PHARMACO-EPIDEMIOLOGICAL STUDIES ON ANTIDEPRESSANT USE IN OLDER ADULTS

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PHARMACO-EPIDEMIOLOGICAL STUDIES ON ANTIDEPRESSANT USE IN OLDER ADULTS

Pharmaco-epidemiologische Studies over Antidepressiva Gebruik in Ouderen

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CHAPTER 1

GENERAL INTRODUCTION

The first antidepressant drug was introduced on the market in the early 1950's. Since then, several antidepressants have been developed. Antidepressants drugs can be categorized into different classes, with the most important classes being tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs) and other antidepressants. These classes act on different neurons in the central nervous system. For example, SSRIs specifically act on serotonergic neurons, whereas TCAs act on multiple neurons, such as adrenergic, dopaminergic and serotonergic neurons.

Antidepressants were originally marketed as a pharmacological compound for the treatment of depression. However, in recent years, antidepressants are also increasingly prescribed for other indications, such as sleep disorders, chronic/neuropathic pain and anxiety disorders. This increasing number of indications likely contributes to the worldwide increase in antidepressant use[1-7], which was confirmed in a number of studies[3, 4, 8, 9]. Compared with the use of TCAs, the use of SSRIs has increased more rapidly over the years, which is mainly due to their relatively mild adverse effect profile. Furthermore, monitoring of plasma levels is not required with SSRIs, as is the case with TCAs[10, 11].

There are multiple reasons why use of antidepressants in elderly patients are of special interest. For example, elderly have, on average, more comorbidities and are more likely to use concomitant drugs than younger patients. Also, drug metabolism in the liver and renal clearance of a drug are impaired in elderly. These factors make elderly a high-risk group to develop adverse drug reactions[11-13]. However, elderly are often excluded from premarketing studies. Therefore, information on the safety of use in elderly is limited. For this reason, post-marketing studies in elderly are pivotal to determine the safety of use of certain pharmacological compounds in this specific population.

Antidepressant drug use and adverse drug reactions

In addition to common and well-defined adverse drug reactions related to antidepressant drug use, such as vomiting, sexual dysfunction and dizziness, a number of adverse drug reactions are less consistently described in the literature, and thus at most only possibly related to the use of an antidepressant drug. A number of such adverse drug reactions are described below, and will be discussed in more detail in subsequent chapters.

The neurotransmitter serotonin plays an important role in the biology and treatment of depression. In addition to a role in psychiatric conditions, serotonin affects also energy metabolism and weight control[14]. The inhibition of serotonin

reuptake from the synaptic cleft by SSRIs has therefore been postulated to result in an unhealthier metabolic phenotype. For example, studies have reported an association between the use of SSRIs and an increased body weight[15, 16] and increased LDL cholesterol level[15, 17, 18]. However, the association between depression itself and body weight and LDL cholesterol complicates the interpretation of the results from these studies. In most of these studies, a treated-only design was used. With this design, patients with depression are followed in time after initiation of the pharmacological treatment. The study outcomes (e.g. body weight and serum LDL cholesterol level) were measured at baseline and after a period of time. However, with this design, a drug effect and an effect induced by a relief in depressive symptoms cannot be separated. For this reason, the pharmacological effect of antidepressants, and specifically SSRIs, on metabolic health remains unclear.

The use of drugs that have the potential to prolong the heart-rate corrected QT interval (QTc) is often restricted (e.g., a lower daily dose is recommended in high-risk patients), as this condition is associated with a higher risk of sudden cardiac death[19, 20]. Of the antidepressant drugs, a large number of TCAs and SSRIs have been associated with QTc-prolongation[21, 22]. However, there are reasons to question whether all described antidepressants truly give prolongation of the QT interval. For example, the formula used to correct the QT interval for the heart rate (notably the formula developed by Bazett [23]) is suboptimal, as an association between heart rate and the calculated QTc is still present. More specifically, the QTc is overestimated in individuals with a high heart rate and underestimated in individuals with a low heart rate[24, 25]. As TCAs exert anticholinergic properties, and thus increase the heart rate, QTc-prolonging properties of TCAs could be overestimated. Because of the limitations of Bazetts formula, the American Food and Drug Administration recommends the use of so-called "thorough QT/QTc studies", in which also other heart-rate correction methods are used beside the method developed by Bazett for the development of new drugs[26]. However, to date, the rationale of the thorough QT/QTc study has not been applied in post-marketing studies[27, 28], which could give valuable new insights into drug safety of existing drugs.

Antidepressants, and specifically SSRIs, have been reported in *in-vitro* studies to inhibit the activation and aggregation of blood platelets[29, 30]. Therefore, it has been postulated that the use of antidepressant drugs might decrease the risk of MI, but also increase the risk of bleedings [31]. However, population studies on the association between antidepressants and MI reported contradictory results. The interpretation of the results of the conducted studies might have been complicated by the association between depression and myocardial infarction[32-37]. Therefore, it remains unclear whether the effect found in *in-vitro* experiments has any clinical relevance.

The examples described above imply that there are still gaps in the knowledge about the safety of use of antidepressants. Therefore, additional studies on the safety of antidepressants are warranted considering the limitations of previously conducted studies and used study outcomes.

Genetics and antidepressant drug use

Failure of antidepressant therapy is common in the treatment of depression[38], and is partly genetically inherited[39-41]. However, despite modern technologies to identify genetic variants on the genome, only very few well-replicated genetic regions have so far been reported to be associated with drug response or adverse drug reactions of antidepressant drugs. In candidate gene studies, in which a predefined genetic variant was tested, genetic variation in genes encoding drug metabolizing enzymes (e.g., *CYP2D6*) and efflux pumps (e.g., *ABCB1*)[42] as well as genes encoding the serotonin transporter (e.g., *SLC6A4*) and receptor (e.g., *HTR2A*)[42, 43] have been reported to be associated with antidepressant drug response. However, although a large number of other candidate genes have been studied as well, only few were well-replicated in other study populations. Also, genome-wide association studies, in which common genetic variants throughout the whole genome are tested, did not report statistically significant results[44-47].

All studies that have been reported to date used a treated-only design. As already discussed in the paragraph on adverse drug reactions related to antidepressant use, the same limitations of that study design are applicable here. Again, the natural course of the depression cannot be separated from the effect of the drug on depressive symptoms. This complicates the quest to identify common genetic variation related to antidepressant drug response. Therefore, more research with alternative study designs is warranted.

Aim and outline of this thesis

In this thesis, we aim to study the use of antidepressants in the overall Dutch population and to investigate unintended effect related to antidepressant drug use in older adults. Furthermore, we aimed to study the role of genetic regions in antidepressant response and antidepressant adverse drug reactions. Addressing these objectives will contribute to a better understanding of antidepressants-related unintended effects in an older population as well as to obtain more insights into the biology involved in antidepressant drug treatment.

In **chapter 2**, we report the prevalence and incidence of antidepressant use as well as the indications to prescribe antidepressants for in the Netherlands over a 15-year period.

In **chapters 3, 4 and 5**, we focus on unintended effects related to treatment with antidepressant drugs. In **chapter 3.1**, we study the association between use of antidepressants and body mass index, and additionally we focus on a possible difference between males and females. In **chapter 3.2**, we assess the association between use of antidepressants and serum lipid levels, and study the possible effect modification by common genetic variation in the *HTR2A* gene. In **chapter 3.3**, we investigate the association between antidepressants and insulin levels among non-diabetics and insulin dependency among diabetic patients. In **chapter 4.1**, we reassess the association between TCAs and QTc prolongation. In this study, we use different methods to correct the QT interval for heart rate. In **chapter 4.2**, we assess the association between SSRIs, and specifically citalopram, and QTc. In **chapter 4.3**, we study the association between antidepressants and heart rate variability, being a measure of the balance between the sympathetic and parasympathetic autonomous nervous system. In **chapter 5.1**, we investigate the association between use of antidepressants and the risk of incident myocardial infarction, and in **chapter 5.2**, we study the association between the use of antidepressants and the presence of cerebral microbleeds.

In **chapter 6**, we investigate the role of common genetic variation in outcomes of antidepressant treatment. In **chapter 6.1**, we study common genetic variation in the *ABCB1* gene, which encodes for P-glycoprotein, in relation to early switching and discontinuation of antidepressant therapy. In **chapter 6.2**, we study the use of drug-gene interaction models on depressive symptoms in population-based studies as an alternative to conventional designs to identify common genetic variants associated with antidepressant treatment response.

In **chapter 7**, we discuss the main results of the conducted studies described in this thesis. Furthermore, methodological considerations and future directions for research will be discussed. The main results are summarized in **chapter 8**.

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CHAPTER 2

ANTIDEPRESSANT DRUG UTILIZATION

CHAPTER 2.1

PRESCRIPTION AND INDICATION TRENDS OF ANTIDEPRESSANT DRUGS IN THE NETHERLANDS BETWEEN 1996 AND 2012: A DYNAMIC POPULATION-BASED STUDY

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Purpose: Antidepressant drug use increases worldwide. It is pivotal to closely monitor the use of antidepressants and to determine in what subpopulations the rise is most substantial. In a Dutch primary care database, we aimed to investigate the (sex- and age-specific) prevalence and incidence of antidepressant prescription and to monitor the indication of incident prescriptions over a 17-year period (1996 – 2012).

Methods: This study, embedded in the Integrated Primary Care Information database, included all patients aged 10 years or older. Per calendar year, prevalence and incidence of antidepressant drug prescription were calculated by drug class (tricyclic antidepressants, selective serotonin reuptake inhibitors (SSRIs) and others), sex and age. The indication of incident prescriptions (e.g. depression, anxiety, sleep disorders and neuropathic pain) was determined based on the International Classification of Primary Care codes.

Results: In total, 1.49 million patients were included. For all antidepressants together, the prevalence increased over time. However, incident prescription of specifically SSRIs decreased from 2000 onwards. During the study period, incidence and prevalence were higher in older and female patients. The increase in prevalence and the decrease in incidence were more pronounced in females than in males. Furthermore, antidepressants were increasingly prescribed for indications as neuropathic pain and sleep disorders instead of depression.

Conclusions: In Dutch primary care, prevalent prescription of antidepressants continued to increase, but incident prescription of particular SSRIs decreased from 2000 onwards. In later years, antidepressants were less frequently prescribed for depression-related indications in incident users.

Introduction

Antidepressant drug use increased substantially during the last decades[1-5]. The rise in antidepressants can be explained by an increasing number of indications (e.g. neuropathic pain and sleep disorders) and by a lower threshold to prescribe antidepressants[3, 4, 6, 7]. The rise in use was shown to be most pronounced for the selective serotonin reuptake inhibitors (SSRIs)[4, 5, 8]. Compared with the older tricyclic antidepressant (TCAs), SSRIs have a milder adverse drug reaction profile, have a lower risk of severe consequences in case of overdosing, and do not require continuous monitoring of blood levels in a number of indications[5, 8-10]. Although increasingly prescribed, it was shown in some studies that incident antidepressant drug use only increased until the year 2000. Thereafter, the number of new users of antidepressants stabilized or even decreased[4, 11]. The discrepancy between patterns of prevalence and incidence of antidepressant use was most likely caused by an increased recurrent antidepressant use and increased treatment duration over time[2, 12].

Although antidepressant use was higher in the older aged, an excessive rise in antidepressant use by the older aged was not demonstrated consistently[4, 8, 11, 13]. Nevertheless, there are concerns whether the indication to prescribe antidepressants is always justified in elderly[14-17]. This patient population is at higher risk for drug-drug interactions and adverse drug reactions, as they have a lower drug metabolism, a lower renal clearance, have more co-morbidities and use more medications concomitantly[10, 18, 19]. To minimize the risk of adverse drug reactions in the elderly, an addendum of the multidisciplinary guidelines for general practitioners and medical specialists was published in 2008. In this guideline, it was recommended to use SSRIs or nortriptyline, instead of the other TCAs[20]. However, it is unclear to what extent general practitioners adhere to these guidelines.

The increase in antidepressant use in the last decades was also observed in the Netherlands[2, 5, 12, 21]. However, most studies did yet not investigate whether there was increased prescription of antidepressants in certain subpopulations (e.g., females or elderly), which is pivotal to estimate what subpopulations are specifically at risk to develop particular antidepressant-related adverse effects. For example, it is unknown whether the preference of the TCA nortriptyline for the treatment of depression in elderly[20] is also reflected by a faster increase in prescription in this subpopulation. In addition, although antidepressants are prescribed for an increasing number of indications, the indication of antidepressant use over time is poorly studied. We aimed to investigate the (sex- and age-specific) prevalence and incidence of antidepressant drug prescription in a population-based study

of specifically middle-aged and elderly in the Netherlands. Besides, we aimed to investigate indications of incident antidepressant drug prescription over time.

Methods

Setting of the Integrated Primary Care Information database

This study was conducted within the Integrated Primary Care Information (IPCI) database. A more detailed description about this database has been published elsewhere [22]. In summary, this database is a research database containing the electronic medical records of general practitioners (GP) and currently comprises more than 1.5 million patients in the Netherlands. The IPCI database was initiated in 1992 and expanded greatly since then. With regard to demographic factors such as age and sex, the distribution of the IPCI population is representative of the overall Dutch population [23]. Research in the IPCI database is optimal in several ways. First, all Dutch inhabitants are registered with a GP practice, limiting selection bias. Second, in the Dutch healthcare system, the GP acts as a gatekeeper between primary and secondary care. Medical records of GPs therefore also contain information from secondary care. And finally, participating GP practices are not allowed to use additional paper-based medical records which makes the electronic database more complete.

The IPCI database contains information on demographics, symptoms, diagnoses, referrals, clinical and laboratory findings, hospitalizations and drug prescriptions. Information on drug prescriptions comprises the brand name, generic name, number of pills/capsules/solution, prescribed daily dose, and the Anatomical Therapeutic Chemical (ATC) classification code [24]. The IPCI database follows the European Union guidelines on the use of medical data for medical research and has been validated for the use of pharmacoepidemiological research [22]. The current study was approved by The Scientific and Ethical Advisory Board of the IPCI project (project number: 12/12).

Study population

For the current study we included all patients aged 10 years and older with at least 1 year of medical history from the IPCI database. Patients, who had a follow-up of at least 1 year, were followed from start of study (January 1, 1996) until death, loss to follow-up or end of the study period (December 31, 2012), whichever came first. For the calculation of the incidence rate, patients were censored at the date of their first antidepressant drug prescription.

Study outcome

An antidepressant drug prescription was defined based on the (four-digit) ATC code "N06A". Antidepressants were classified into antidepressant drug classes (ATC code: TCA, "N06AA"; SSRI, "N06AB"; other, "N06AX"). Amitriptyline (ATC code: "N06AA09") and nortriptyline (ATC code: "N06AA10") were identified based on the individual complete (seven-digit) ATC codes. Changes in ATC coding were taken into account. For all these categories both prevalence and incidence rates were calculated.

The yearly prevalence of antidepressant prescriptions was calculated by dividing the total number of patients with at least one antidepressant drug prescription by the total number of person years in a calendar year. Because of data conversion in different health care software systems in 2006, prevalence rates could not be calculated correctly in this year. For this reason prevalence rates in this year were interpolated based on the average of the surrounding years.

To calculate the incidence of antidepressant prescription, we divided the total number of patients who were prescribed an antidepressant for the first time during follow-up by the total number of person years (PY) within a calendar year. We defined a prescription as incident when no antidepressant drug prescription was identified in the 1 year medical history prior to study inclusion. For this analysis, no interpolation was necessary, as interpolated results and actual calculated results were similar in 2006.

The indication of incident antidepressant prescriptions was based on International Classification of Primary Care (ICPC) codes[25]. We selected only those indications for which we expected to have sufficient numbers in middle-aged and elderly patients. The following indications were considered when identified within 90 days before or after the first antidepressant prescription: depression (ICPC code: P03, P76), anxiety (P01, P74), sleep disorders (P06), neuropathic pain (N94), and psychosis and schizophrenia (P71, P72, P98). When more than one indication was recorded within 90 days around initiation of incident antidepressant therapy, the incident antidepressant prescription was considered to be prescribed for "multiple indications."

Co-factors

Prevalent and incident antidepressant prescription were studied over time, and stratified by sex and different age strata. Strata were defined as such that a sufficient number of patients were available in all strata. The following age strata were defined: 10 – 19, 20 – 39, 40 – 59, 60 – 79, and ≥ 80 years. For the analysis on amitriptyline and nortriptyline, we applied a different stratification, as the number of patients treated with amitriptyline or nortriptyline ≥ 80 years was too low. This age stratum was

combined with the patients between the age of 70 and 79 years (denoted hereafter as elderly). This group was compared with patients between the age of 50 and 69 (denoted hereafter as middle-aged), to compare the patterns in middle-aged and elderly patients.

Data analysis

The characteristics of the study population were studied by calculating the number of patients in an age group stratified by sex. During follow-up patients were allowed to switch age groups; therefore, these numbers exceeded the total number of patients included in the study.

The prevalence and incidence rates were calculated per calendar year. Results were presented by calculating the average of the rates per pair of calendar years as in some of the subanalyses, an insufficient number of prescriptions was available. Rates were calculated for all antidepressants together, separately per drug class and separately for amitriptyline and nortriptyline.

We stratified the analyses additionally by sex and age strata. The comparison of prevalence and incidence rates over time between different age categories and between males and females was conducted using linear regression analysis. As the number of patients increased over time, we weighted the analyses for the total number of person years in two consecutive years. The comparisons were statistically tested by including a multiplicative interaction term between the calendar year and the studied factor (for example, is a rise or decrease in prevalent or incident prescribing specific for a particular subpopulation). Analyses were conducted with SPSS statistical software (version 21.0, IBM Corporation, Armonk, NY). A two-sided p-value below 0.05 was considered statistically significant.

To study the indications of incident antidepressant prescriptions over time, the total number of known indications within a pair of calendar years, as extracted from the study population, was considered as 100%. The contribution of an individual indication was calculated as a percentage relative to the total number of identified indications.

Results

Population characteristics

In total, approximately 1.49 million patients had an age above 10 years during follow-up and were included in the study (Table 1). The study population comprised slightly more females than males. Females were also more frequently older than 70 years.

Table 1 Characteristics of the study population

	Females	Males
	N = 767,492	N = 724,870
Age, years, N (%)		
10 – 19	122,914 (16.0)	127,358 (17.6)
20 – 29	148,862 (19.4)	137,251 (18.9)
30 – 39	150,756 (19.6)	146,658 (20.2)
40 – 49	161,353 (21.0)	159,925 (22.1)
50 – 59	139,100 (18.1)	137,416 (19.0)
60 – 69	112,033 (14.6)	109,267 (15.1)
70 – 79	75,537 (9.8)	64,483 (8.9)
≥80	52,391 (6.8)	29,913 (4.1)

The sum of the percentages exceeds the 100% and exceeds total number of patients, as participants could change age category during follow up.

Prevalent antidepressant prescriptions

From 1996 to 2012 (Figure 1A), prevalent antidepressant prescription doubled (from 35.5 per 1,000 patients in 1996-1997 to 69.8 per 1,000 patients in 2012). The absolute rise in prevalence of SSRIs was largest and increased from 18.2 per 1,000 patients in 1996-1997 to 38.4 per 1,000 patients in 2012. In addition, prevalence of TCAs remained stable and the prevalence of other antidepressants almost tripled over time.

In the sex-stratified analysis (Figure 1B), prevalence was higher and increased more over time in females than in males (p-value = 0.005). In addition, although prevalence was higher in the older aged (Figure 1C), the increase in prevalent prescription of antidepressants was similar over the age strata (p-values > 0.18). This remained similar when stratified by drug class (data not shown). In addition, patients aged 70 years and older (the elderly) were prescribed more amitriptyline and nortriptyline compared to patients between the age of 50 and 69 years (the older adults; Figure 1D). In older adults, the rise of prevalent prescription of nortriptyline was less pronounced than the rise of prevalent prescription of amitriptyline (p-value = 0.003). However, the increase in prevalent prescription was similar for amitriptyline and nortriptyline in the elderly (p-value = 0.51).

