onderwerpen die aan de orde komen en belangrijk kunnen zijn, met name in farmacogenetisch onderzoek, zijn het risico op bias als analyses niet gecorrigeerd worden voor verschillen in de verdeling van potentiële confounders in de genotype groepen en het risico op selectiebias als de kans om geïncludeerd te worden in het onderzoek afhankelijk is van het genotype. We presenteren een aantal richtlijnen die gebruikt kunnen worden voor verdere discussie over methodologie in farmacogenetische studies.

Het beschikbare bewijs van de relatie tussen 5-HTTLPR en STin2 en SSRI respons hebben wij beschreven in een systematische review (hoofdstuk 3). We waren in staat om negen studies te identificeren die de relatie tussen polymorfismen in de serotonine transporter en de respons op een SSRI beschrijven in blanke of Aziatische patiënten met depressie. SSRI respons werd uitgedrukt als een scorevermindering op de Hamilton Rating Scale for Depression (HAM-D) of de Montgomery and Ashberg Depression Rating Scale (MADRS) of als het percentage responders op een antidepressivum. Hoewel de individuele studiebevindingen heterogeen waren, lieten de gecombineerde resultaten zien dat blanke patiënten met het 5-HTTLPR s/s genotype een lagere scorevermindering hadden op de HAM-D na behandeling met een SSRI gedurende 4 weken in vergelijking met patiënten met een s/l of l/l genotype, gewogen gemiddelde afname in HAM-D score: 26.2 % (s/s) versus 51.5% (s/l) en 50.2% (l/l). De resultaten waren vergelijkbaar na een SSRI behandeling gedurende 6 weken, gewogen gemiddelde afname in HAM-D score: 40.7% (s/s) versus 56.0% (s/l) en 52.4% (l/l). De enige studie bij blanke patiënten die SSRI respons uitdrukt als het percentage responders concludeert ook lagere aantallen responders in de groep patiënten met het s/s of het s/l genotype na een behandeling met een SSRI gedurende 6 weken: s/s 70.4% responders en s/l 75.5% responders tegen 87.5% responders bij het l/l genotype ($p = 0.029$). Ondanks de criteria waar studies aan moesten voldoen om geïncludeerd te worden in de systematische review, was er sprake van aanzienlijke heterogeniteit tussen de individuele studies wat betreft populatie karakteristieken, interventie, uitkomstmaat en validiteit. Het is waarschijnlijk dat de diversiteit van de studiedesigns heeft bijgedragen aan de heterogeniteit in de resultaten. Door deze heterogeniteit, was het, na het bekijken van al het beschikbare bewijs, niet mogelijk om een definitief antwoord te geven op de vraag of farmacogenetica gebruikt zou kunnen worden om voorafgaand aan behandeling patiënten te selecteren met een hoge kans op SSRI non-respons.

Meerdere factoren die belangrijk zijn bij de behandeling van depressie zijn beschreven in relatie tot het serotonine transporter genotype (hoofdstuk 4, 5 en 6) om te evalueren of farmacogenetica een aanvulling zou kunnen zijn in de behandeling van depressie. In hoofdstuk 4 beschrijven we de invloed van 5-HTTLPR en STin2 op het effect van een behandeling met SSRI's in 214 depressieve patiënten. Patiënten met het 5-HTTLPR s/s of s/l genotype leken een verhoogd risico te hebben op een SSRI non-respons. Dit risico was echter niet statistisch significant; Odds Ratio (OR) 1.27 (95%-BI 0.44-3.72) voor het s/s genotype en OR 1.86 (95%- BI 0.71-4.88) voor patiënten met het s/l genotype. Voor STin2 lieten de resultaten geen duidelijke associatie zien met SSRI non-respons en het 10/10 of 10/12 genotype; de OR was 1.07 (95%- BI 0.40-2.87) en 0.73 (95%- BI 0.32-1.68) voor respectievelijk het 10/10 en het 10/12 genotype.