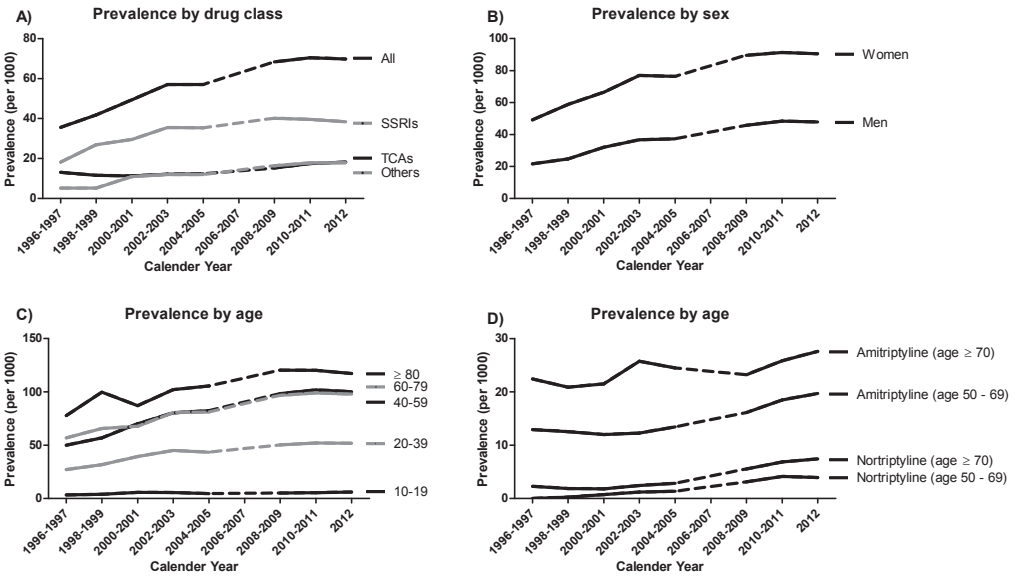

Figure 1 **Prevalence of antidepressant drug prescription over time**

A) Prevalence of antidepressant use by drug class. **B)** Prevalence of antidepressants in males and females. **C)** Prevalence of antidepressant drug prescription by age strata. **D)** Prevalence of amitriptyline and nortriptyline in patients between age 50 and 69 and above 69 years. Data presented as the number of patients with at least one prescription in a calendar year per 1000 patients. The dotted part of the line represents the period in which prevalence could not be estimated properly.

Incident antidepressant prescription

Until 2000, incident prescription of antidepressant drugs increased (Figure 2A), but decreased thereafter (from 22.6 per 1,000 PY in 2000-2001 to 17.1 per 1,000 PY in 2012). Of the different antidepressant drug classes, the decrease in incident prescription of SSRIs was strongest, whereas incident prescription of TCAs increased in more recent years and was highest in 2012 (4.3 per 1,000 PY).

Females had a higher incidence of antidepressant drug prescription than males (Figure 2B). The decrease in incidence from 2000 to 2001 onwards was more pronounced in females than in males (p-value = 0.014). Furthermore, the incidence of antidepressant prescription was highest in patients >80 years, but trends over time were similar across the age strata (Figure 2C). From 2006 onwards (Figure 2D), incident prescription of amitriptyline and nortriptyline increased both in older adults and elderly, but the difference between the two remained similar over time for both age strata (p-values > 0.16).

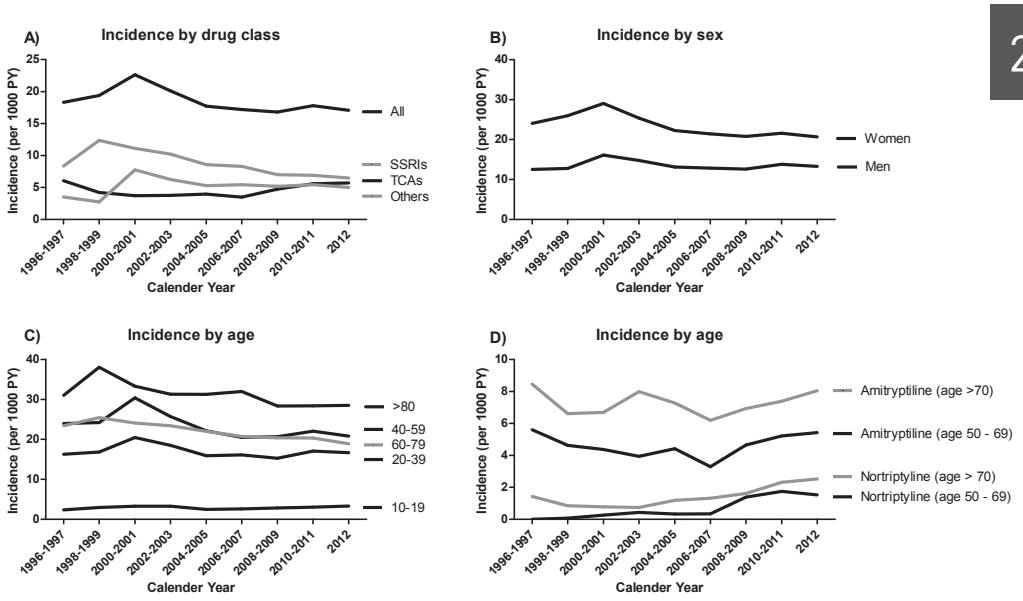

Figure 2 **Incidence of antidepressant drug prescription over time.**

A) Incidence of antidepressant use by drug class. **B)** Incidence of antidepressants in males and females. **C)** Incidence of antidepressant drug use by age strata. **D)** Incidence of amitriptyline and nortriptyline in patients between age 50 and 69 and above 69 years. Data presented as the total number of incident users per 1000 person years within a calendar year.

Indication of prescribing

Indications registered by ICPC codes were recorded for 41% of the incident prescriptions during the study period, which decreased from 68% in 1996-1997 to 40 percent in 2012. In this subpopulation, antidepressants were mostly prescribed for depression-related disorders. However, this percentage decreased from 65% in 1996 to 47% in 2012 (Figure 3A). In more recent years, antidepressants were increasingly prescribed for sleep disorders and neuropathic pain. The relative decrease of prescribing antidepressants for depression-related indications was least visible for SSRIs (Figure 3B). Furthermore, depression-related indications accounted only for a minor percentage (18% in 2012) of incident TCA prescriptions (Figure 3C). TCAs were mostly prescribed for sleep disorders and neuropathic pain (both 25 – 30% in 2012). However, this was different for nortriptyline, which was mostly prescribed for depression-related indications (31% in 2012; results not presented in the figure). Other antidepressants were also increasingly prescribed for indications like anxiety and sleep disorders (Figure 3D). Compared with middle-aged adults, elderly were more often prescribed antidepressants for indications different than depression (data not shown).

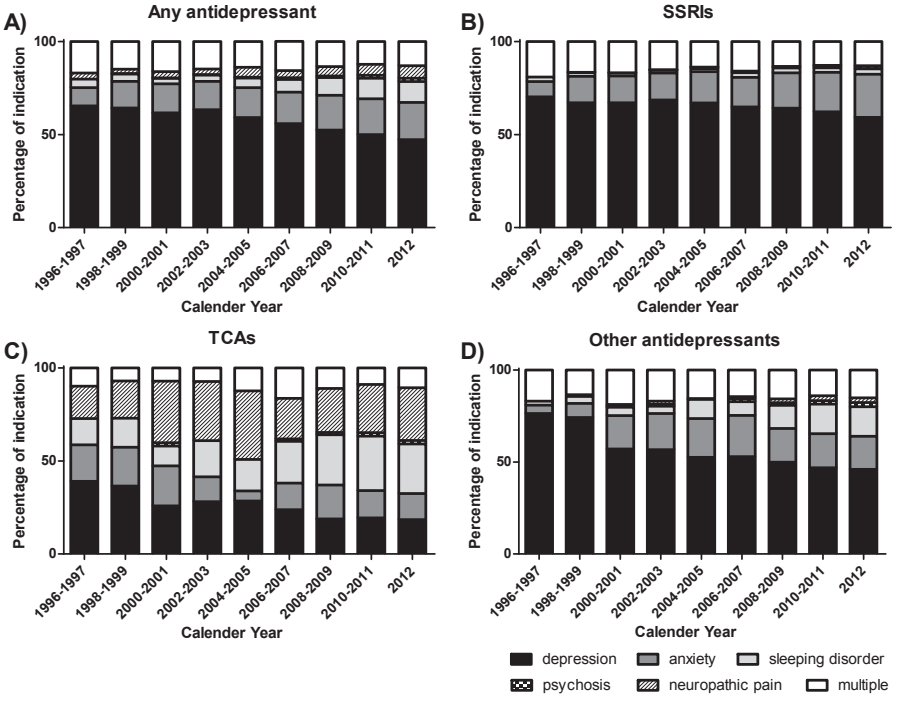

Figure 3 **Indication of antidepressant prescribing over time.**

A) Indication trends of all antidepressants taken together. **B)** Trends of indications of selective serotonin reuptake inhibitors. **C)** Indications trends of tricyclic antidepressants. **D)** Indications trends of other antidepressants. Data presented as the percentage of drugs prescribed for that indication.

Discussion

Within this dynamic population-based study, we observed that prevalent antidepressant drug prescription increased between 1996 and 2012, although incident prescription of particular SSRIs decreased from 2000 onward. In females, the increase in prevalence and the decrease in incidence of antidepressant drug prescription were more pronounced over time. In addition, the prevalence and incidence of antidepressant prescription were higher in the older aged. However, the rise in prevalence and incidence of antidepressant prescription was not different for a specific age-group. Over time, antidepressant drugs (in particular TCAs and other antidepressants) were increasingly prescribed for other indications than depression-related indications.

The 1994 edition of the guidelines by the Dutch College of General Practitioners stated that pharmacological treatment is only preferred for severe cases of depression[26]. However, the revised guidelines, published in 2003, were more reserved regarding the use of pharmacological treatment as first choice. This guideline preferred to start with non-pharmacological treatment[27]. Within our study population, we observed that the prevalence of antidepressants, in particular SSRIs and other antidepressants, increased. At least for the group of other antidepressants, the preference of venlafaxine and mirtazapine by the multidisciplinary guidelines (version 2008) might have contributed to the increase in prevalence of this drug class[20, 28], although the introduction of more antidepressants in this drug class on to the market may have contributed as well. In addition, our findings are in agreement with other studies conducted in Western populations[4, 11, 12]. Furthermore, we observed that from 2000 onward, there is a trend towards a lower incidence of antidepressant prescription, which is in line with other studies[4, 11]. This was in particular the case for the SSRIs. The revised treatment guideline from 2003, which was more reserved to initiate pharmacological treatment, might have contributed to the modest decrease in incident use[27].

The discrepancy between prevalence and incidence trends of antidepressant drug use is also described by other studies and is most likely explained by an increase in recurrent use and treatment duration[2, 12]. The difference in prevalent and incident prescription over time between males and females has not yet been reported. The indication depression to prescribe antidepressants for might explain the difference in trends between males and females, although we cannot exclude that also other indications (e.g., premenstrual syndrome) contributed to the observed difference between males and females[29, 30].

Elderly patients are of special interest, as this population has more co-morbidities and is at higher risk of polypharmacy, which increases the risk of drug-drug interactions and adverse drug reactions[10, 18, 19]. Some of the previously conducted studies showed that the increase in antidepressant drug use was mainly in elderly when compared to middle-aged adults [8, 13]. However, within our study population, the increase in prevalence and the decrease in incidence were similar across different age strata. Similar results were found in a study conducted in British Colombia[4]. Since 2008, the interdisciplinary guidelines for the treatment of depression recommend nortriptyline instead of amitriptyline for an elderly patient diagnosed with depression[20], as nortriptyline has less cardiac side effects than other TCAs. However, no larger increase in prevalence and incidence over time was observed for nortriptyline relative to amitriptyline, which indicates that there was no clear preference for nortriptyline. The difference in indications to prescribe amitriptyline and nortriptyline might explain part of the inconsistency with the

treatment guidelines. For example, amitriptyline is preferred by the guidelines of the Dutch College of General Practitioners as a treatment for neuropathic pain[31, 32]. In addition, nortriptyline was prescribed more often for depression-related indications than all TCAs together, which fits the preference by the treatment guidelines for the treatment of depression in the elderly[20].

This study has a few strengths and limitations. A strength was the large sample size and the representativeness of the overall Dutch population[23]. The latter strength was supported by the similarity of our numbers with prescription numbers from the total Dutch population, as collected by the Dutch healthcare insurances[33]. This study was conducted in a general practice database. Thus, we were dependent on the quality of registering patient information by the GP. For this reason, the indication of incident antidepressants can be misclassified. In addition, the definition of an incident prescription was based on non-use during a 1-year period prior to study inclusion. Patients could still use prior to this period for which we did not have information. Furthermore, we used ICPC codes to define the indication of incident antidepressant drug prescriptions, but this could only be defined for a minor proportion of the total population (about 41%). A manual validation of free text of the patient records would have been the best approach, but because of the large sample size, this was not feasible. However, the percentage of registered indications was similar as published before in a different Dutch population[34]. However, their time-window around the first antidepressant drug prescription was larger than used in our study. Nevertheless, the proportion of missing indications of use is unlikely to be related to the type of antidepressant and thus would not have influenced the results.

In conclusion, we observed that the prevalence of antidepressant prescription increased over time, but incident antidepressant prescription, and in particular incident SSRI prescription, decreased from 2000 onward in Dutch primary care. These trends over time were different for males and females, but not for different age strata. In addition, the proportion of antidepressants prescribed for depression-related indications decreased during the study period.

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CHAPTER 3

ANTIDEPRESSANT USE, EFFECTS ON BODY WEIGHT, AND UNINTENDED ENDOCRINOLOGIC EFFECTS

CHAPTER 3.1

SEX-SPECIFIC ASSOCIATION OF ANTIDEPRESSANT USE AND BODY WEIGHT IN A POPULATION-BASED STUDY IN OLDER ADULTS

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OBJECTIVE: This study aimed to investigate the association between antidepressant use and body weight in a population-based study in older adults.

METHODS: All participants (N = 7,269) from the prospective Rotterdam Study with data on anthropometrics and current depressive symptoms were studied (data collected between September 1993 and December 2011). The association between antidepressant use, derived from pharmacy records, and change in body mass index (BMI) between repeated examination rounds were analyzed. We considered current depressive symptoms (assessed by questionnaire) and baseline BMI (for the change in BMI analysis only) as important covariates. Additional analyses were stratified by sex and restricted to long-term use (≥90 days) and by level of binding affinity to the serotonin reuptake transporter (denoted as hSERT antidepressants).

RESULTS: Participants who used Selective Serotonin Reuptake Inhibitors (SSRIs, N = 198) had a larger increase in BMI compared to non-users (+0.74 and + 0.23 kg/m^2, respectively, p-value <0.001) between repeated examination rounds. No change in BMI was observed for tricyclic antidepressants (N = 146) and other antidepressants (N = 57) compared to non-users. Weight gain was only observed in females who were treated for ≥90 days with hSERT antidepressants or SSRIs, and not in males (p-value for interaction = 0.002).

CONCLUSION: Within our study of older adults, hSERT antidepressants were associated with an increased body weight in females, which is supported by the biological function of serotonin in weight control and the differences in serotonergic signaling between males and females.

Introduction

In the biology and treatment of psychiatric diseases, such as depression, the neurotransmitter serotonin is of great interest. The transmission of serotonin across neurons in the central nervous system, but more specifically in the prefrontal cortex, plays an important role in experiencing emotion. Also, besides being a treatment target for depression, the transmission of serotonin across neurons also has an important role in, for example, energy metabolism and weight control[1]. The involvement of serotonin in mechanisms other than emotion was clearly shown in genetic studies. For example, genetic variation in the serotonin receptor and the serotonin reuptake transporter, as well as the availability of serotonin receptors on the membrane of neurons, are associated with body weight[1-4]. Also, the efficiency of serotonergic signaling across the membrane of neurons is different for males and females. In females, serotonin has a higher level of binding potential to the postsynaptic 5-HT_{1A} receptor and a lower level of binding potential to the serotonin reuptake transporter as compared to males[5, 6]. This difference might (partly) explain the already observed difference between males and females in antidepressant treatment response[7-10]. Taken together, these studies suggest that drugs (including antidepressants and other psychotropic drugs), that affect the bioavailability of serotonin in neurons, might be able to increase body weight, but also that the increase in body weight is different for males and females.

Several studies aimed to study the effect of antidepressant use on body weight. However, a limited sample size might explain why results were often contradictive between these studies[11-14]. Results of a meta-analysis showed that the use of paroxetine and amitriptyline for a duration of at least 4 months was associated with an increased body weight[12]. These studies included patients who were diagnosed with a major depressive disorder and who were treated with antidepressants and who were followed over time. As depressive symptoms are often accompanied by a reduction in appetite, these studies were not able to disentangle whether the observed weight gain was caused by a relief in depressive symptoms (and therefore an increase in appetite) or by the antidepressant drug itself.

Based on the biological findings, it can be hypothesized that antidepressants with a high binding affinity to the serotonin transporter, like Selective Serotonin Reuptake Inhibitors (SSRIs), could increase body weight. Because of the limitations of previously conducted studies, we performed a population-based study comprising older adults with body weight and current depressive symptoms being measured at repeated examination rounds. Within this study population, we studied the association between antidepressants, by drug class and level of binding affinity

to the serotonin transporter, and body mass index (BMI), stratified by sex and treatment duration.

Methods

Research setting

For the current study, we used data from the prospective Rotterdam Study, which aims to investigate the incidence of, and risk factors for, several age-related diseases. A more detailed description of the design and rationale of the study is published elsewhere[15, 16]. From 1990 to 1993, all inhabitants aged 55 years and older from a district (Ommoord) located in Rotterdam, the Netherlands, were asked to participate in the original cohort (denoted hereafter as RS-I). In total, 7,983 individuals agreed to participate (response rate 78%). An extension of the original cohort was initiated in 2000 (denoted hereafter as RS-II). Within this subcohort, all inhabitants from Ommoord aged 55 years and older, and not already participating in RS-I, were asked to participate in RS-II. In total, 3,011 individuals agreed to participate (response rate 67%). Follow-up examinations were conducted every 4 – 5 years after start at baseline. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands and written informed consent was obtained from all study participants.

Study population

For the current study we included all participants who had their height and weight measured and who had filled in a depression questionnaire during the center visits. In total, participants from RS-I could at most have 4 measurements, while participants from RS-II had a maximum of 3 measurements.

Exposure

More than 99% of the participants have their drug prescriptions filled at one of the seven regional pharmacies, which are fully computerized. From January 1 1991 onwards, complete prescription data is available, which included the Anatomical Therapeutical Chemical (ATC)-code [17], the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and the product name of the drug. Exposure to an antidepressant drug was defined as current if the center visit date fell within a prescription episode, calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. Antidepressant use was subdivided in Tricyclic antidepressants (TCA, N06AA), SSRIs (N06AB)

and other antidepressants (N06AX). Participants without an antidepressant drug prescription at a center visit were considered as non-users, and used as the reference population. We also defined an antidepressant exposure category based on a low dissociation constant to the serotonin reuptake transporter (0 – 1 nmol/L), and thus antidepressants with a high level of binding affinity (denoted as hSERT antidepressants). With this reclassification of antidepressants, the specific role of serotonin modulation by antidepressants could be studied best[18]. This included the antidepressants clomipramine, fluoxetine, paroxetine and sertraline[19, 20]. The duration of treatment at the time of the study center visit was computed by calculating the cumulative number of prescription days. Long-term exposure was defined as the cumulative use of any antidepressant of more than 90 days. The prescribed dose was calculated relative to the defined daily dose[17].

Outcome definition

For the current study, we defined the following outcomes, namely BMI, waist-to-hip ration (WHR), height and weight for cross-sectional analyses and the change in BMI between repeated examination rounds for the longitudinal analyses. Length (in cm) and weight (in kg) were measured by research nurses during all follow-up visits. WHR was measured (in cm) by research nurses, except at the second center visit of RS-I. BMI was calculated by dividing the weight (in kg) by the length (in meters) squared. For the longitudinal analysis we calculated the difference in BMI between the repeated examination rounds.

Co-variables

For the current study, we considered age, sex, current smoking status, use of alcohol, depressive symptoms and concomitant use of antipsychotics as potential confounding factors. For the analyses on weight, length was also considered as a potentially confounding factor, as antidepressants, and mainly SSRIs, are suspected to decrease bone mineral density and might therefore ultimately decrease height and therefore lead to increased BMI[21]. Current smoking status (yes/no) and alcohol use were obtained using interview questionnaires. As different questionnaires were used to assess habitual alcohol intake for every examination round, the median intake per round was used to define low and high alcohol use. Antipsychotic treatment (yes/no) at the examination round use was determined using pharmacy records (ATC-code: N05A). Depressive symptoms were screened with a Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D). The questionnaire gives a score, ranging between 0 and 60, with higher scores indicative of more depressed feelings. A score of above 16 was considered as an indicator for a potential depression[22, 23]. In case of the analyses on the change in BMI, also the baseline BMI was considered.

Statistical analyses

As we had multiple measurements of the study outcomes from most study participants, analyses were performed using repeated measurement analyses (Generalized Equation Estimation). Co-variables, as described above, were only included in the analysis when they reached statistical significance (p-value < 0.05) in a multivariable analysis with BMI. For the analysis on weight, we included height (in meters) squared in the model to assess whether a difference in BMI was independent from height. We used pair-wise comparisons to assess the associations between non-use and the different antidepressant drug classes (TCAs, SSRIs and others) and BMI, and change in BMI between repeated examination rounds. These analyses were also stratified by treatment short- and long-term treatment and by the presence or absence of clinically relevant depressive symptoms (CES-D score > 16). Within the non-treated population, also the difference in BMI was compared between depressed and non-depressed participants.

The analysis on BMI and change in BMI between repeated examination rounds were additionally stratified by sex for SSRIs, TCAs and hSERT antidepressants. Other antidepressants were not taken into account due to a limited number of participants. All analyses were additionally restricted to long-term drug exposure. Statistical interaction between sex and the drug exposure on BMI or change in BMI was assessed by including an interaction term between them in the multivariate model.

All statistical analyses were performed using SPSS (version 20.0, IBM Corporation, Armonk, NY). P-values below 0.05 were considered statistically significant.

Results

Baseline characteristics

In total, 7,269 participants (16,331 measurements) from both RS cohorts had at least one BMI measured at the center visits and had information about co-variables (table 1). Time intervals between examination rounds were on average 5.1 years (SD = 1.1). Mean age of the study population was 68.9 years (SD = 8.1) and comprised of 57% females. The mean BMI was 27.0 kg/m^2 (SD = 4.0) with 19.5 percent being obese (BMI >30 kg/m^2). At baseline, TCAs were used by 1.1 percent, SSRIs by 1.7 percent and other antidepressants by 0.3 percent of the participants.

Among non-users, BMI was 0.17 kg/m^2 (95%CI: 0.06 – 0.29; p-value = 0.002) lower in participants with clinically relevant depressive symptoms compared to non-depressed participants.

Table 1 Characteristics of the study population

	First visit characteristics (N = 7,269)	
Age (years), mean (SD)	68.9	(8.1)
Females, N (%)	4,142	(57.0)
Body Mass index (kg/m^2), mean (SD)	27.0	(4.0)
Length (centimeters), mean (SD)	167.3	(9.3)
Weight (kilograms), mean (SD)	75.5	(12.9)
Body Mass Index >30 kg/m^2, N (%)	1,420	(19.5)
Waist to hip ratio, mean (SD)	0.92	(0.10)
Depression (CESD > 16), N (%)	662	(9.1)
Current smoking (yes/no), N (%)	1,492	(20.5)
High alcohol use (yes/no)[a], N (%)	3,525	(48.5)
Medication use, N (%)		
Tricyclic antidepressants (N06AA)	80	(1.1)
Selective Serotonin Reuptake Inhibitors (N06AB)	120	(1.7)
Other antidepressants (N06AX)	20	(0.3)
Antipsychotic drugs (N05A)	30	(0.4)

Abbreviations: N, number of participants; SD, standard deviation; CESD, Center for Epidemiological Studies Depression Scale. Data presented as the means or percentages of the different variables at the first examination round that was included in this study. [a] High alcohol use was defined as having an alcohol use above the median of the study population.

Association between antidepressant drug use and anthropometric measures

Participants treated with SSRIs (N = 288) had a higher BMI compared to control participants (28.4 and 27.9 kg/m^2, respectively, p-value = 0.017; table 2). A similar result was obtained when this analysis was repeated with weight (79.3 and 78.1 kg, respectively for SSRI users and controls, p-value = 0.017). When we stratified for treatment duration, a higher BMI in SSRI users was only observed in participants that used SSRIs for a period of at least 90 days (28.5 and 27.9 kg/m^2, respectively, p-value = 0.005), whereas no association was observed for the participants that used SSRIs for a period less than 90 days (27.7 and 27.9 kg/m^2, respectively, p-value = 0.68). BMI was similar for participants using an TCA compared to non-users (28.0 and 27.9 kg/m^2, respectively, p-value = 0.65). The difference between TCA use and non-use did not materially change when we stratified by treatment duration. The use of other antidepressants was also not associated with BMI or weight. WHR was

Table 2 **Association between different antidepressant drug classes and anthropometric measures**

	Non-use (ref)		SSRI			TCA			Others		
	Mean	95% CI	Mean	95% CI	P-Value	Mean	95% CI	P-Value	Mean	95% CI	P-Value
No. of measurements		15,782		288			193			68	
Dosage[a]		NA		1.03			0.52			0.78	
BMI (kg/m^2)	27.9	26.9 – 29.0	28.4	27.3 – 29.4	**0.017**	28.0	27.0 – 29.1	0.59	27.7	26.6 – 28.9	0.58
Weight (kg)	78.1	73.2 – 82.9	79.3	75.4 – 83.2	**0.017**	78.4	74.7 – 82.0	0.65	77.3	73.9 – 80.8	0.49
Height (cm)	167.9	167.3 – 168.6	167.9	167.0 – 168.7	0.52	167.7	166.8 – 168.7	0.84	167.3	166.0 – 168.6	0.29
Waist to Hip ratio	0.92	0.91 – 0.94	0.92	0.91 – 0.94	0.70	0.93	0.91 – 0.94	0.60	0.91	0.88 – 0.93	0.095
<90 days treatment											
No. of measurements		15,782		36			25			9	
Dosage[a]		NA		0.90			0.39			0.71	
BMI (kg/m^2)	27.9	26.9 – 28.9	27.7	26.5 – 29.0	0.68	28.1	26.8 – 29.4	0.70	26.8	25.0 – 28.7	0.21
Weight (kg)	78.1	73.8 – 82.4	77.7	74.3 – 81.2	0.76	78.7	75.0 – 82.4	0.69	74.5	69.5 – 79.6	0.13
Height (cm)	168.2	167.5 – 168.8	168.7	167.5 – 170.0	0.32	167.2	165.8 – 168.7	0.17	167.7	165 – 170.0	0.67
Waist to Hip ratio	0.92	0.91 – 0.94	0.92	0.89 – 0.95	0.79	0.93	0.90 – 0.96	0.53	0.86	0.81 – 0.90	**0.002**
≥90 days treatment											
No. of measurements		15,782		252			168			59	
Dosage[a]		NA		1.05			0.54			0.79	
BMI (kg/m^2)	27.9	26.9 – 28.9	28.5	27.5 – 29.5	**0.005**	28.0	27.0 – 29.1	0.66	28.0	26.8 – 29.1	0.91
Weight (kg)	78.1	73.8 – 82.4	79.7	76.1 – 83.2	**0.005**	78.4	75.1 – 81.7	0.68	77.9	74.5 – 81.3	0.86
Height (cm)	167.8	167.5 – 168.2	167.9	167.5 – 168.4	0.59	167.9	167.4 – 168.4	0.63	167.5	166.8 – 168.2	0.27
Waist to Hip ratio	0.92	0.91 – 0.94	0.93	0.91 – 0.94	0.79	0.92	0.90 – 0.94	0.89	0.92	0.89 – 0.94	0.60

Abbreviations: BMI, body mass index; CI, Confidence interval; NA, not applicable; SSRIs, Selective Serotonin Reuptake Inhibitors; TCAs, Tricyclic antidepressants. [a]Calculated by dividing the prescribed daily dosage with the defined daily dosage. Data presented as the estimated means with the 95 percent confidence interval. Analyses were adjusted for age, gender and depression (CES-D score above 16), concomitant use of anti-psychotics, smoking (yes/no) and alcohol use (low/high). Weight was additionally adjusted for the height squared to assess which component in BMI was responsible for the association.

significantly lower in users of other antidepressants for a period less than 90 days, but sample size of this group was low (N = 9). For all antidepressant drugs, no association was observed with height. In addition, the estimates for SSRIs did not differ when the analyses were stratified for the presence or absence of clinically relevant depressive symptoms (results not shown).

Association between antidepressant drug classes and change in BMI between repeated examination rounds

Between repeated examination rounds, participants who used a SSRI had a larger increase in BMI as compared to participants who did not use an antidepressant (+0.74 and +0.23, respectively, p-value <0.001; table 3). In addition, an increase in BMI for treatment durations that lasted longer than 90 days at the consecutive visits was also observed (+0.74 and + 0.24, respectively, p-value <0.001). There was no difference in BMI between users of TCAs and other antidepressants compared to non-use. Participants treated with other antidepressants for less than 90 days had a decreased BMI (-1.38 and +0.24, respectively, p-value = 0.014, N = 6). No difference in change in BMI was observed for both TCA treatment duration categories and long-term use of other antidepressants.

Sex-stratified analyses

In females (figure 1), the use of SSRIs was associated with a significant larger increase in BMI between repeated examination rounds as compared to participants who did not use antidepressants (+0.685 kg/m^2). No increase in BMI was observed in male SSRI users (-0.144 kg/m^2, p-value for interaction between sex and SSRI use on BMI = 0.002). A similar result was observed when restricting to participants who used an SSRI for at least 90 days (p-value for interaction = 0.006). Although females tended to have a larger increase in BMI when using an TCA as compared to controls, this was not statistically significant, nor was it significantly different from males (p-value for interaction = 0.13). This was similar when this analysis was restricted to a treatment duration of at least 90 days. Similar to SSRIs, also hSERT antidepressants were only associated with increased BMI in females. The difference in association between hSERT antidepressants and BMI between males and females was statistically significant (-0.054 and +0.757 kg/m^2, respectively for males and females, p-value for interaction = 0.005). Also when we restricted for treatment duration that lasted for more than 90 days (p-value for interaction = 0.026). Similar results were obtained for BMI (results not shown).

Table 3 **Association between different antidepressant drug classes and change in BMI in a longitudinal analysis**

	Non use		SSRI			TCA			Others		
	Mean	95% CI	Mean	95% CI	P-Value	Mean	95% CI	P-Value	Mean	95% CI	P-Value
Number of measurements		12,593		198			146			57	
Mean defined daily dosage		NA		1.02			0.50			0.74	
BMI change (kg/m^2)	0.23	-0.18 – 0.63	0.74	0.28 – 1.20	**<0.001**	0.32	-0.16 – 0.80	0.52	+0.11	-0.47 – 0.70	0.60
<90 days treatment											
Number of measurements		12,593		25			22			6	
Mean defined daily dosage		NA		0.88			0.38			0.54	
BMI change (kg/m^2)	0.24	-0.11 – 0.59	0.78	0.07 – 1.50	0.091	0.13	-0.61 – 0.87	0.75	-1.38	-2.70 – -0.07	**0.014**
≥90 days treatment											
Number of measurements		12,593		173			124			51	
Mean defined daily dosage		NA		1.03			0.52			0.76	
BMI change (kg/m^2)	0.24	-0.11 – 0.59	0.74	0.32 – 1.16	**<0.001**	0.38	-0.06 – 0.83	0.33	+0.34	-0.22 – 0.90	0.66

Abbreviations, BMI, body mass index; CI, Confidence interval; NA, not applicable; SSRIs, Selective Serotonin Reuptake Inhibitors; TCAs, Tricyclic antidepressants. Data presented as the estimated means with the 95 percent confidence interval. Analyses were adjusted for age, gender and depression (CES-D score above 16), concomitant use of anti-psychotics, smoking (yes/no), alcohol use (low/high) and the BMI of the previous examination round. Change in BMI per center visit depicts the estimated mean change in BMI between repeated examination rounds center visits.

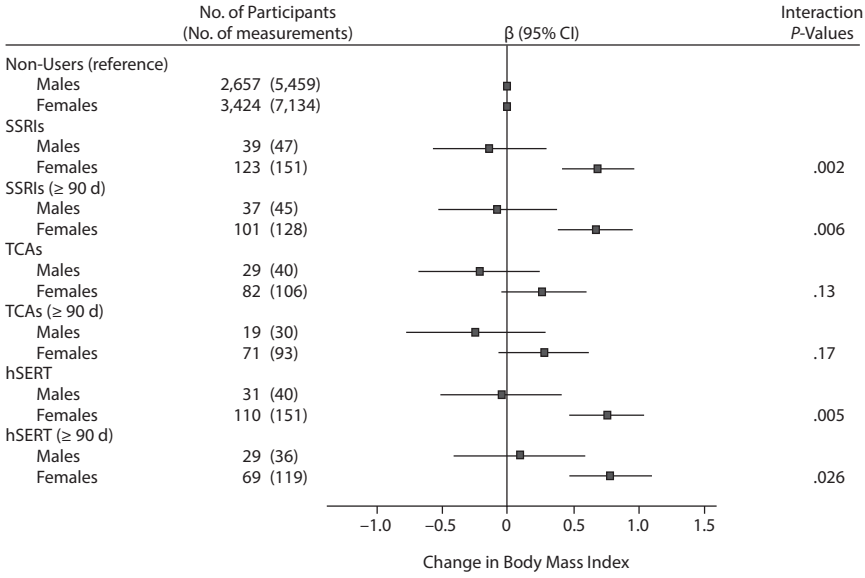

Figure 1 **Association antidepressants and change in body mass index stratified by sex**

Abbreviations: CI, confidence interval. hSERT, high affinity to the serotonin reuptake transporter (namely: clomipramine, fluoxetine, paroxetine and sertraline). SSRIs, Selective Serotonin Reuptake Inhibitors. TCAs, Tricyclic antidepressants. Analysis performed in all treatment durations and restricted to treatments longer than 90 days. Analyses were adjusted for age, gender and depression (CES-D score above 16), concomitant use of anti-psychotics, smoking (yes/no) and alcohol use (low/high) and the baseline BMI. All data presented as the mean difference in change in BMI between users of antidepressants and non-users.

Discussion

In this population-based study we observed that SSRI was associated with an, on average, approximate 0.5 kg/m^2 higher BMI compared to non-use. This observation, which was independent of the baseline BMI, was also reflected in a larger increase in BMI (0.5 kg/m^2) between repeated examination rounds. The increase in BMI was only observed when participants were using SSRIs for a period of at least 90 days. When we performed this analysis on weight, the use of SSRIs was associated with a 1.2 kg higher weight compared to non-users, independent of height. In addition, use of SSRIs was not significantly associated with height. These associations were not observed in participants that used TCAs or other antidepressants. This would suggest that TCAs and other antidepressants do not cause weight gain or that the

prescribed dosage is too low to observe a significant effect. However, in both cases this would mean that, at least in an older population, TCA and other antidepressant use in daily practice does not lead to weight gain. When the analyses were stratified by sex, an increased BMI in SSRI and hSERT users was only observed in females and not in males.

Most of the previously published studies were clinical trials in which depressive patients were followed from start of antidepressant therapy to the end of the study[11-14]. The main limitation of these studies include that they were not able to disentangle weight gain induced by the drug with weight gain induced by the relief in depressive symptoms. To our knowledge, only one study has investigated the association between the use of SSRIs and obesity in a population-based study[24]. Similar to our study, the authors showed that the use of SSRIs as well as use of SSRIs with a high affinity to the serotonin reuptake transporter (paroxetine and sertraline) were associated with an increased risk on obesity[19]. Although the authors adjusted for depression and anxiety, the chance that these results were caused by confounding by indication cannot be ruled out, as there might, for example be a preference to prescribe SSRIs to people with a higher BMI. Confounding by indication is therefore also a risk in our cross-sectional analyses. However, compared with the previous population-based study, we were able to take into account the treatment duration. As the effect was only observed with a treatment duration of more than 90 days, it was more likely that the increase was due to the drug instead of the underlying indication. In addition, the risk of confounding by indication was further minimized by our longitudinal study design, in which we studied the change in BMI between repeated examination rounds. Other differences between our study and the previously reported clinical trials include the usage of antidepressants for other indications than depression (which are not associated with weight), the ability to adjust for depressive symptoms and the inclusion of participants with depression but that were not treated with antidepressant drugs. In addition, the findings of our study are in line with findings on the biological role of serotonin in energy metabolism and weight control[1]. Genetic polymorphisms in the serotonin reuptake transporter gene[3, 4] and the postsynaptic serotonin receptor[2], which affect the efficiency of serotonergic signaling, are associated with an increased risk of obesity.

To our knowledge, so far, only one study, observed that females had a larger gain in weight compared to males when using SSRIs. However, as the data of the study outcomes was self-reported, the authors suggested that this might be due to bias[25]. The observed difference between males and females, within our study, might be caused by factors that affect the bioavailability of serotonin in neurons[5, 6]. Influencing this pathway by SSRIs or hSERT antidepressants might result in a more enhanced signaling in females compared to males. However, these studies

were performed in a much younger population, in which females were still pre-menopausal[5, 6]. Translating these results to our aging population, and thus post-menopausal population, should therefore be done with caution. A second biological explanation might include leptin and the leptin receptor, which are key players in the central regulation of energy metabolism[26]. Genetic variation in genes encoding for the leptin and leptin receptor were only associated with BMI in females, and not in males[27-31]. These genetic variations were also associated with the response to antidepressant drug response[32], although this study did not stratify on sex. Future research should be performed to disentangle the exact mechanism that gives a different SSRI-induced increase in weight between males and females.

Main strengths of the present study include the longitudinal design of part of the analyses, the use of pharmacy records instead of drug exposure defined by interview and the availability of data on depressive symptoms. Using the longitudinal design with the adjustment for the baseline BMI and adjustment for current depression, we were able to reduce a possible effect of confounding by indication in our results. This strategy allowed us to disentangle weight gain by relief in depressive symptoms with a drug-induced effect. Using the pharmacy records to define drug exposure, we were able to calculate the duration and to rule out recall bias and information bias. Also, our study did not harm from selection bias, as participants of the Rotterdam Study were included regardless there health status. This study, however, had a limited number of participants on short term antidepressant exposure. However, because also no trend of an effect was observed for short-term treatment, the effect of long-term treatment with SSRIs and hSERT antidepressants is likely to be causal. With this limited number, we were not able to study the effect of antidepressant drug dosage on weight gain. The time period between two examination rounds (4 – 5 years) in which we calculated the change in BMI is rather long. However, this time period is similar for the treated and untreated group, and potential misclassification would be non-differential. In addition, because of the nonrandomized design of the study, residual confounding might still be present. One possible confounding factor, which we did not take into account, might be food intake. However, because serotonin increases appetite, dietary intake is most probable an intermediate rather than a confounding factor.

In conclusion, the data from the current study showed that, in older adults, the use of SSRIs and hSERT antidepressants for a longer period was associated with a higher BMI and a larger increase in BMI between repeated examination rounds, notably in females only. The difference in SSRI-induced higher BMI between males and females might be explained by the difference in serotonergic signaling. Future studies should focus on disentangling the mechanism explaining SSRI- and hSERT-induced weight gain in females.