Interactie tussen allel en geslacht was borderline significant, p = 0.065. Analyses gestratificeerd voor geslacht lieten zien dat vrouwelijke patiënten met het 5-HTTLPR s-allel een verhoogd risico hadden op SSRI non-respons (OR 3.54 95%- BI 1.05-11.92), terwijl dit voor mannen niet het geval leek te zijn (OR 0.29 95%- BI 0.04-2.34). Stratificatie voor leeftijd (≤ 44 en > 44 jaar) liet zien dat voor patiënten ≤ 44 jaar het risico op SSRI non-respons verhoogd was bij het 5-HTTLPR s-allel (OR 9.34, 95%- BI 1.41-61.98), maar dat dit niet het geval was voor patiënten > 44 jaar oud (OR 1.13, 95%- BI 0.32-3.98). We concludeerden dat, hoewel de overall invloed van het serotonine transporter genotype klein was, de invloed van genotype in bepaalde subgroepen patiënten in de praktijk een belangrijke rol zou kunnen spelen bij de behandeling van depressie.

In hoofdstuk 5 wordt een studie beschreven naar de associatie tussen het serotonine transporter genotype en het voorkomen van bijwerkingen tijdens een behandeling met SSRI's. De resultaten van deze studie laten zien dat patiënten met het 5-HTTLPR s/l genotype een verhoogd risico lijken te hebben op het krijgen van bijwerkingen tijdens behandeling, OR 1.42 (95%- BI 0.43-4.74). Patiënten met het 5-HTTLPR s/s genotype lijken een verhoogd risico te hebben op het krijgen van bepaalde bijwerkingen, maar niet op alle bijwerkingen. Het risico op bijwerkingen bij patiënten met het s/s of s/l genotype lijkt het grootste te zijn voor algemene bijwerkingen (hieronder vallen dermatologische reacties, gewichtsverandering en vermoeidheid); OR 1.73 95%- BI 0.78-3.84 voor het s/s genotype en OR 2.37 95%- BI 1.13-4.96 voor het s/l genotype. Voor het STin2 polymorfisme zijn de resultaten minder eenduidig maar overall lijkt het STin2 genotype geen duidelijke invloed te hebben op het krijgen van bijwerkingen tijden een behandeling met SSRI's. Alleen voor bijwerkingen van het centraal/perifeer zenuwstelsel werd een verlaagd risico geobserveerd voor patiënten met het 10/10 genotype. Afgaand op onze resultaten, lijkt het serotonine transporter genotype betrokken te zijn bij de ontwikkeling van bijwerkingen tijdens SSRI behandeling. Deze resultaten moeten echter gerepliceerd worden voordat deze informatie daadwerkelijk gebruikt zou kunnen worden in de dagelijkse psychiatrische praktijk.

Patiënten met een depressie rapporteren vaak somatische comorbiditeit, comorbiditeit zou ook van invloed kunnen zijn op het behandelingseffect. In hoofdstuk 6 beschrijven we de associatie tussen het serotonine transporter genotype en somatische comorbiditeit, en met name pijnlijke comorbiditeit, bij depressie. In onze studie rapporteert 51% van de patiënten een of meerdere somatische aandoeningen. Nagenoeg al deze aandoeningen konden worden aangemerkt als pijnlijke aandoeningen. We observeerden een verhoogd risico op somatische comorbiditeit bij patiënten met het s/s genotype, 2.74 (95% BI 1.04-7.19). Dit risico veranderde nauwelijks als de analyses beperkt werden tot de mensen met pijnlijke comorbiditeit (OR 2.47, 95%- BI: 0.93-6.52). Voor niet-pijnlijke comorbiditeit konden geen OR's berekend worden omdat de aantallen hiervoor te klein waren. Patiënten met het STin2 10/10 of 10/12 genotype leken een verlaagd risico te hebben op somatische comorbiditeit tijdens de behandeling; OR 0.40 (95%- BI: 0.18-0.89) voor het 10/12 genotype en OR 0.68 (95%- BI: 0.24-1.95) voor het 10/10 genotype. Ook deze risico's veranderen nauwelijks als mensen met niet-pijnlijke comorbiditeit uitgesloten werden van de analyses; OR 0.43 95% BI 0.19-0.96 voor het 10/12 genotype en OR 0.74 95% BI 0.26-2.16 voor het 10/10 genotype.