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CHAPTER 3.2

ANTIDEPRESSANT USE AND SERUM LIPID LEVELS IN A POPULATION-BASED STUDY IN OLDER ADULTS

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Under revision

Background: Antidepressants with a high serotonin reuptake transporter affinity may increase serum lipid levels, specifically low density lipoprotein (LDL) cholesterol, but evidence is scarce. We aimed to investigate the association between antidepressants and serum lipid levels in a population-based study in older adults.

Methods: We included participants from the prospective Rotterdam Study with data on lipid levels (total, LDL, and high density lipoprotein cholesterol, and triglycerides). We classified antidepressants based on the binding affinity to the serotonin reuptake transporter (low/intermediate-, and high-affinity antidepressants). We compared lipid levels from users of the different groups with lipid levels from non-users of antidepressants. As a method to infer potential causality, we studied effect modification of this association by the 102C>T polymorphism in the *HTR2A* (*5-hydroxytryptamine [serotonin] receptor 2A)* gene, which is associated with antidepressant drug response and metabolic outcomes.

Results: Compared with non-users (N = 6,438), LDL cholesterol level was only higher (mean: 2.9 versus 3.1 mmol/L, respectively; p-value = 0.05) in users of high-affinity antidepressants (N = 89). For the other lipids, no higher levels were observed in users of high-affinity antidepressants. The mean difference in serum LDL cholesterol level between non-use and use of high-affinity antidepressants was largest for the participants carrying the CC genotype compared to the other genotypes (notably 0.47 mmol/L), suggesting effect modification by this polymorphism (p-value for interaction = 0.03).

Conclusion: Antidepressants with a high serotonin reuptake transporter affinity were associated with higher LDL cholesterol levels, which was modified by common genetic variation in the *HTR2A* gene.

Introduction

It is widely recognized that pharmacological intervention with statins is effective in lowering the level of low density lipoprotein (LDL) cholesterol in serum and decreasing the risk of cardiovascular events and mortality[1, 2]. Several other drugs, however, might have the potential to increase the LDL cholesterol level, such as antidepressants that specifically target serotonin signaling (e.g., paroxetine and sertraline)[3-5].

Many antidepressants inhibit a transport channel for the reuptake of serotonin from the synaptic cleft of serotonergic neurons. Serotonin is a biological substance which is involved in a number of metabolic processes, including glucose metabolism and processes such as appetite and weight control[6]. Two often studied genes involved in serotonin signaling are *SLC6A4* (solute carrier family 6 [neurotransmitter transporter], member 4) and *HTR2A* (5-hydroxytryptamine [serotonin] receptor 2A, G protein-coupled) genes, which encode the serotonin transporter and a serotonin receptor, respectively. Genetic variation in these genes, namely the serotonin-transporter-linked polymorphic region (5-HTTPLR) in the *SLC6A4* gene and the 102C>T polymorphism the *HTR2A* gene, are often studied in relation to antidepressant treatment [7]. For example, carriers of the long variant of the 5-HTTPLR polymorphism have a higher risk of developing cardiovascular diseases[8] and type 2 diabetes mellitus[9] than carriers of the short variant. Moreover, carriers of the long variant have higher serum LDL cholesterol levels[10-13], but also a better antidepressant drug response[7] than short-variant carriers. Similarly, C-allele carriers of the 102C>T polymorphism in the *HTR2A* gene have a better antidepressants drug response, a lower high density lipoprotein (HDL) cholesterol level, and a higher risk of the metabolic syndrome than T-allele carriers[7, 14-18]. These findings indicate a role of serotonin in lipid metabolism.

Based on these studies, we hypothesized that antidepressants with a high binding affinity to the serotonin transport may influence serum lipid levels, specifically the level of serum LDL cholesterol[3, 5]. However, evidence from *in-vivo* studies in humans that support this hypothesis is scarce. Therefore, in this population-based study of older adults, we aimed to study the association between antidepressant use, classified based on binding affinity to the serotonin reuptake transporter, and serum lipid levels. Furthermore, we aimed to investigate whether this association was modified by the 102C>T polymorphism in the *HTR2A* gene, as the 5-HTTPLR polymorphism in the *SLC6A4* gene was not available for this study population.

3.2

Methods

Setting of the Rotterdam Study

This study was conducted in the prospective population-based Rotterdam Study cohort, which aims to investigate the occurrence and risk factors of age-related diseases and conditions. The design and rationale of the Rotterdam Study has been described in more detail elsewhere[19, 20]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were asked to participate in the first subcohort (RS-I). In total, 7,983 individuals agreed to participate (response rate 78%). From 2000 to 2001, all inhabitants who became 55 years of age or moved into the district were asked to participate in the second subcohort (RS-II), of whom 3,011 individuals agreed (response rate 67%). Follow-up examinations were conducted every 4 to 5 years after baseline measurements. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act: Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all study participants.

Study population and design

For the present study, we included all participants from the Rotterdam study with data available on fasting serum lipid levels, which were measured in one or two examinations rounds per participant. Lipid measures with missing information on co-variables were excluded.

Antidepressants exposure assessment

From January 1st 1991 onwards, more than 99% of the participants had their drug prescriptions filled at one of seven fully computerized pharmacies in the Ommoord district in Rotterdam. The available pharmacy data included the Anatomical Therapeutic Chemical (ATC)-code[21], the dispensing date, the total amount of tablet/capsules per dispensing, the prescribed daily number of tablets/capsules, the total dosage and the product name of the drug. A participant was exposed to an antidepressant drug if the date of serum measurement fell within a dispensing episode. A dispensing episode was calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. Treatment duration at the date of serum measurement was computed by calculating the cumulative number of dispensing days. A gap of 30 days between two consecutive dispensings was allowed to consider it as a single dispensing episode of continuous use. We classified antidepressant drugs based on their affinity for the serotonin transporter. This classification was based on the dissociation constant to the serotonin transporter: >10 nmol/L as low,

1 – 10 nmol/L as intermediate and 0 – 1 nmol/L as high-binding affinity[22-24]. Because the number of serum lipid measures during which antidepressants with low and intermediate affinity to the serotonin reuptake transporter were used was low, these two groups were combined (hereafter denoted as non-high-affinity antidepressants).

Study outcome measures

Serum measurements of total cholesterol, HDL cholesterol and triglycerides concentrations, were conducted with an automated enzymatic procedure (Hitachi analyzer, Roche Diagnostics, Washington, DC). LDL cholesterol levels were calculated using the Friedewald formula[25].

A LDL cholesterol level >4.0 mmol/L (≈160 mg/dL) was defined as high, and participants with high LDL cholesterol were considered cases. This cutoff was based on guidelines of the American Heart Association and the American College of Cardiology[26].

102C>T HTR2A gene polymorphism assessment

The 102C>T polymorphism (rs6313) within the *HTR2A* gene was extracted from whole genome data, which was imputed with MACH software 1.0.15 with 1000 genomes as the reference set[27]. Surrounding SNPs, used for imputation, were genotyped using the Infinium II HumanHap 550K Genotyping BeadChip® (Illumina, San Diego, CA, USA) according to the instructions of the manufacturer. Quality control and results of the genotyping were published elsewhere[28]. We calculated the genotype frequencies by estimating the genotype based on the imputed values (e.g., 1-5 – 2, as homozygous C-allele carriers).

Co-variables

The following co-variables were considered as possible confounders for the association between antidepressants and serum lipid levels: age, sex, body mass index (BMI), depression score, use of lipid-lowering agents, glucose-lowering agents and antipsychotics. All co-variables were assessed at the same measurement round as the lipid measurements. Length and weight were measured at the study center. BMI was calculated with the following formula: weight (in kilograms) / height-squared (in meters). Depressive symptoms were screened for with the Dutch version of the Center for Epidemiological Studies Depression Scale score (CES-D). This previously validated questionnaire gives a score, ranging between 0 and 60, with higher scores indicative of more depressed feelings. A score above 16 can be considered as an indication for clinically relevant depressive symptoms[29, 30]. Current use of lipid-lowering agents (ATC-code: C10), glucose-lowering agents (ATC-code:

A10), and antipsychotics (ATC-code: N05A) at the date of examination was based on pharmacy dispensing records.

Statistical analyses

Study characteristics were stratified by antidepressant drug treatment group and assessed at baseline, defined as the first lipid measurement of a participant in the present study.

As part of the study population had two serum lipid level measurements available (≈26%), all statistical analyses were performed using repeated measurement analyses. Linear mixed models were used for continuous outcomes and generalized estimating equations were used for the dichotomous outcome "high LDL cholesterol (yes/no)". For the analyses on the continuous outcomes, we compared serum lipid levels of non-users with serum lipid levels collected during antidepressant drug use. We adjusted the analyses for age, sex, BMI, CES-D score and the use of glucose-lowering drugs, lipid-lowering drugs and antipsychotics. In a first analysis we conducted a pairwise comparison between the drug groups separately, with non-users as reference. Secondly, we conducted a trend analysis, which depended on the binding-affinity groups, with non-users as reference. Serum triglyceride levels were not normally distributed and therefore log-transformed prior to the analyses. For presentation purposes, we presented the results as the estimated (geometric) means. In two separate sensitivity analyses, we excluded lipid measurements during which antidepressants exposure was shorter than 90 days and during which lipid-lowering agents were used.

To study the physiological role of the 102C>T polymorphism in the *HTR2A* gene on serum lipid levels, we assessed the association between the 102C>T polymorphism and serum lipid measures in genotypic models. This was conducted for the total population and for non-users of antidepressants. Next, we investigated whether genotypes of the 102C>T polymorphism modified the association between high-affinity antidepressants and serum lipid measurements. Therefore, we investigated the mean difference in lipid levels in users of the different antidepressant groups compared with non-users with the same genotype. In this analysis, non-users and homozygous carriers of the T allele were considered as reference. We investigated additive models and adjusted for all co-variables. Statistical interaction was tested by including a multiplicative interaction term between genotype and antidepressant drug group. In addition, we tested the interaction separately for non-high and high affinity antidepressants. For these analyses, we excluded all lipid measures during which lipid-lowering agents were used.

We used IBM SPSS Statistics (version 21.0, IBM Corp., Somers, NY, USA) for all analyses. Two-sided p-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics of the study population

A total of 6,691 lipid measurements with data on co-variables were available. As LDL cholesterol level could not be calculated with the Friedewald formula when the level of triglycerides was higher than 4.5 mmol/L[25], a total of 47 measurements were excluded. This resulted in a total sample of 6,644 measurements from 5,283 participants (Table 1). At baseline, participants using non-high (N = 88) and high affinity antidepressants (N = 71) were more frequently female, had a slightly higher BMI and had a higher CES-D score than non-users of antidepressants.

The 102C>T polymorphism was not determined in 590 participants (=11.1%). The allele frequencies of the polymorphism were not different between the three groups, and the polymorphism was in Hardy-Weinberg equilibrium (p-value > 0.05).

Association between antidepressants and serum lipid levels

LDL cholesterol level was statistically significantly higher in high-affinity antidepressant users, when compared to non-users (Table 2, 2.9 versus 3.1 mmol/L, respectively; p-value = 0.05), equivalent to a 6.9 percent difference. In addition, LDL cholesterol level was statistically significantly higher if the binding affinity to the serotonin reuptake transporter was higher (p-value for trend = 0.03). The level of triglycerides was significantly higher in users of non-high-affinity antidepressant (p-value = 0.03) than in non-users, but not in users of high-affinity antidepressants. No statistically significantly differences in total and HDL cholesterol level were observed between the different groups. These results did not materially change when serum lipid levels during which antidepressants were used for less than 90 days and lipid-lowering agents were used, were excluded (results not shown).

A total of 1,950 out of 5,402 LDL measurements were above 4.0 mmol/L after exclusion of LDL cholesterol measurements taken during use of lipid-lowering agents. Compared to non-users of antidepressants, LDL cholesterol was 1.60 times more likely above 4.0 mmol/L in users of high-affinity antidepressants, but this was marginally not statistically significant (95% confidence interval (CI): 0.99 – 2.57). No increased risk of high LDL cholesterol was observed for non-high-affinity antidepressants compared to non-users of antidepressants (odds ratio = 0.93; 95%CI: 0.59 – 1.48).

Table 1 Baseline characteristics of the study population

	Non-use of antidepressants	Use of non-high affinity antidepressants	Use of high-affinity antidepressants
	N = 5,124	N = 88	N = 71
Age (years), mean (SD)	72.6 (6.5)	73.5 (6.3)	72.5 (5.4)
Women, N (%)	2,910 (56.8)	62 (70.5)	50 (70.4)
BMI (kg/m^2), mean (SD)	27.0 (4.0)	27.4 (4.3)	27.3 (4.3)
CES-D, median (IQR)	2.0 (0.0 – 6.0)	8.0 (2.0 – 17.0)	5.0 (2.0 – 10.0)
Lipid-lowering agents, N (%)	667 (13.0)	15 (17.0)	9 (12.7)
Glucose-lowering agents, N (%)	255 (5.0)	7 (8.0)	1 (1.4)
Antipsychotics, N (%)	14 (0.3)	1 (1.1)	1 (1.4)
102C>T, minor allele frequency	0.42	0.40	0.45
CC, N (%)	1492 (33.1)	35 (35.7)	27 (32.9)
CT, N (%)	2227 (49.3)	47 (48.0)	37 (45.1)
TT, N (%)	794 (17.6)	16 (16.3)	18 (22.0)

Abbreviations: N, number of participants; SD, standard deviation; BMI, body mass index; CESD, Center of Epidemiological Studies Depression Scale; *HTR2a*, 5-hydroxytryptamine (serotonin) receptor 2A; IQR, inter quartile range.

Association between antidepressants and serum lipid levels, stratified by 102C>T genotype

After exclusion of measurements which were taken during the use of lipid-lowering agents as well as those from individuals for whom we did not have data on genetics, a total of 4,850 measurements remained for analyses.

The level of triglycerides and LDL cholesterol was higher in homozygous carriers of the C allele compared with the T allele (p-values = 0.02 and 0.04, respectively). No association was observed with total and HDL cholesterol. These results remained similar after exclusion of antidepressant users.

A graphical presentation of the interaction between the 102C>T polymorphism and use of antidepressants on serum LDL cholesterol levels is displayed in Figure 1. The mean difference in serum LDL cholesterol level between non-use and use of high-affinity antidepressants was largest for the participants carrying the CC genotype (estimated difference: 0.47 mmol/L), indicative of interaction between this polymorphism and use of high-affinity antidepressants (p-value for interaction = 0.03). No statistical interaction was observed for the other studies

Table 2 **Association between antidepressants and mean serum lipid levels**

	Non-use of antide-pressants (N = 6,438)*	Use of non-high affinity antidepressants (N = 117)		Use of high-affinity antidepressants (N = 89)		
	Mean (95% CI)	Mean (95% CI)	P-value†	Mean (95% CI)	P-value†	P-value (trend)
Total cholesterol (mmol/L)	5.0 (4.8 – 5.2)	5.1 (4.9 – 5.4)	0.14	5.1 (4.9 – 5.4)	0.24	0.09
LDL cholesterol (mmol/L)	2.9 (2.8 – 3.1)	3.0 (2.8 – 3.2)	0.29	3.1 (2.9 – 3.3)	**0.05**	**0.03**
HDL cholesterol (mmol/L)	1.5 (1.4 – 1.5)	1.4 (1.3 – 1.5)	0.39	1.4 (1.3 – 1.5)	0.18	0.12
Triglycerides (mmol/L)‡	1.3 (1.2 – 1.4)	1.4 (1.3 – 1.6)	**0.03**	1.3 (1.2 – 1.5)	0.91	0.36

Abbreviations: N, number of serum lipid measurements in the stratum; CI, confidence interval; LDL, Low Density Lipoprotein; HDL, High Density Lipoprotein. * reference population in the analyses. † Indication of statistical significance as compared to the reference population (non-use). ‡ Displayed as the geometric mean with the 95% confidence interval. Analyses adjusted for age, sex, CES-D score, body mass index, concomitant use of lipid lowering agents, glucose-lowering agents and antipsychotics.

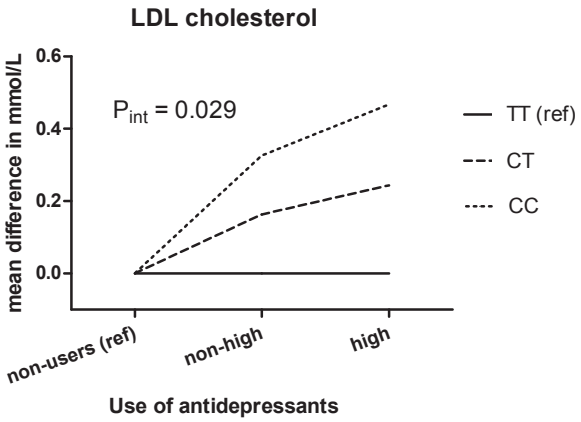

Figure 1 **Association between the use of antidepressants and LDL cholesterol level stratified by 102C>T genotype**

Abbreviation: P_{int}, P-value for interaction for the association between antidepressants and low density lipoprotein cholesterol levels across 102C>T genotypes. Participants using cholesterol lowering agents were excluded. Analyses adjusted for age, sex, CES-D score, body mass index, concomitant use of lipid lowering agents, glucose-lowering agents and antipsychotics.

lipid measures: total cholesterol, HDL cholesterol and triglycerides (p-values for interaction > 0.05).

Discussion

Within this population-based study in older adults, users of antidepressants with a high affinity to the serotonin reuptake transporter had a higher LDL cholesterol level than non-users, equivalent to a 6.9 percent difference. The level of triglycerides was significantly higher in users of non-high affinity antidepressants. No significant association was observed for other lipid levels. In addition, we observed that the association between antidepressants and LDL cholesterol levels was modified by the genotype of the 102C>T polymorphism in the *HTR2A* gene. The difference in LDL cholesterol between non-use and use of high-affinity antidepressants was largest for CC genotype carriers (estimated difference: 0.47 mmol/L).

To date, only a few studies investigated the association between antidepressants and lipid levels. These studies had a limited sample size, were closely monitored, or had hypercholesterolemia as a study outcome[3-5]. The effect size of high-affinity antidepressants was similar as what was observed previously in users of paroxetine, which is a high-affinity antidepressant[3, 5]. We did not find evidence in the literature that the use of non-high-affinity antidepressants was associated with a higher triglyceride level[3, 5]. As there was no association with high-affinity antidepressants, it is possible that this was a spurious finding.

Because of our study design, we were not able to infer possible causality of the association between use of high-affinity antidepressants and LDL cholesterol levels. Effect modification by the 102C>T polymorphism in the *HTR2A* gene could provide arguments in favor of a causal relationship. This polymorphism was previously shown to be associated with antidepressant drug response[7] and metabolic outcomes[8, 14, 17]. In our study population, the C allele was associated with a lower LDL cholesterol level, which, to our knowledge, hasn't been reported before. The rationale of the effect modification is similar as to Mendelian Randomization[31]. The concept of Mendelian Randomization has been an effective tool to study whether an epidemiological association is causal or not[31, 32]. Within our study population, the presence of effect modification by the 102C>T polymorphism suggests that the association between use of serotonin reuptake inhibiting antidepressants and LDL cholesterol levels may be causal. Additional evidence of a causal relationship between serotonin reuptake inhibiting antidepressants and LDL cholesterol levels, could have been provided by the 5-HTTPLR polymorphism in the *SLC6A4* gene, because of its effects on antidepressant drug response and metabolic outcomes[10-13].

However, because this polymorphism was not available in our study population, additional research is required to provide further evidence whether the observed association is causal or not.

The clinical consequence of the increase in LDL cholesterol levels by antidepressants with a high affinity to the serotonin transporter is complicated by the possible role of LDL cholesterol in the onset of depression[13, 33, 34]. In short, chronic depletion of cholesterol was observed to reduce the binding capacity of ligands to the 5-Hydroxytryptamine (Serotonin) receptor 1A (HTR1A) receptor, although the expression level was unchanged[35-37]. This might indicate that higher LDL cholesterol levels increases binding affinity of the HTR1A receptor and may therefore improve the therapeutic response in patients treated for depression[35-37]. Consequently, one might question whether the increase in LDL cholesterol by antidepressants with the potential to inhibit serotonin reuptake is a negative or a beneficial effect[38]. However, although the differences in LDL cholesterol are small on a population level, individual patients may have a larger increase in antidepressant-induced LDL cholesterol levels than others. For example, in participants with the CC genotype, the difference in LDL cholesterol between non-use and use of high-affinity antidepressants was already 0.47 mmol/L. Furthermore, one might question whether the increase in LDL cholesterol level is still favorable in patients with high LDL levels who have a history of cardiovascular disease.

This study has strengths and limitations. A strength is the population-based setting, minimizing the risk of potential selection bias. Besides, we were able to correct for the presence of depressive symptoms. However, our study was limited by the low number of antidepressant users. Therefore, we were not able to study the association between individual antidepressant drugs and LDL cholesterol levels.

Use of antidepressants with a high affinity to the serotonin reuptake transporter was associated with a higher level of LDL cholesterol, when compared to non-users in a population-based cohort study in older adults. Carriers of the CC genotype of the 102C>T polymorphism in the *HTR2A* gene had the largest difference in LDL cholesterol. The effect modification of this association by the 102C>T polymorphism in the *HTR2A* gene might suggest that this association is causal, although more studies are warranted.

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CHAPTER 3.3

SELECTIVE SEROTONIN REUPTAKE INHIBITORS DECREASE PANCREATIC INSULIN SECRETION IN OLDER ADULTS AND INCREASE THE RISK OF INSULIN DEPENDENCY IN TYPE 2 DIABETES PATIENTS

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Objective: Selective serotonin reuptake inhibitors (SSRIs) may decrease insulin secretion, but evidence from population studies is scarce. We investigated the association between SSRIs and markers for glucose-insulin homeostasis in a non-diabetic older population. Furthermore, we studied the association between SSRI use and insulin dependency in a diabetic population of older adults.

Research Design and Methods: This study was embedded in the prospective population-based Rotterdam Study cohort. In non-diabetic participants, glucose and insulin levels and the homeostasis model assessment for insulin sensitivity and secretion were compared between users of SSRIs and non-users of any antidepressant. In diabetic patients using oral glucose-lowering agents, the risk of insulin dependency, defined as the start of insulin treatment, was compared between users of SSRIs and non-users of any antidepressant.

Results: In non-diabetic participants, SSRI users (n = 64) had a statistically significantly (p-values < 0.05) lower level of serum insulin (8.7 and 9.9 mU/L, respectively), a lower degree of insulin resistance (2.1 and 2.4%, respectively) and less insulin secretion (88 and 101%, respectively), but a similar level of serum glucose, compared with non-users of antidepressants (n = 5,505). Within diabetic patients, >90 days of consecutive use of SSRIs was associated with a 2.17 times higher risk (95% confidence interval: 1.02 – 4.60) to start insulin treatment than non-use of antidepressants.

Conclusions: Use of SSRIs was associated with lower insulin secretion in non-diabetics and an increased risk of insulin dependency in type 2 diabetics in older adults. However, additional studies are required to confirm our results.

Introduction

Depression has been associated with an increased risk of type 2 diabetes[1-3], obesity[4] and metabolic syndrome[5]. Independently of depression, use of antidepressants, specifically use of selective serotonin reuptake inhibitors (SSRIs), has also been associated with an increased risk of type 2 diabetes[6-8]. Interestingly, use of SSRIs was not associated with higher levels of glucose[7] and it therefore remains unclear how SSRIs increase the risk of type 2 diabetes independently of glucose. For this reason, one of the hypotheses is that the use of SSRIs has direct effects on the onset of type 2 diabetes[7].

There is increasing evidence that serotonin is involved in the secretion of insulin. Mice deficient for tryptophan hydroxylase 1, an enzyme essential for peripheral serotonin production, had lower levels of serotonin in serum and a lower pancreatic insulin secretion[9]. Antagonizing the serotonin reuptake channel on pancreatic beta cells inhibits insulin secretion and activates apoptotic mechanisms in murine beta-cell models[10]. Furthermore, the few studies conducted in humans showed that use of SSRIs result in lower insulin secretion[11, 12].

Based on these studies, it is biologically plausible that SSRIs decrease insulin secretion, and might therefore be a mechanism underlying the previously observed association between SSRIs and increased risk of type 2 diabetes. Consequently, type 2 diabetes patients on SSRIs might also have a higher risk to develop insulin dependency, a condition associated with an increased risk of mortality[13,14]. In the present study, we aimed to investigate the association between SSRI use and glucose-insulin homeostasis in a non-diabetic older population. Furthermore, we aimed to study the association between the use of SSRIs and the risk of insulin dependency in a population of older adults with type 2 diabetes.

Research Design and Methods

Study setting

We embedded the present study in the prospective population-based Rotterdam Study cohort, which was designed to investigate the incidence of, and risk factors for, several age-related diseases. A more detailed description of the design and rationale of the study has been published elsewhere[15, 16]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from a district (Ommoord) located in Rotterdam, the Netherlands, were asked to participate in the original cohort (denoted as RS-I). In total, 7,983 individuals agreed to participate (response rate 78%). An extension of the original cohort was initiated in 2000 (denoted as RS-II). Within

this cohort, all inhabitants from Ommoord aged 55 years and older, and not already participating and invited in RS-I, were asked to participate in RS-II. In total, 3,011 individuals agreed to participate (response rate 67%). In 2006, a second extension (RS-III) was initiated. A total of 3,932 inhabitants from the same district agreed to participate (response rate 65%). These inhabitants were not already participating in the other cohorts, were not invited before, and were 45 years or older. Follow-up examinations were conducted every 4 – 5 years after baseline examination. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all study participants.

Study population and designs

The research questions were addressed in two study populations. First, we conducted a cross-sectional analysis on the association between use of SSRIs and markers of glucose-insulin homeostasis. Fasted glucose and insulin levels were measured simultaneously during one visit round, namely during the third round of RS-I (1997 – 1999; N = 4,797) and the first round of RS-II (2000 – 2001; N = 3,011). No measurements were available for RS-III. Participants with diabetes at the date of examination were excluded. Diagnosis of diabetes was based on current treatment with oral glucose-lowering agents or insulin, a measured fasting glucose > 6.9 mmol/L at the date of examination, or a previous diagnosis of diabetes made by the general practitioner or medical specialist. Non-diabetic participants were excluded when they were non-fasted at the moment of blood draw or had missing information on co-variables.

Second, we conducted a follow-up study in all persons with a history of diabetes at baseline and in persons with incident diabetes during follow-up who were using oral glucose-lowering drugs without insulin. These diabetics were followed until a first prescription for insulin, death, or end of the study period, whichever came first. These cases of insulin dependency were matched to all eligible diabetic participants in the three cohorts (in this analysis, RS-III was included) at the same calendar date (denoted hereafter as the index date). For every matched set, on the index date the exposure status to antidepressants in each case and its corresponding controls was assessed as described below.

Antidepressant drug exposure

From January 1st, 1991 onwards, more than 95% of the study participants had their drug prescriptions filled at one of the fully computerized regional pharmacies. Dispensing data included the Anatomical Therapeutic Chemical (ATC) code[17], the

dispensing date, the total number of drug units per prescription, the prescribed daily number of units, and the product name of the drug. The duration of a dispensing was calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. A participant was considered as a current SSRI user (ATC code: N06AB) when the date of examination or the index date fell within an antidepressant dispensing episode. Participants were classified as past SSRI user if they previously filled a TCA, SSRI or other antidepressant drug prescription, but were not current user at the date of examination or index date. For comparison to the effect of SSRIs, we also defined current and past use of tricyclic antidepressants (ATC code: N06AA) and other antidepressants (ATC code: N06AX). Participants who did not use any antidepressant drug during the study period were considered as non-user.

Study outcomes

Fasted glucose (in mmol/L) was measured enzymatically using a hexokinase method (Boehringer Mannheim, Mannheim, Germany). Fasted insulin (in mU/L) was measured in serum on a Modular Analytics E170 analyzer using a Cobas Roche electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany). Both measurements were performed using the standard manufacturer's instructions. Peripheral insulin resistance was assessed using the homeostatic model assessment (HOMA-IR; [glucose (mmol/L) * insulin (mU/L)] / 22.5). The homeostatic model assessment was also used to estimate pancreatic beta cell function (HOMA-β; [20 * insulin (mU/L)] / [glucose – 3.5])[18, 19].

Insulin dependency was defined as the initiation of insulin treatment (ATC code: A10A) in type 2 diabetes patients already using oral glucose-lowering agents (ATC code: A10B) as add-on therapy, or as therapy switch.

Co-variables

The following co-variables were considered as possible confounders in the analyses, in addition to age and sex: body mass index (BMI), depression, number of concomitantly used oral glucose-lowering agents, and prevalent use of glucose-lowering agents (latter two only for the analysis on initiation of insulin treatment). For the analysis on insulin dependency, co-variables were determined time-dependently (e.g., the measurement closest to the index date was used for analyses). Length and height were measured by trained research nurses at the study center. Body mass index (BMI) was calculated by dividing the weight (in kg) by the height (in squared meters). The clinical diagnosis of depressive symptoms, syndromes and disorders was defined based on reports from physicians and psychiatrists, which has been described in more detail elsewhere[20]. This data was only available for RS-I,

RS-II, and not for RS-III. In short, research nurses screened parts of the records of the general practitioner in which a possible case of depression/depressive symptoms is discussed. Two independent researchers reviewed all possible cases using records from the general practitioner. These records included the observations made by the general practitioner, but also letters with diagnoses from medical specialists. When there was no consensus on a diagnosis, a psychiatrist was consulted who made the final decision. Because of a low number of cases with depressive syndromes and disorders at a particular moment during follow-up, we combined these together with depressive symptoms in one variable, hereafter denoted as depression. Prevalent use of oral glucose-lowering agents refers to the participants treated with oral glucose-lowering agents without insulin at the date of inclusion in the Rotterdam Study.

Statistical analyses

For the population of non-diabetic participants, study characteristics were assessed at the date of examination. For the population of participants using oral glucose-lowering agents, study characteristics were assessed at baseline of the Rotterdam Study (for prevalent users) or at the date of the first oral glucose-lowering agent dispensing (for incident users).

The cross-sectional analyses on measures of glucose-insulin homeostasis were conducted with linear regression analyses. We compared the measures of glucose-insulin homeostasis between users of SSRIs, users of TCAs, and non-users of antidepressants. Users of other antidepressants were not studied as the number of users was too low (n = 15). Insulin levels and the homeostatic model assessment were not normally distributed and therefore log-transformed. Estimated means of these measures were back transformed on a normal scale as a geometric mean. Analyses were adjusted for age, sex, BMI and depression (yes/no). In a sensitivity analysis, we compared the measures of glucose-insulin homeostasis between users of SSRIs and TCAs. In addition, we studied the association between past use of antidepressants and the measures of glucose-insulin homeostasis. Together with statistical adjustment for depression, these two sensitivity analyses were conducted to reduce the possibility that our findings were observed because of confounding by indication.

The analyses on the risk of insulin dependency were conducted using conditional logistic regression models. Because the number of cases using other antidepressants was low, results are not presented for this drug class (n = 2). Analyses were adjusted for age, sex, BMI, prevalent use of glucose-lowering agents, and number of different concomitantly used oral glucose-lowering agents. All analyses on current use of the different antidepressant drug classes were repeated in which the first 90 days of consecutive treatment was defined as non-use. This was done as it is not expected

that insulin dependency is an acute effect of SSRI use. Furthermore, we restricted the analyses to RS-I and RS-II, in which we had information available on depression, and statistically adjusted for the presence of depression. For the assessment of the association between past use of an antidepressant drug group, never use of that drug group was used as reference. In an additional analysis, we excluded prevalent users of oral glucose-lowering agents from the population of treated diabetic patients.

We used IBM SPSS Statistics (version 21.0, IBM Corp., Somers, NY, USA) for all analyses. Two-sided p-values less than 0.05 were considered statistically significant.

Results

Characteristics of the study populations

A total of 5,571 non-diabetic participants were included in the cross-sectional analysis on measures of glucose-insulin homeostasis, and a total of 1,677 diabetic participants were included in the analysis on the risk of insulin dependency (Table 1). In short, both groups had a similar percentage of women and comprised older people (mean age approximately 70 years). Antidepressant use was approximately 2 times as frequent in the diabetic group as in the non-diabetic group.

Glucose-insulin homeostasis

The estimated means of the measures of glucose-insulin homeostasis for non-users, TCA users, and SSRI users are presented in Table 2. The level of glucose was comparable between the group of non-users and the groups of users of TCAs and SSRIs. However, the level of insulin was statistically significantly lower in users of SSRIs than in non-users of antidepressants (8.7 and 9.9 mU/L, respectively; p-value = 0.02), and in users of TCAs (8.7 and 10.7 mU/L, respectively; p-value = 0.01). TCAs users and non-users had a comparable level of insulin (p-value = 0.24). Users of SSRIs had a statistically significantly lower HOMA-IR than non-users of antidepressants (2.1 and 2.4%, respectively; p-value = 0.03), and users of TCAs (2.1 and 2.6%, respectively; p-value = 0.01). Moreover, SSRI users had a statistically significantly lower HOMA-β than non-users of antidepressants (88 and 101%, respectively; p-value = 0.01), and users of TCAs (88 and 105%, respectively; p-value = 0.03). No statistically significant difference in HOMA-IR and HOMA-β was observed between non-users of antidepressants and TCA users.

We observed no statistically significant difference between never-users of antidepressants and past users of SSRIs or TCAs in these outcomes (results not shown).

Table 1 Characteristics of the study population

	Non-diabetic participants (N = 5,571)	Diabetic participants (N = 1,677)
Age, years (SD)	69.1 (8.2)	72.3 (9.7)
Females, N (%)	3,212 (57.7)	944 (56.3)
BMI, kg/m^2 (SD)	26.7 (3.9)	29.1 (4.4)
Depression, N (%)	69 (1.2)	32 (1.9)
Glucose-lowering agents, N (%)		
Biguanides	NA	661 (39.4)
Sulfonamides urea derivatives	NA	1,067 (63.6)
Prevalent use	NA	508 (30.3)
Antidepressant use, N (%)		
TCAs	64 (1.1)	35 (2.1)
SSRIs	87 (1.6)	61 (3.6)
Others	15 (0.3)	13 (0.8)

Abbreviations: N, number of participants; SD, standard deviation; BMI, body mass index; TCAs, tricyclic antidepressants; SSRIs, Selective Serotonin Reuptake Inhibitors; NA, not applicable.

Table 2 Association between use of antidepressants and glucose-insulin homeostasis

	Non-use (N = 5,505)	TCA use (N = 64)		SSRI use (N = 87)		
	Mean (95% CI)	Mean (95% CI)	P-Value	Mean (95% CI)	P-Value	P-Value*
Glucose (mmol/L)	5.5 (5.5 – 5.6)	5.6 (5.5 – 5.7)	0.37	5.5 (5.4 – 5.6)	0.83	0.41
Insulin (mU/L)	9.9 (9.4 – 10.5)	10.7 (9.4 – 12.1)	0.24	8.7 (7.8 – 9.8)	0.02	0.01
HOMA-IR (%)	2.4 (2.3 – 2.6)	2.6 (2.3 – 3.0)	0.21	2.1 (1.9 – 2.4)	0.03	0.01
HOMA-ß (%)	101 (95 – 107)	105 (93 – 119)	0.47	88 (79 – 99)	0.01	0.03

Abbreviations: N, Number of participants; SSRI, Selective Serotonin Reuptake Inhibitors; TCA, tricyclic antidepressants; 95% CI, 95% confidence interval; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HOMA-ß, homeostasis model assessment-estimated beta cell function. Data presented as the estimated mean with 95% confidence interval. Analyses adjusted for age, sex, depression (yes/no) and body mass index. *P-value refers to the level of statistical significance of the comparison of the outcomes between users of SSRIs and TCAs.

Insulin dependency

Of the 1,677 diabetic patients, 304 started insulin treatment during follow-up (Table 3). Compared with non-use of antidepressants, current use of SSRIs was associated with a 1.81 (95% CI: 0.86 – 3.71) times higher risk to initiate insulin treatment, although this was not statistically significant. Current use of TCAs was associated with a 1.40 (95% CI: 0.67 – 2.96) times higher risk to initiate insulin treatment which was also not statistically significant. When we defined current exposure of antidepressants after 90 days of consecutive treatment, current use of SSRIs was associated with a significantly 2.17 (95% CI: 1.02 – 4.60) times increased risk of starting insulin treatment. Past use of TCAs and SSRIs was not associated with an increased risk of initiating insulin treatment.

These results did not materially change after statistical adjustment for depression in the sub-cohorts of which we had data available on depression. Moreover, the point estimate did not materially change when prevalent users of glucose-lowering agents were excluded from the analyses (results not shown).

Table 3 Association between antidepressant and start of insulin in diabetic patients

	Percentage*	Number of cases	HR (95% CI)
Current use, unrestricted			
Non-use of antidepressants	-	287	1.00 (reference)
TCAs	2.7	8	1.40 (0.67 – 2.96)
SSRIs	3.3	9	1.81 (0.89 – 3.71)
Current use, restricted to >90 days treatment			
Non-use of antidepressants	-	288	1.00 (reference)
TCAs	2.2	8	1.90 (0.89 – 4.06)
SSRIs	2.8	8	2.17 (1.02 – 4.60)
Past use			
Never use of antidepressants	-	215	1.00 (reference)
TCAs	12.0	40	0.94 (0.65 – 1.38)
SSRIs	10.1	32	0.99 (0.65 – 1.51)

Abbreviations: 95%CI, 95% confidence interval; HR, hazard ratio; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Analyses adjusted for age, sex, prevalent use of glucose-lowering agents at start of the Rotterdam Study (yes/no), the number of concomitantly dispensed glucose-lowering agents, body mass index, and use of other antidepressants. Antidepressants drug groups were studied in one single statistical model. *As we studied the associations with time-varying exposure analysis, controls could contribute more than once to the computations before they were censored or became a case. For this reason, exposure is reported as a percentage.

3.3

Discussion

Our study yielded two different findings. First, we observed that current use of SSRIs was associated with a lower level of insulin, lower pancreatic insulin secretion (assessed with HOMA-β), and lower peripheral insulin resistance (assessed with HOMA-IR) than non-users of antidepressants in people without type 2 diabetes. These outcomes remained lower in users of SSRIs when compared with users of TCAs. Second, we observed that in people with type 2 diabetes current use of SSRIs for more than 90 consecutive days was associated with an approximately 2 times higher risk to start insulin treatment than in non-users of antidepressants.

This study is in agreement with the existing literature conducted in *in-vitro* and mouse models. Serotonin inhibits the secretion of insulin from pancreatic beta cells[9]. Pancreatic beta cells express both the serotonin transporter and the vesicular monoamine transporter, channel proteins capable of transporting serotonin into the cells, and which can be antagonized by SSRIs[9, 21, 22]. In line with this, SSRIs decrease insulin secretion in murine pancreatic beta cells[10]. The few studies conducted in humans also observed a lower level of insulin in serum in users of SSRIs, similar to what we observed in our study[11, 12]. SSRIs may also promote apoptosis of pancreatic beta cells[10], which suggests also a long-term effect of SSRI use. However, we did not observe an association between past use of SSRIs and the different study outcomes. Therefore, our study did not provide evidence of long-term effects on glucose-insulin homeostasis by SSRIs.

To our knowledge, the association between use of SSRIs and insulin dependency has not been studied before. The higher risk of starting insulin treatment associated with use of SSRIs might be clinically relevant, as this condition is associated with a higher risk of mortality[13, 14]. Thus, our data might suggest that progression of type 2 diabetes during the use of SSRIs is accelerated. Furthermore, the combination of a higher risk of depression in type 2 diabetes patients[1, 2], the higher frequency of antidepressant use in type 2 diabetes patients[23], and the increasing prevalence of type 2 diabetes[24] make our findings relevant to an increasing population.