Onze resultaten wijzen erop dat de aanwezigheid van somatische aandoeningen in depressie gerelateerd is aan het serotonine transporter genotype. Aangezien somatische aandoeningen het behandelingseffect negatief kunnen beïnvloeden, is het mogelijk dat eerder geobserveerde associaties tussen het serotonine transporter genotype en SSRI respons mede verklaard kunnen worden door de aanwezigheid van somatische comorbiditeit.

In hoofdstuk7 presenteren we een besliskundig model waarin de huidige behandelingsstrategie waarbij alle depressieve patiënten een SSRI krijgen vergeleken wordt met een alternatieve behandelingsstrategie waarin de keuze voor een antidepressivum bepaald wordt door de uitkomst van een genetische test op 5-HTTLPR. De resultaten geven aan dat het gebruik van een genetische test in de behandeling van depressie ervoor zorgt dat het percentage patiënten in remissie na 6 tot 12 weken behandeling, stijgt. Na een behandeling van 6 weken zou 60% van de patiënten in remissie zijn volgens de huidige strategie waarin alle patiënten een SSRI ontvangen. Na 12 weken behandeling zou dit getal 76.7% zijn. In de strategie waarin een genetische test gebruikt wordt om de keuze voor een antidepressivum te bepalen en waarbij patiënten met het s/s of s/l genotype een SNRI krijgen, zou 64.6% van de patiënten in remissie zijn na een behandeling van 6 weken. Na 12 weken behandeling zou dit 79.5% zijn. Als patiënten met het s/s of s/l genotype een TCA zouden krijgen na de genetische test zou 64.6% van de patiënten in remissie zijn na 6 weken en 83.2% na 12 weken. De robuustheid van het model hebben we geëvalueerd in sensitiviteitsanalyses, de conclusie van het model was dat genetisch testen de voorkeur verdiende boven de huidige behandelingsstrategie ongeacht veranderingen in de baseline waardes. Wij concluderen dat de implementatie van een genetische test mogelijk het aantal patiënten in remissie verhoogt en het aantal mensen dat voortijdig stopt met de behandeling verlaagt, waardoor naar verwachting, de overall gezondheidskosten kunnen dalen.

De conclusies van de afzonderlijke studies worden bijeengevoegd in hoofdstuk 8. In dit proefschrift leveren wij additioneel bewijs voor de hypothese dat het serotonine transporter genotype invloed heeft op verschillende aspecten van de behandeling van depressie (respons, bijwerkingen en comorbiditeit). We observeerden een associatie tussen het serotonine transporter genotype en SSRI respons, het vóórkomen van bijwerkingen en de aanwezigheid van somatische comorbiditeit in depressieve patiënten. Daarnaast lieten we in een besliskundig model zien dat de implementatie van een genetische test in de psychiatrische praktijk zou kunnen zorgen voor een hoger aantal patiënten in remissie na de start van een behandeling met antidepressiva. Uiteindelijk kunnen wij concluderen dat farmacogenetica een belangrijke en relevante bijdrage zou kunnen leveren aan een doelmatige behandeling van depressie.

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ABOUT THE AUTHOR

Kim Maria Smits was born on 13 September 1979 in Heerlen, The Netherlands. After completing secondary school (VWO) at the Rombouts College in Brunssum in 1997, she studied Health Sciences at Maastricht University. During her study, she fulfilled two internships. The first was carried out at the department of Epidemiology in collaboration with the department of Pathology, Maastricht University, in which she studied the association between K-ras mutations and folate intake in colorectal cancer patients from the Netherlands Cohort Study on diet and cancer. The second was performed at the Ospedale Maggiore, IRCCS-Molecular and Genetic Epidemiology Unit, Milan, Italy, where she studied the association between GSTM1 genotype and smoking in colorectal cancer and the influence of metabolic gene polymorphisms (CYP1A1, GSTM1, GSTT1, NAT2 and GSTP1) on tobacco consumption in healthy controls which resulted in two publications. She graduated in Biological Health Sciences in 2001. From February 2002 till February 2006, she worked as a PhD student at the department of Epidemiology at Maastricht University, on a research project examining the influence of serotonin transporter genotype on several aspects of treatment with selective serotonin reuptake inhibitors in depression. This project resulted in the present thesis. Since February 2006, she has been working as an assistant professor in Epidemiology at the department of Epidemiology, Maastricht University.