Although SSRIs have been associated with an increased risk of type 2 diabetes[6-8], no difference in glucose levels has been observed between users of SSRIs and non-users of antidepressants[7], similar as found in our study. This might indicate that the increased risk of type 2 diabetes in users of SSRIs is independent of fasting glucose levels. However, it remains unclear whether glucose disposal rate is also different between participants using SSRIs and participants using no antidepressants. Low insulin secretion, independently from peripheral insulin sensitivity, has also been associated with a higher risk of type 2 diabetes[25, 26]. Therefore, the underlying mechanism of the association between SSRIs and type 2 diabetes might include a

lower insulin secretion rather than a lower peripheral insulin sensitivity. Contrary, use of SSRIs was also associated with increased peripheral insulin sensitivity. One explanation for this finding might be that the higher peripheral insulin sensitivity is required to preserve glucose-insulin homeostasis.

The present study has a number of strengths. First, the analyses on the start of insulin treatment were conducted using time-varying co-variables and exposures. Using this method, the exposure to antidepressants during follow-up was analyzed more accurately than with conventional statistical models, as logistic regression models. Study participants of the Rotterdam Study cohort were not selected on health condition, which minimized selection bias. Second, exposure to antidepressants and glucose-lowering drugs (oral and insulin) were defined on the basis of prospectively collected automated pharmacy records, which minimized the risk of information bias. And last, we adjusted for the presence of depression. Potential effects of depression on the study outcomes, as for example on immunology[27], did not confound our findings. A drug effect was also supported by our observation that insulin secretion was lower in users of SSRIs than in users of TCAs. However, our study also has a few limitations. First, we had only few participants treated with SSRIs at the moment they became a case (n = 8). Second, data on the diagnosis of depression/depressive symptoms was not available for RS-III at the time of the study. And third, assessment of insulin resistance and insulin secretion by pancreatic beta cells were based on homeostatic model assessment formulas, instead of an euglycemic clamp test. Because both formulas showed a high correlation with measures obtained by euglycemic clamp tests, interpretation of the study results is not majorly different[18, 19]. Possible measurement errors by the formulas are most likely non-differential between treated and untreated participants.

In conclusion, we found that SSRI use was associated with decreased secretion of insulin in pancreatic beta cells and with an increased risk of insulin dependency in type 2 diabetes patients. Although biologically plausible, these findings should be investigated in more detail using larger samples in future studies to confirm our result.

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CHAPTER 4

ANTIDEPRESSANT USE AND UNINTENDED EFFECTS ON ELECTROPHYSIOLOGY

CHAPTER 4.1

ASSESSING PROLONGATION OF THE HEART-RATE CORRECTED QT INTERVAL IN USERS OF TRICYCLIC ANTIDEPRESSANTS: ADVICE TO USE FRIDERICIA RATHER THAN BAZETT'S CORRECTION

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4.1

A prolonged heart-rate corrected QT interval (QTc) increases the risk of sudden cardiac death. Some methods of heart-rate correction (notably Bazett) overestimate QTc in people with high heart rates. Studies suggest that tricyclic antidepressants (TCAs) can prolong the QTc and increase heart rate. Therefore, we aimed to study whether TCA-induced QTc prolongation is a false-positive observation due to overestimation at high heart rates. For this, we included 12,734 participants from the prospective population-based Rotterdam Study, with a total of 27,068 electrocardiograms (ECGs) of which 331 during TCA use. Associations between use of TCAs, QTc, and heart rate were studied with linear repeated measurement analyses. QT was corrected for heart rate according to Bazett (QTc_{Bazett}), Fridericia ($QTc_{Fridericia}$) or a correction based on regression coefficients obtained from the Rotterdam Study data ($QTc_{Statistical}$). On ECGs recorded during TCA use, QTc_{Bazett} was 6.5 milliseconds (95% confidence interval: 4.0 – 9.0) longer and heart rate was 5.8 beats per minute (95% confidence interval: 4.7 – 6.9) faster than during nonuse. $QTc_{Fridericia}$ and $QTc_{Statistical}$ were not statistically significantly longer during TCA use than during non-use. Furthermore, QTc_{Bazett} was similar for ECGs recorded during TCA use and nonuse after statistical adjustment for heart rate. According to our results, TCA use appears not to be associated with QTc prolongation. Therefore, the current advice of regulatory authorities to restrict use of these drugs and to do regular check-ups of the QTc may need to be revised. Other formulas, like Fridericia's, might be preferred.

Introduction

A prolonged heart-rate corrected QT interval (denoted hereafter as QTc) on the electrocardiogram (ECG) is associated with an increased risk of torsade de pointes and sudden cardiac death[1, 2]. QTc prolongation can be hereditary, environmentally, or drug-induced[2]. Drug-induced QTc prolongation is a frequent reason for withdrawal of a drug from the market[3]. In recent years, a large number of cardiac and non-cardiac drugs have been associated with QTc prolongation[4].

The QT interval and heart rate are highly correlated. Therefore, several formulas have been developed to correct the QT interval for heart rate. Both in clinical practice and in research, the formula by Bazett is used most frequently[5-7]. However, this correction is suboptimal because the resulting QTc is not completely independent of heart rate[6, 8]. More specifically, Bazett's QTc is overestimated in individuals with a high heart rate, and underestimated in individuals with a low heart rate[6, 8]. Nevertheless, QTc corrected with the formula of Fridericia is also overestimated in individuals with higher heart rate, but to a lesser extent than with Bazett's[8]. Therefore, the American Food and Drug Administration recommends that studies assessing the QTc-prolonging effects of new drugs (thorough QT/QTc studies) should not use Bazett's formula alone[7] but also other correction formulas, such as Fridericia's[9] or correction formulas based on regression coefficients[10-12].

One of the drug groups thought to prolong the QTc are tricyclic antidepressants (TCAs)[13-16]. However, since TCAs also increase heart rate due to anticholinergic effects[17], it is possible that the increase in QTc can be explained by a higher heart rate. The objective of our study was to reassess the association between use of TCAs and QTc using different heart-rate correction methods in a population-based cohort.

Methods

Study setting

Our study was part of the Rotterdam Study, a prospective population-based cohort of middle-aged and elderly participants. The design and rationale of the Rotterdam Study is described in detail elsewhere[18, 19]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were asked to participate. In total, 7983 individuals agreed to participate (response rate 78%). The original cohort was extended in 2000. In this first subcohort, all inhabitants who turned 55 years of age or moved into the district (matching the age criterion) were asked to participate, of whom 3011 individuals

agreed (response rate 67%). A second extension of the cohort was initiated in 2006. In this subcohort, inhabitants from the same district aged 45 years and older were invited to participate. In total, 3932 inhabitants agreed to participate (response rate 65%). Follow-up examinations were conducted every 4 to 5 years, with a current maximum of 5 center visits per participant. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands. Written informed consent was obtained from all study participants.

Study population

We included all participants with at least one ECG recorded in the period that drug-dispensing data was available (January 1, 1991 – December 31, 2011). ECGs on which atrial fibrillation or pacemaker rhythm was determined were excluded from the study.

Assessment of TCA use

At study entry, more than 99% of the participants had their drug prescriptions filled at one of the seven regional pharmacies. Drug dispensing at these pharmacies was fully computerized. Dispensing data included the product name, the anatomical therapeutic chemical (ATC) code, dispensing date, total amount of tablets/capsules per prescription and dispensed daily number of tablets/capsules. Dispensing episodes were calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. If the date of a center visit during which the ECG was recorded fell within a dispensing episode of TCA use (ATC code: N06AA), the participant was considered as a current user.

ECG measurements

A standard 12-lead resting ECG was recorded with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florance, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS) program, which has been described previously and validated and applied extensively[20-24]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with use of template matching techniques[22]. The duration of the QT interval was measured by the MEANS program from the start of the QRS complex to the end of the T wave, which is described more extensively elsewhere[25]. The median RR and average QT interval was computed after exclusion of RR intervals that immediately preceded or followed premature ventricular complexes[25].

The following methods were used to correct the QT interval for heart rate: Bazett's formula (QTc_{Bazett}: QT / [formule])[5], Fridericia's formula ($QTc_{Fridericia}$: QT / [formule])[9], and one based on regression coefficients measured in the Rotterdam Study ($QTc_{Statistical}$), which was similarly done elsewhere[11]. A prolonged QTc_{Bazett} was defined as a QTc >450 ms for men and >470 ms for women. The method of the regression-based model will be discussed in the statistical analyses paragraph. Heart rate in beats per minute (bpm) was calculated by dividing 60,000 by the RR interval in milliseconds (ms).

Covariables

The following covariables were considered additionally to age and sex: body mass index (BMI), use of drugs that affect the heart rate and/or QTc, heart failure, hypertension, myocardial infarction and diabetes mellitus. Weight and height were measured at the study center at the date of ECG examination. BMI was calculated with the following formula: weight (in kilograms) / height^2 (in meters). Use of drugs associated with an altered heart rate was determined on the date of ECG examination, and was based on the pharmacy dispensing records. These drugs were beta-blockers (ATC code: C07), verapamil (ATC code: C08DA01), diltiazem (ATC code: C08DB01), and digoxin (ATC code: C01AA05). Of the QTc-prolonging drugs, we only considered those having probable QTc-prolonging properties. Probable QTc-prolonging drugs are generally thought to increase the risk of torsade de pointes. This list contains drugs from various classes, including several antibiotics and antipsychotics[4]. TCAs on this list were not taken into account in this variable. The diagnosis of heart failure was based on typical signs and symptoms confirmed by objective evidence of cardiac dysfunction[26]. The presence of hypertension at the center visit was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or use of blood-pressure lowering medication with the indication hypertension. Myocardial infarction was adjudicated based on a combination of symptoms, ECG measurements and enzyme markers[26]. The presence of diabetes mellitus was defined as use of glucose-lowering drugs, a non-fasting glucose level of more than 11.0 mmol/L, or a fasting glucose level of more than 6.9 mmol/L.

Statistical analyses

Characteristics of the study population were studied at baseline, which was defined as the first eligible ECG recording of a participant included in the study irrespective of the TCA-use status.

As most participants had multiple ECG recordings, analyses were conducted with linear mixed models to correct for within-person correlations between visits.

We tested covariance structures and selected the one with the lowest Akaike's information criterion.

We based $QTc_{Statistical}$ on the regression coefficients of the independent variables heart rate and heart rate squared as computed in the Rotterdam Study. Heart rate squared was included in the regression model to take into account the nonlinear relationship between heart rate and QT interval. $QTc_{Statistical}$ was calculated with the following formula: QT – 3.984323*(60 – heart rate) + 0.014126*(3600 – heart rate squared).

We analyzed the data in two ways: cross-sectional and longitudinal. In the cross-sectional analysis all ECGs made during TCA use were compared with ECGs made during nonuse, with respect to QTc_{Bazett}, $QTc_{Fridericia}$, $QTc_{Statistical}$ and heart rate. After assessing the effects of TCAs as a class, we analyzed the effect of individual drugs. We also stratified the analysis based on tertiles of heart rate. For the longitudinal analysis, we studied the within-person changes in QTc_{Bazett}, $QTc_{Fridericia}$, $QTc_{Statistical}$ and heart rate in between two consecutive visits (an ECG pair). The within-person changes were calculated by subtracting the first measurement from the second measurement. We classified the following exposure classes in an ECG pair: no TCA use at both visits (none-none), use of TCAs only at the second visit (none-TCA), use of TCAs only at the first visit (TCA-none), and use of TCAs at both consecutive visits (TCA-TCA). For the analyses, we compared the within-person changes between exposure classes using none-none as the reference. All analyses were adjusted for age and sex. Other covariables were included if they changed the effect size by a minimum of 10%.

In the sensitivity analysis we excluded all ECGs in which left bundle branch block, right bundle branch block, second and third degree atrioventricular block, left ventricular hypertrophy (according to Sokolow-Lyon criteria) or QRS duration > 120 milliseconds, were detected as well as ECGs recorded during use of beta-blocking agents, verapamil, diltiazem and digoxin.

For every analysis, we used SPSS (version 21.0, IBM Corp., Somers, NY, USA). Two-sided p-values below 0.05 were considered statistically significant.

Results

Baseline characteristics

Baseline characteristics of the study population are presented in Table 1. We included 12,734 participants with a total of 27,048 ECGs. At baseline, the mean age of the study population was 65.1 years (standard deviation (SD) = 9.8), and 58.1% of

Table 1 Baseline characteristics of the study population

Characteristics	Total population N=12,734
Number of ECGs	27,068
ECGs per participant, median (range)	2 (1 – 5)
Age in years	65.1±9.8
Females	7,402 (58.1)
Body mass index in kg/m^2	26.9±4.2
QTc_{Bazett} in milliseconds	430±25
Prolonged QTc_{Bazett} in men(>450 ms)	714 (13.4)
Prolonged QTc_{Bazett} in women (>470 ms)	445 (6.0)
$QTc_{Fridericia}$ in milliseconds	421±22
$QTc_{Statistical}$ in milliseconds	422±21
QRS in milliseconds	101±18
Diabetes mellitus	967 (7.6)
Hypertension	6,761 (53.1)
History of myocardial infarction	952 (7.4)
History of heart failure	261 (2.0)
Left ventricular hypertrophy	391 (3.1)
Left bundle branch block	193 (1.5)
Right bundle branch block	315 (2.5)
2nd or 3rd degree atrioventricular block	12 (0.1)
Beta blockers	1,666 (13.1)
Verapamil	83 (0.7)
Diltiazem	156 (1.2)
Digoxin	157 (1.2)
Probable QTc-prolonging drugs*	271 (2.1)

Abbreviations: ECG: electrocardiogram; QTc_{Bazett}: heart-rate corrected QT interval according to Bazett's formula; $QTc_{Fridericia}$: heart-rate corrected QT interval according to Fridericia's formula; $QTc_{Statistical}$: heart-rate corrected QT interval using linear regression coefficients observed in the Rotterdam Study. Presented data refer to the first eligible center visit with an ECG measurement of a participant.
Data presented as mean±standard deviation or number of participants (percentage), or as indicated otherwise.
* Tricyclic antidepressants defined as probable QT prolonging drugs were excluded.

the participants were women. Mean QTc_{Bazett} was 430 ms (SD = 25), $QTc_{Fridericia}$ 421 ms (SD = 22), $QTc_{Statistical}$ 422 ms (SD = 21) and mean heart rate was 69 bpm (SD = 11).

Association between TCAs, QTc and heart rate

Of the tested covariance matrices, the first-order autoregressive covariance structure with homogenous variances had the lowest Akaike's information criterion, which we therefore used in the rest of the study for all analyses.

The results of the cross-sectional analyses are presented in Table 2. Compared to ECGs made during nonuse, QTc_{Bazett} was 6.5 ms (95% confidence interval (CI): 4.0 – 9.0 longer in ECGs made during TCA use. However, when heart rate was added to the statistical model, the difference was no longer statistically significant. Furthermore, neither $QTc_{Fridericia}$ nor $QTc_{Statistical}$ were significantly different when comparing ECGs made during TCA use with ECGs made during nonuse. These results remained similar when we added heart rate to the statistical model.

When we stratified on tertiles based on heart rate, QTc_{Bazett} was 4.4 ms (95%CI: 1.0 – 7.7) longer in ECGs made during use than in ECGs made during nonuse, but only in the highest tertile (heart rate > 73 bpm). In all tertiles, there was no association between TCA use during ECG recording and $QTc_{Fridericia}$ and $QTc_{Statistical}$. When stratified on individual TCAs, QTc_{Bazett} was statistically longer than nonuse during use of the following TCAs: imipramine, amitryptiline, nortriptyline and maprotiline. However, only maprotiline use of showed a significantly longer $QTc_{Fridericia}$ or $QTc_{Statistical}$ than nonuse.

Heart rate was 5.8 bpm (95%CI: 4.7 – 6.9) faster on ECGs recorded during TCA use than on ECGs recorded during nonuse. Of the individual TCAs, heart rate was significantly faster on ECGs recorded during use of imipramine, amitriptyline, nortriptyline and maprotiline.

Results did not materially change when ECGs were excluded that were made in participants with cardiac pathology and that were made during use of heart-rate affecting drugs.

Longitudinal analyses: changes in QTc and heart rate

A total of 13,885 ECG pairs were available with a median time interval between two consecutive visits of 4.3 years (interquartile range: 2.4 – 5.0). In the TCA exposure class none-TCA, QTc_{Bazett} was 4.7 ms (95%CI: 0.5 – 9.0) higher at the second ECG (Figure 1A) than the none-none class. Conversely, in the TCA exposure class TCA-none, QTc_{Bazett} was lower at the second ECG (-7.1 ms, 95%CI: -11.8 – 2.3) than at the first ECG. These associations were neither observed for $QTc_{Fridericia}$ (Figure 1B) nor for $QTc_{Statistical}$ (Figure 1C). In the same TCA exposure classes, heart rate was higher at the second ECG than at the first ECG when TCAs were used during the second

Table 2 **Cross-sectional association between use of tricyclic antidepressants and QT interval using different heart-rate correction methods and heart rate**

		QTc_{Bazett}		$QTc_{Fridericia}$		$QTc_{Statistical}$		Heart rate	
	N	β in ms	95%CI	β in ms	95%CI	β in ms	95%CI	β in bpm	(95%CI)
No TCA use	26,737	ref		ref		Ref		Ref	
Any TCA	331	6.5	4.0 – 9.0†	0.6	-1.5 – 2.8	0.9	-1.2 – 3.0	5.8	4.7 – 6.9†
Amitriptyline	200	5.5	2.3 – 8.8†	-0.5	-3.3 – 2.3	-0.2	-3.0 – 2.5	5.8	4.4 – 7.3†
Maprotiline	56	10.8	4.9 – 16.7†	5.1	0.0 – 10.2*	5.0	0.1 – 9.9*	5.7	3.0 – 8.4†
Clomipramine	31	3.6	-4.0 – 11.1	0.5	-0.6 – 7.0	0.6	-5.7 – 6.9	3.0	-0.3 – 6.4
Imipramine	17	11.3	0.7 – 21.9*	0.5	-8.7 – 9.7	0.7	-8.1 – 9.6	10.9	6.0 – 15.7†
Nortriptyline	16	13.0	1.8 – 24.2*	1.9	-8.1 – 11.9	2.4	-7.3 – 12.1	11.5	6.7 – 16.3†
Doxepin	7	-7.8	-24.3 – 22.8	-3.4	-17.6 – 10.8	-3.2	-16.7 – 10.4	-5.0	-12.8 – 2.7
Dosulepin	4	-2.2	-27.2 – 22.7	-2.2	-24.2 – 19.6	-1.8	-22.9 – 19.3	-0.7	-12.1 – 10.7

Abbreviations: bpm: beats per minute; CI: confidence interval; ECG: electrocardiogram; ms: milliseconds; N: number of ECGs; QTc_{Bazett}: heart-rate corrected QT interval according to Bazett's formula; $QTc_{Fridericia}$: heart-rate corrected QT interval according to Fridericia's formula; $QTc_{Statistical}$: heart-rate corrected QT interval using linear regression coefficients observed in the Rotterdam Study; TCA: tricyclic antidepressant. Data presented as the mean adjusted difference in milliseconds of the QTc or beats per minute of heart rate between TCA users and nonusers. Analyses are adjusted for age and sex. * P-value < 0.05; † P-value <0.001.

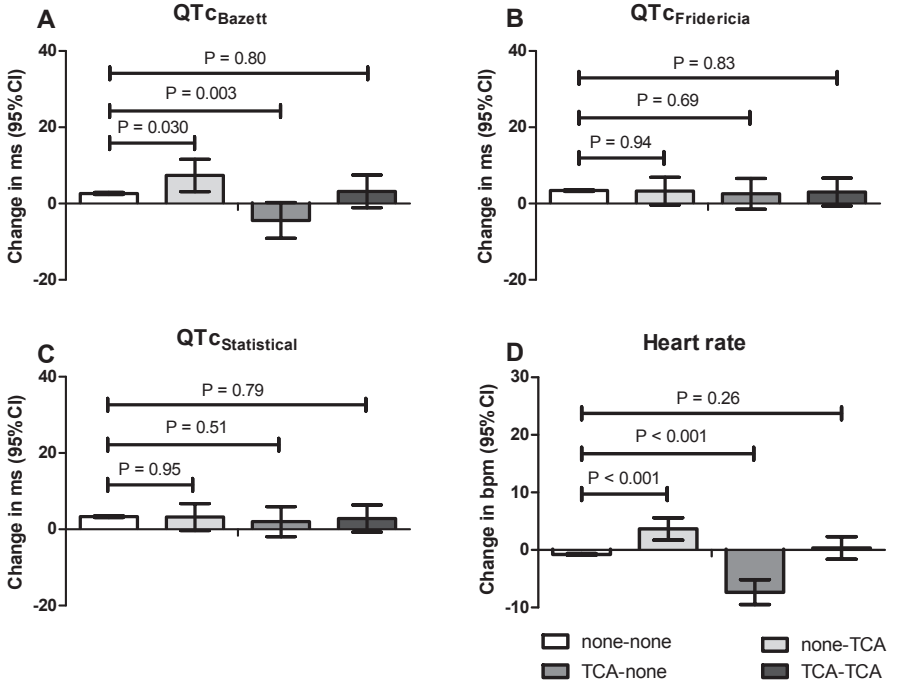

Figure 1 **Changes in intervals between repeated center visits.**

None-none: no use of tricyclic antidepressants (TCAs) at both consecutive visits; None-TCA: used a TCA at the second of two consecutive visits; TCA-none: used a TCA at the first of two consecutive visits: TCA-TCA, used TCAs at both consecutive visits. **A)** Change in QTc_{Bazett}. **B)** Change in $QTc_{Fridericia}$. **C)** Change in $QTc_{Statistical}$. **D)** Change in heart rate. Data presented as the mean change in milliseconds with 95% confidence interval between consecutive center visits. Analyses were adjusted for age and sex.

ECG, and heart rate was lower at the second ECG than at the first ECG when TCAs were used during the first ECG.

Results did not materially change when we excluded ECGs made in participants with cardiac pathology and ECGs made during use of drugs affecting the heart rate.

Discussion

In the present study, use of TCAs was associated with a prolonged QTc according to Bazett's formula, but not with a significant increase in QTc when using Fridericia's formula or when QT was corrected for heart rate using regression coefficients

obtained from the included ECGs of the present study. Furthermore, TCA use was not associated with prolongation of QTc_{Bazett} when we additionally adjusted for heart rate. Of the individual TCAs, only use of maprotiline showed a significantly higher QTc with the correction methods other than Bazett's, although the effect size with these methods was lower than with Bazett. QTc_{Bazett} was only higher in ECGs made during TCA use than in ECGs made during nonuse in the tertile with the highest heart rates. Results were similar in the longitudinal analyses of QTc interval and heart rate. In addition, use of TCAs was associated with a faster heart rate of 5.8 bpm.

Because the association between TCAs and QTc_{Bazett} was no longer significant when additionally adjusted for heart rate, the higher heart rate is likely the cause of the overestimation of the TCA-induced QTc prolongation. Therefore, the results of our study indicate that the previously described TCA-induced prolongation of QTc_{Bazett} is possibly observed because of overestimation instead of a real drug effect. This is supported by our finding that TCA-induced prolongation of QTc_{Bazett} was only observed in participants with the highest heart rates (i.e. upper tertile). Furthermore, of the individual TCAs, we observed the largest difference between QTcBazett and other correction methods for those TCAs with the largest effect on heart rate.

The association between TCA use and QTc_{Bazett} prolongation has been consistently observed in cohort studies[13-15]. In addition, a number of case reports on TCA-induced QTc prolongation have been published[16]. Although these case reports did not support our findings directly, it should be noted that these case studies reported TCA-induced QTc prolongation in patients with severe cardiac pathologies or in patients with a TCA overdose[16]. As further support of our findings, no association was observed in population-based studies between use of TCAs and sudden cardiac death, which is one of the hard endpoints associated with QTc prolongation[27, 28]. However, this does not imply that use of TCAs is without risk with respect to cardiovascular end points. A higher heart rate has previously been associated with an increased risk of cardiac conditions like heart failure and overall cardiovascular mortality[29-31]. As use of TCAs is associated with an increased risk of cardiovascular mortality[32], detrimental cardiovascular effects of TCAs can be caused by an increase in heart rate rather than a prolonged QT interval. TCAs have also been associated with an increased risk of torsade de pointes[16]. Because the correlation between QTc prolongation and torsade de pointes is modest at best, this may still be in line with our findings[33].

Bazett's formula is the most often used method to correct the QT interval for heart rate in both clinical practice and in research[6, 7]. The two other studied correction formulas are less dependent on heart rate after correction, as point estimates did not materially differ when additionally adjusted for heart rate in the

statistical model. This indicates that these formulas are less prone to overestimation of QTc in persons with high heart rates. To study the safety of TCAs with respect to QTc prolongation, these formulas might be more appropriate. The correction formula based on regression coefficients would be the best, as the coefficients were obtained from the same population as the one we used for the analyses, and therefore this formula would correct for heart rate optimally. However, such a model is specific for a study population, and thus the clinical use is limited[8]. Heart-rate correction with Fridericia's formula might be preferred in patients taking heart-rate increasing drugs, as it is also easily calculated and results were similar with respect to the model based on regression coefficients.

A strength of our study is the analysis of longitudinal changes in QTc and heart rate. The cross-sectional analysis is more prone to be confounded by a contraindication, as participants with a longer QTc interval are less likely to be prescribed TCAs. Also, the cross-sectional analysis cannot infer causality between TCAs and QTc prolongation. Although the longitudinal analysis is still observational, it can provide additional arguments in favor of a causal relationship. As similar results were obtained with cross-sectional and longitudinal analyses, our findings are more likely due to the drug rather than due to other unmeasured factors. Another strength of our study is the MEANS algorithm to calculate QT interval and heart rate. This algorithm works systematically and automatically and has been evaluated extensively[21-23]. Finally, information bias was limited as pharmacy-dispensing data was recorded irrespective of disease state. Our study also has some limitations. In our study, only a limited number of participants were using TCAs. Second, the median time interval between two examination rounds is 4.3 years, which might be too lengthy for the calculation of changes in QTc intervals. Therefore, it is still possible that there is an acute QTc-prolonging effect of TCA use, which is visible with all heart-rate correction models. However, our results indicate that long-term treatment with TCAs is not accompanied by QTc prolongation. And last, being an observational study, our study is subjected to confounding. However, none of the considered covariables changed the effect sizes substantially.

In conclusion, the results of our study suggested that TCA-induced prolongation of QTc_{Bazett} might be the result of overestimation that occurs in persons with high resting heart rates. TCA prescribing should nonetheless still be done with caution due to the associated increase in resting heart rate. When the QT interval was corrected for heart rate with Fridericia's formula or with a model based on regression coefficients, no increase in QTc was observed. Reported effects on the QTc interval should be reconsidered for heart-rate increasing drugs. For such drugs Fridericia's correction might be preferred. The advice of regulatory authorities to restrict use of these drugs and to do regular check-ups of the QTc, may need to be revised.

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CHAPTER 4.2

USE OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS AND THE HEART-RATE CORRECTED QT INTERVAL IN A REAL-LIFE STETTING: THE POPULATION-BASED ROTTERDAM STUDY

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4.2

Aims: Selective serotonin reuptake inhibitors (SSRIs), specifically citalopram and escitalopram, are thought to give QTc prolongation, although studies showed contradictory results. Nevertheless, a maximum citalopram dosage of 20 mg in high-risk patients (e.g. >60 years of age) is recommended. We aimed to investigate the association between use of (individual) SSRIs and QTc in a population-based study in older adults.

Methods: This study, which was part of the prospective Rotterdam Study (period: 1991-2012), included participants with up to 5 electrocardiograms (ECGs). We used linear mixed models to compare QTcF (QT corrected according to Fridericia) measured during use of individual SSRIs with QTc measured during non-use of any antidepressant. For citalopram, analyses were additionally restricted to a maximum dosage of 20 mg in participants aged 60 years and older.

Results: We included 12,589 participants with a total of 26,620 ECGs of which 436 ECGs were made during SSRI use. The mean QTcF was similar during use of any drugs from the SSRI class and during non-use. After stratifying to individual SSRIs, ECGs recorded during use of citalopram had the longest QTcF compared with ECGs recorded during non-use (+12.8 ms; 90% CI: 7.5 – 18.2). This result remained similar in the analysis comprising participants aged 60 years and older with a maximum prescribed daily dosage of 20 mg of citalopram .

Conclusions: Although no SSRI class effect was observed, use of citalopram was associated with a longer QTcF, even after considering the recommended restriction. Other SSRIs may not give a clinically relevant QTcF prolongation.

Introduction

Sudden cardiac death accounts for approximately 50% of cardiovascular mortality and 20% of all deaths[1-3]. Prolongation of the heart-rate corrected QT interval (hereafter denoted as QTc) may induce ventricular arrhythmias, which is one of the causes of sudden cardiac death [4-6]. Risk factors for QTc prolongation include female sex, old age, certain genetic variants (e.g., in the *NOS1AP* gene), and use of certain drugs[5, 7-10].

One of the drug classes for which QTc prolonging properties are currently under debate, are selective serotonin reuptake inhibitors (SSRIs). *In-vitro* studies reported that a large number of the individual SSRIs have the potential to block the hERG channel[11-14], which could consequently increase the QTc. In line with this, many SSRIs are already listed as having QTc prolonging properties[15, 16], which suggests that SSRIs as a class might increase QTc. However, in a large cross-sectional study, conducted in an electronic health-care database, only citalopram and escitalopram were associated with a longer QTc[17]. On the other hand, in a meta-analysis of randomized clinical trials on SSRI-induced QTc prolongation, the use of fluvoxamine and sertraline were also associated with QTc prolongation, although effect sizes were smaller than with citalopram and escitalopram[18]. In 2011, the American Food and Drug Administration (FDA) announced that the daily dosage of citalopram should be restricted to a maximum of 40 mg daily in healthy adults and 20 mg maximum in high-risk patients (e.g., >60 years of age)[19, 20]. However, the safety of use has not yet been determined within the limits of these restrictions.

Within the Rotterdam Study, the association between the use of different psychotropic drugs, including SSRIs, and QTc prolongation has been studied previously[21]. However, no significantly longer QTc was observed in users of any of the individual SSRIs as well as SSRIs as a class, which was probably due to the low number of users. After this study was conducted, the Rotterdam Study cohort was further extended, more follow-up data became available[22], and SSRIs were increasingly dispensed[23]. Furthermore, the FDA now recommends the use of QTc corrected with Fridericia (hereafter denoted as QTcF) instead of Bazett, because the latter overestimates the QTc in participants with higher heart rates[24-26]. Therefore, we aimed to reinvestigate the association between SSRIs as a class and QTcF as well as the association between individual SSRIs and QTcF in this population-based cohort of middle-aged and older adults, and aimed to study QTcF prolongation within the limits of the FDA recommendations.

4.2

Methods

Research setting

The present study was embedded in the prospective Rotterdam Study, which aims to investigate the occurrence of, and risk factors for different age-related diseases. The design and rationale of the Rotterdam Study was described in more detail elsewhere[22, 27]. In short, the original cohort was initiated with a baseline visit between 1990 and 1993, and included inhabitants aged 55 years and older, who lived in the Ommoord district in Rotterdam, the Netherlands. In total, this cohort consisted of 7,983 individuals (response rate 78%). From 2000 to 2001, participants who became 55 years of age or older, or moved into the district were asked to participate in an extension of the first cohort, which included 3,011 individuals (response rate 67%). From 2006 until 2008 a second extension was initiated with a total of 3,932 inhabitants from the same district, aged 45 and older (response rate 65%). After baseline assessment, follow-up examinations were conducted every 4-5 years.

The Rotterdam Study was approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study) executed by the Ministry of Health, Welfare and Sports of the Netherlands. All study participants provided written informed consent.

Study population

We included all participants from the Rotterdam Study with up to 5 electrocardiogram (ECG) measurements and for whom we have had access to pharmacy dispensing records. ECGs that showed atrial fibrillation or a pacemaker rhythm were excluded from the analysis. ECG recordings during which participants were using other antidepressants than SSRIs were excluded as well.

SSRI exposure assessment

Fully computerized pharmacy dispensing records were available from January 1st 1991 onwards for more than 99% of the study population. Dispensing records included Anatomical Therapeutic Chemical (ATC) code, dispensing date, total amount of tablets/capsules per prescription, prescribed daily number of tablets/capsules, and product name of the drug. Dispensing episodes were calculated by dividing the total number of filled tablets/capsules by the prescribed daily number. If the date of the ECG assessment fell within a dispensing episode of SSRI use (ATC-code: N06AB), the participant was considered a current SSRI user. The use of individual SSRIs was based on the full, 7-digit, ATC-code. The prescribed daily dosage was standardized by dividing the prescribed daily dosage by the defined daily dosage (PDD / DDD ratio)[28].

Electrocardiogram assessment

A standard 12-lead resting ECG was recorded with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS) program, which has been described previously and validated and applied extensively[29-33]. MEANS determines common onsets and offsets for all 12 leads together on one representative averaged beat, with the use of template matching techniques[31]. The duration of the QT interval was measured from the start of the QRS complex to the end of the T wave, which is described in more detail elsewhere[34]. The median RR and mean QT interval were computed after exclusion of beats that immediately preceded or followed premature ventricular complex[35].

Heart rate in beats per minute (bpm) was calculated by dividing 60,000 by the RR interval in milliseconds (ms). As QT and heart rate are highly correlated, QT intervals were corrected for heart rate (expressed as the RR interval) using the formula by Fridericia (QTcF: QT/ [formule])[36], which is advised by authorities[26, 37].

Co-variables

We considered the following factors as potentially confounding factors at the date of ECG recording, in addition to age and sex: body mass index (BMI), use of drugs that affect the heart rate and/or QTc, diabetes mellitus, hypertension, myocardial infarction, and heart failure. Weight and height were measured at the study center. BMI was calculated as weight (in kilograms) / height^2 (in meters). Use of drugs associated with an altered heart rate were determined on the date of ECG examination, and was based on the pharmacy dispensing records. The following drugs were considered heart-rate altering: beta blockers (ATC code: C07), verapamil (ATC code: C08DA01), and diltiazem (ATC code: C08DB01). Definite QTc prolonging drugs are generally thought to increase the risk of torsade de pointes. This group of drugs comprises, among others, a number of antibiotics and antipsychotics[16]. SSRIs on this list were not taken into account for this variable. Diabetes mellitus was defined as use of glucose-lowering drugs (ATC code: A10), a non-fasting glucose level of more than 11.0 mmol/L, or a fasting glucose level of more than 6.9 mmol/L. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or use of blood-pressure lowering medication for the indication hypertension[38]. History of myocardial infarction was defined based on a combination of enzyme markers indicative of the presence of myocardial infarction, ECG measurements, and symptoms. This information was retrieved from medical files[39]. Heart failure was assessed based on typical signs and symptoms confirmed by objective cardiac dysfunction[39].

Statistical analyses

Study characteristics were determined at baseline, defined as the first eligible ECG recordings of the included participants.

We used linear mixed models to adjust for within-person correlations between repeated center visits. We tested several covariance structures, and selected the one with the lowest Akaike Information Criterion (AIC)[40].

To address the research question, we studied the effect of SSRIs on cross-sectional examinations of QTcF, QT (uncorrected for heart rate), and heart rate. These analyses assessed the difference of QTcF, QT and HR between the ECGs recorded during SSRI use and ECGs recorded during non-use. These analyses were done for SSRIs as a single exposure group as well as for individual SSRIs.

In a second analysis, we assessed whether the within-person change in QTcF between two consecutive ECG examination rounds was dependent on the use of (individual) SSRIs. The change in QTc was calculated according to the following formula: $QTcF_{visit(t+1)} - QTcF_{(visit\ t)}$. As participants could have had up to 5 ECG recordings included in the study, participants could have up to 4 ECG pairs. The effect of SSRI exposure on QTcF was studied at the second examination of an ECG pair, and explored in two ways. First, use of (individual) SSRIs was considered irrespective of the participants' exposure status during the first examination of an ECG pair. Second, use of (individual) SSRIs was considered when the first examination of an ECG pair was recorded during non-use of antidepressants. To interpret prolongation of QTcF attributed to the use of SSRIs, results were presented as beta with respect to the ECGs pairs with non-use of antidepressants during the second visit (for both exposure definitions). These analyses were conducted for SSRIs as a single drug exposure group as well as for the individual SSRIs.

Our basic statistical model was adjusted for age and sex. In additional analyses, all potential covariables were included in the statistical model to study whether they confounded the association between SSRIs and QTcF.

We also conducted analyses in different subsamples. First, we restricted the analyses to ECGs of participants without cardiac conduction disorders (left or right bundle branch block, or second or third degree atrioventricular block) and/or left ventricular hypertrophy. Second, we restricted analyses to ECGs recorded in participants aged 60 years and older and with a maximum citalopram dose of 20 mg per day. These restrictions are in line with the recommendations of the FDA[19, 20].

All analyses were conducted using IBM SPSS Statistics (version 21.0, IBM Corp., Somers, NY, USA). The results were presented as the effect of (individual) SSRIs on ECG measures compared with ECGs or ECG pairs recorded during non-use of antidepressants with the 90% confidence interval. No correction was made for multiple testing.

Results

Baseline characteristics

Table 1 reports the general characteristics of the study population at baseline. In total, 12,589 participants were included with a total of 26,620 ECGs. At baseline, 57.9% was female and the mean age was 65.2 years (standard deviation (SD) = 9.8). The mean heart rate was 70 beats per minute (bpm) and the mean QTcF was 421 milliseconds (ms).

Table 1 Characteristics of the study population

	Total population N = 12,589
Number of ECGs	26,620
ECGs per participant, median (range)	2 (1 – 5)
Females, N (%)	7,294 (57.9)
Age in years, mean (SD)	65.2 (9.8)
Body mass index (kg/m^2), mean (SD)	26.9 (4.1)
Heart rate in bpm, mean (SD)	70 (11)
QTc in ms, mean (SD)	421 (22)
Diabetes mellitus, N (%)	636 (5)
Hypertension, N (%)	6,645 (53)
Myocardial infarction, N (%)	930 (7)
Heart failure, N (%)	257 (2)
Right bundle branch block, N (%)	314 (2.5)
Left bundle branch block, N (%)	190 (1.5)
2nd or 3rd degree atrioventricular block, N (%)	12 (0.1)
Left ventricular hypertrophy, N (%)	386 (3.1)
Beta-blocking agents, N (%)	1,625 (12.9)
Verapamil	80 (0.6)
Diltiazem	156 (1.2)
Definite QTc-prolonging drugs, N (%)*	261 (2)

Abbreviations: bpm, beats per minute; ms, milliseconds; N, Number of participants; SD, Standard deviation; ECG, electrocardiogram. Data presented at the first eligible ECG recording per participant. * Selective Serotonin Reuptake Inhibitors were not taken into account in this variable.

Cross-sectional analyses

Of the tested covariance matrices, the "heterogeneous first-order autoregressive covariance structure" had the lowest AIC, which we therefore used for all analyses.

Table 2 presents the differences in QTcF between ECGs recorded during SSRI use with ECGs recorded during non-use. Compared with ECGs recorded during non-use, ECGs recorded during SSRI use had a 2.9 ms longer QTcF (90% CI: 1.3 – 4.5) and a 6.2 ms longer uncorrected QT interval (90% CI: 4.0 – 8.4). Furthermore, heart rate was 1.7 bpm slower (90% CI: 0.9 – 2.5) on ECGs recorded during use of SSRIs than on ECGs recorded during non-use of antidepressants.

Of the different studied individual SSRIs, ECGs recorded during citalopram use had a 12.8 ms longer QTcF (90% CI: 7.3 – 18.2) and a 19.5 longer uncorrected QT interval (90% CI: 11.9 – 27.1) than ECGs recorded during non-use of antidepressants. Heart rate was 3.7 bpm lower on ECGs recorded during citalopram than on ECGs recorded during non-use of antidepressants. Besides escitalopram, which was only prescribed during 5 ECGs, the upper limit of the 90% confidence interval was not higher than 10 ms for the other studied SSRIs (Table 2).

Table 2 Association between SSRIs and cross-sectional assessments of the heart-rate corrected QT (QTcF) interval

	N	PDD / DDD *	DDD in mg†	QTcF		QT		Heart rate	
				ß in ms	90% CI	ß in ms	90% CI	ß in bpm	90% CI
Non-user	26,184			-	Reference	-	reference	-	reference
Any SSRI	436			2.9	1.3 – 4.5	6.2	4.0 – 8.4	-1.7	-2.5 – -0.9
Fluoxetine	39	1.1	20	4.5	-0.4 – 9.3	9.4	2.7 – 16.2	-2.6	-5.2 – 0.0
Citalopram	35	1.1	20	12.8	7.3 – 18.2	19.5	11.9 – 27.1	-3.7	-6.5 – -0.9
Paroxetine	263	1.0	20	1.7	-0.4 – 3.7	3.5	0.6 – 6.4	-0.9	-1.9 – 0.2
Sertraline	42	1.2	50	1.7	-3.4 – 6.9	9.2	2.0 – 16.4	-4.1	-6.7 – -1.5
Fluvoxamine	52	1.8	100	1.7	-2.9 – 6.3	5.7	-0.7 – 12.1	-1.8	-4.2 – 0.5
Escitalopram	5	1.0	10	2.7	-11.6 – 16.9	2.5	-17.4 – 22.3	-0.3	-7.3 – 6.7

Abbreviations: ß, mean difference in QTc (corrected with Fridericia's formula; in milliseconds), QT (uncorrected; in milliseconds) or heart rate (in beats per minute) between SSRI users and non-users (reference group); bpm, beats per minute; CI, confidence interval; DDD, defined daily dose; ms, milliseconds; N, number of ECG measurements; PDD / DDD, ratio of the prescribed daily dosage and the defined daily dosage; SSRIs, Selective Serotonin Reuptake Inhibitors. * The mean prescribed daily dosage with respect to the defined daily dosage. Analyses were adjusted for age and sex. Results presented as the difference with respect to non-users of antidepressants. †Data obtained from the World Health Organization[28]

All results remained similar after exclusion of ECGs with cardiac pathologies and additional adjustment for co-variables (e.g., BMI and definite QTc-prolonging drugs). A total of 14 ECGs was recorded during use of citalopram with a maximum daily dosage of 20 mg, and in participants aged 60 years and older. In this subsample, QTcF was 15.5 ms longer (90% CI: 6.8 – 24.1) in citalopram users than in non-users of antidepressants.

Longitudinal analyses

Table 3 shows the changes in QTcF and heart rate associated with different pairs of consecutive ECG recordings. In total, 13,225 pairs of ECGs were available for analyses with a median time interval between two consecutive visits of 4.3 years (interquartile range: 2.4 – 5.0).

ECG pairs with SSRI use at the second recording had no longer QTcF when compared with ECG pairs with non-use during the second recording. The results were similar for both definitions of exposure (beta estimates are 1.6 and 1.1 ms, respectively for the sample irrespective of previous exposure and for the sample with non-use during the previous visit).

Of the individual SSRIs, citalopram use at the second recording of an ECG pair was associated with a 21.5 ms (90% CI: 12.1 – 30.8) longer QTcF than ECG pairs during non-use at the second recording irrespective of antidepressant use during the first round of an ECG pair. The difference in QTcF was somewhat more explicit when no antidepressant use was allowed during the first visit of an ECG pair (28.9 ms; 90% CI: 15.3 – 42.5). No prolonged QTcF was observed for the other SSRIs. For these SSRIs, the upper limit of the 90% confidence intervals were not above 6 ms for both definitions of exposure during the first visit of an ECG pair.

Results remained similar after additional adjustment for the covariables (e.g., BMI and definite QTc-prolonging drugs) and exclusion of ECGs with cardiac pathologies. In participants aged 60 years and older, use of citalopram during the second ECG recording with a maximum daily dosage of 20 mg was associated with a 22.2 ms longer QTcF (90% CI: 11.4 – 33.0). Due to a limited number of users, this analysis was not conducted when no antidepressant use was allowed during the first visit of an ECG pair.

Table 3 **Changes in heart-rate corrected QT (QTcF) interval stratified by exposure changes between two consecutive electrocardiogram (ECG) measurements**

	Exposure definition 1			Exposure definition 2		
	N	change in ms	90% CI	N	change in ms	90% CI
Non-user	13,036	-	reference	12,881	-	Reference
Any SSRI	189	1.6	-0.3 – 3.5	114	1.1	-1.6 – 3.8
Fluoxetine	14	-5.9	-12.9 – 1.2	11	-3.9	-12.6 – 4.9
Citalopram	10	21.5	12.1 – 30.8	5	28.9	15.3 – 42.5
Paroxetine	120	2.2	-0.1 – 4.6	69	1.9	-1.7 – 5.6
Sertraline	19	-4.4	-10.4 – 1.7	12	-5.8	-14.5 – 2.9
Fluvoxamine	24	0.1	-5.2 – 5.4	12	-10.8	-19.3 – -2.3

Abbreviations: ß, mean difference in QTc (corrected with Fridericia's formula; in milliseconds) and heart rate (in beats per minute) between SSRI users and non-users (reference group); bpm, beats per minute; CI, confidence interval; ms, milliseconds; N, number of ECG pairs; SSRIs, Selective Serotonin Reuptake Inhibitors. Escitalopram could not be studied because of a too low number of ECGs at which participants were using. None-users at both ECG assessments were used as the reference. All analyses were adjusted for age and sex. Results are presented as the difference in change with respect to paired ECGs of non-use. Exposure definition 1 refers to the use of (individual) SSRIs at the subsequent visit irrespective of use at the previous visit. Exposure definition 2 refers to the use of (individual) SSRIs at the subsequent visit when no antidepressants were used at the previous visit.

Discussion

In our study population of middle-aged and elderly participants, QTcF during use of SSRIs as a class was not different from the QTc during non-use of antidepressants. In the analyses where individual SSRIs were studied separately, only use of citalopram was consistently associated with a longer QTc in the cross-sectional and longitudinal analysis. This result was similarly observed in a subsample of participants aged 60 years and older who were prescribed citalopram with a maximum daily dose of 20 mg.

The association between use of SSRIs as one exposure class and QTc has been studied in our study population before, but with a smaller sample size[21]. Nevertheless, the observed difference between ECGs recorded during use of SSRIs and during non-use of antidepressants in the present study population was considered too small to be of any clinical importance by regulatory authorities[41]. Furthermore, the upper limits of the confidence intervals were not above 5 ms. Therefore, our study

adds to the evidence that SSRIs as a class, including drugs that share a similar pharmacological target, do not or at best minimally prolong the QTcF.

For the individual SSRIs, this study is in line with a large cross-sectional study that used electronic health care records[17], but is at variance with other, often smaller, studies[18, 42]. Except for fluvoxamine, all other SSRIs have been listed to have QTc-prolonging properties[15, 16], and have been observed to have the potential to block the HERG channel[11-14]. This discrepancy might be explained by variation in patient populations or by a too low number of users of specific SSRIs in some of these studies, including ours. However, except for escitalopram which was only prescribed during 5 ECG recordings, all other SSRIs had an upper limit of the confidence interval below 10 ms. For paroxetine, the SSRI with the largest number of users, the upper limit of the confidence interval was below 5 ms, which means that there is a 90% probablility that paroxetine has no QTc-prolonging properties[41]. For the other SSRIs, this means that there is at most moderate QTcF prolongation, which is considered as minimally clinically relevant[41]. Thus, despite the fact that most SSRI have been observed to block the hERG channel[11-14], in our naturalistic setting, no QTcF prolongation was observed for most SSRIs that was of clinical importance. Nevertheless, we cannot rule out that SSRIs like fluoxetine and sertraline have some QTcF increasing effects since the number of users was limited and the upper limited of the 90% confidence interval in the cross-sectional analysis was above 5 ms.

In August 2011, the FDA issued a warning concerning citalopram. The FDA recommended a maximum citalopram dosage of 40 mg per day in normal patients and 20 mg in high-risk patients (e.g., >60 years of age)[19, 20]. In the present study, we additionally restricted analyses to ECGs recorded in participants aged 60 years and older to assess the association between a citalopram-dosing regimen of maximum 20 mg per day and QTcF. In the cross-sectional analysis, we observed a 15.5 ms longer QTcF on ECGs during citalopram use than on ECGs during no use of antidepressants. In the longitudinal analysis an approximately 20 ms longer QTcF was observed in participants aged 60 years and older with a citalopram dosage equal or less than 20 mg per day. As also the lower limit of the confidence interval was above 10 ms, this result may suggest that there is a considerably increased risk of arrhythmias[41]. However, in contrast to what our data suggest, the clinical importance of the restrictions for citalopram are under debate, as patients treated with daily dose of citalopram of at least 40 mg have not been associated with an increased risk of sudden cardiac death or cardiac mortality[43, 44].

Our study has a number of strengths. First, we were able to study the within-person change in QTcF dependent on the use of individual SSRIs because for most participants, more than one ECG was available. Although the longitudinal analysis is still observational, it can provide additional arguments in favor of a causal

relationship. As similar results were observed in the cross-sectional and longitudinal analysis with respect to the effect of citalopram use on QTcF measures, the observed effects seems to be caused by the drug instead of by other factors. Second, pharmacy dispensing data were registered prospectively and irrespective of disease status, which minimizes the risk of information and/or recall bias. And finally, all ECGs were processed using the MEANS program. MEANS calculates the QT interval and heart rate automatically, which reduces intra- and inter-observer variability in assessment of the QTcF[30-32]. This study also has some limitations. First, some of the individual SSRI drugs were dispensed in low numbers. Therefore, it was not possible to conduct dose-response analyses. Nevertheless, numbers were sufficient to observe that citalopram gave a clinically relevant prolongation in QTcF, and that paroxetine is most likely not associated with any clinically relevant prolongation in QTcF. Second, the median time period between two consecutive ECG recordings was 4.3 years, which is long for the calculation of the change in QTcF interval. Therefore, a possible acute effect of (individual) SSRIs on QTcF cannot be ruled out. Third, due to the non-randomized nature of the data, the analyses are subject to confounding factors. We examined several possible confounders and adjusted or restricted our analyses to eliminate potential confounding as much as possible, and this did not influence the results.

In conclusion, this study showed that, while no SSRI class effect on QTcF was observed, the use of citalopram was associated with a longer QTcF while paroxetine was not associated with a longer QTcF. Even with the recommended dosage restriction in participants aged 60 years and older, still a considerable longer QTcF interval was observed in citalopram users.

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CHAPTER 4.3

ANTIDEPRESSANTS AND HEART-RATE VARIABILITY IN A GENERAL MIDDLE-AGED AND ELDERLY POPULATION: THE ROTTERDAM STUDY

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Background: Tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) may be associated with lower heart rate variability (HRV), a condition associated with increased mortality risk. We aimed to investigate the association between TCAs, SSRIs and HRV in a population-based study.

Methods: In the prospective Rotterdam Study cohort, up to five ECGs per participant were recorded (1991 – 2012). Two HRV variables were studied: standard deviation of normal-to-normal RR intervals (SDNN) and root mean square of successive RR-interval differences (RMSSD). We compared the HRV on ECGs recorded during use of antidepressants with the HRV on ECGs recorded during non-use of any antidepressant. Additionally, we analyzed the change in HRV on consecutive ECGs. Those who started or stopped using antidepressants before the second ECG were compared with non-users on two ECGs.

Results: We included 23,647 ECGs from 11,729 participants (59% women, mean age 64.6 years at baseline). Compared with ECGs recorded during non-use of antidepressants (22,971 ECGs), SDNN and RMSSD were lower in ECGs recorded during use of TCAs (296 ECGs) and SSRIs (380 ECGs). Participants who started using TCAs before the second ECG had a lower HRV, and those who stopped had a higher HRV than consistent non-users (p-value < 0.001) at the time of the second ECG recording. Starting or stopping SSRIs was not associated with HRV changes.

Conclusion: TCAs were associated with a lower HRV in all analyses, indicating a real drug effect. For SSRIs the results are mixed, indicating a weaker association, possibly due to other factors.

Introduction

Heart-rate variability (HRV) refers to the beat-to-beat variability in heart rate. HRV is influenced by the parasympathetic and sympathetic autonomous nervous system. HRV is lowered when parasympathetic nerve activity is decreased or when sympathetic nerve activity is increased[1-3]. A relatively low HRV is associated with an increased risk of all-cause mortality[4-9], cardiac mortality[8, 10], and sudden cardiac death[11].

Use of antidepressants has been associated with a lower HRV in a number of studies[12-18]. Two population-based studies on the relation between antidepressants and HRV[12, 16] reported that use of tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs) is associated with a lower HRV. In both studies, the effect of the TCAs on HRV was more pronounced than that of SSRIs[12, 16]. This is possibly caused by the anticholinergic effects of TCAs[19]. A meta-analysis reported that depression itself – and not antidepressant use – is associated with a lower HRV[17], while the association between depression and HRV was found to be predominantly driven by the use of antidepressants in a different and larger study[16].

To date, only one population-based cohort study has addressed the association between TCAs, SSRIs and HRV[12]. However, this study neither investigated longitudinal effects of antidepressant use on HRV nor a possible dose-response relationship of antidepressants on HRV. These analyses can add to the evidence for a drug effect instead of an effect observed due to residual confounding. In addition, no studies to date have examined individual antidepressants with regard to their association with HRV, as only class effects were considered. Therefore, in order to address these issues, our objective was to investigate the association between TCA use, SSRI use, and HRV in a general middle-aged and elderly population.

Methods

Study setting

This study is part of the Rotterdam Study, a prospective population-based cohort study. The design and rationale of the Rotterdam Study have been described in more detail elsewhere[20, 21]. In short, from 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district in Rotterdam, the Netherlands, were invited to participate in the initial cohort. A total of 7,983 individuals agreed to participate (response rate 78%). In 2000, the cohort was extended by including all inhabitants from the same district who became 55 years or older, or who had moved into the district after the start of the initial cohort. In this extension of the

cohort, 3,011 individuals agreed to participate (response rate 67%). The cohort was additionally extended in 2006 by inviting inhabitants of the same district aged 45 years and older. In total, 3,932 individuals agreed to participate (response rate 65%). Follow-up examinations were conducted approximately every four to five years after baseline examination, with a maximum of five center visits per participant. The Rotterdam Study has been approved by the medical ethics committee according to the Wet Bevolkingsonderzoek ERGO (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sport of the Netherlands. Written informed consent was obtained from all study participants.

Study population and selection of ECGs

ECGs of participants of the Rotterdam Study recorded between January 1st, 1991 and December 31st, 2012 were included. ECGs recorded before January 1st, 1991 were excluded because pharmacy dispensing records were not available. We also excluded ECGs with less than five normal heartbeats and ECGs on which the following pathology was detected: left or right bundle branch block, second degree or third degree atrioventricular block, ventricular hypertrophy according to Sokolow-Lyon criteria, atrial fibrillation. ECGs recorded with a pacemaker rhythm or recorded during the use of monoamine-oxidase inhibitors (ATC code: N06AF/AG) and other antidepressants (ATC code: N06AX) were excluded, because of the low number of prescriptions and heterogeneous pharmacodynamics.

Antidepressant exposure assessment

At study entry, more than 95% of the participants had their drug prescriptions filled at one of the seven fully computerized regional pharmacies, which use one common computer network. Dispensing data was available on a day-to-day basis, which included the anatomical therapeutic chemical (ATC) code, the dispensing date, the total amount of tablets/capsules per dispensing, the prescribed daily number of tablets/capsules, and the product name of the drug. Dispensing episodes were calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. If the date of an ECG recording fell within a dispensing episode of TCAs (ATC code: N06AA) or SSRIs (ATC code: N06AB), the participant was considered as being exposed during that ECG recording. We allowed a carry-over period of 7 days to define current users. For individual antidepressants, the complete, 7-digit ATC code was used. The dosage was defined as the ratio between the prescribed daily dosage by the defined daily dosage (PDD/DDD ratio), as determined by the World Health Organization[22].

Heart-rate variability

A standard 10-second, 12-lead resting ECG was recorded with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Florance, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the standardized modular ECG analysis system (MEANS), which has been described previously and has been validated and applied extensively[23-27].

HRV was calculated based on RR intervals between normal heart beats: RR intervals were excluded if they immediately preceded or followed premature ventricular complexes or premature supraventricular complexes[28]. We selected two of the most commonly used HRV variables; the standard deviation of normal-to-normal RR intervals (SDNN) and the root mean square of successive RR-interval differences (RMSSD)[29, 30].

Depression score

A Dutch version of the Center for Epidemiological Studies Depression (CES-D) scale was used to screen for depressive symptoms. The outcome of this questionnaire is a score ranging between 0 and 60. A higher score indicates more depressed feelings[31, 32]. The CES-D questionnaire was taken at visits from 1993 onwards. Therefore, we adjusted for CES-D score in a subsample analysis, using only those persons and ECGs for which a CES-D score is available.

Covariables

The following covariables were considered: age, sex, smoking status, body-mass index (BMI), RR interval, hypertension, prevalent coronary heart disease, prevalent diabetes mellitus, heart failure, use of beta-blockers, use of verapamil and use of diltiazem. All covariables were determined at the date of ECG recording. Smoking status (current smoker or non-smoker) was determined by home interview. BMI was calculated by dividing weight by the squared height, with weight in kilograms and height in meters, which were both measured at the study center. Heart rate was taken into account in the form of the RR interval as recorded on the ECG (heart rate equals 60,000 / RR interval), because both HRV measures are also based on the RR interval. Blood pressure was measured twice in sitting position at the upper right arm. The average of these measurements was used. Hypertension was defined as a systolic blood pressure of 140 mmHg or higher, a diastolic blood pressure of 90 mmHg or higher, or the use of blood pressure lowering medication for the indication hypertension. Coronary heart disease was defined as a prevalent myocardial infarction or as a history of coronary artery bypass graft surgery (CABG) or percutaneous coronary intervention (PCI). Myocardial infarction was adjudicated based on a combination of symptoms, ECG measurements, and enzyme markers

indicative for the presence of myocardial infarction[33]. Diabetes mellitus was defined by the use of blood-glucose lowering drugs, a non-fasting glucose level of more than 11.0 mmol/L, or a fasting glucose level of more than 6.9 mmol/L[34]. The diagnosis of heart failure was based on typical signs and symptoms confirmed by objective cardiac dysfunction[33]. Use of heart-rate affecting drugs (beta-blockers, verapamil, and diltiazem) at the date of ECG recording was based on pharmacy dispensing records.

Statistical analyses

Baseline characteristics were assessed at the first eligible ECG recording for each participant. We log-transformed the HRV measures for all analyses, but results are presented back-transformed to the geometric scale.

We used linear mixed models to take into account the within-person correlation between multiple visits. We fitted models with different covariance matrices and selected the model that had the lowest Akaike's information criterion (AIC). All statistical models were adjusted for age, sex, RR interval, and heart-rate affecting drugs (beta-blockers, verapamil, and diltiazem). In a second model, we included all other available covariables.

In the first set of analyses, we compared the HRV recorded during TCA use and SSRI use with the HRV recorded during non-use of antidepressants. We repeated these analyses for the individual antidepressants if more than 10 exposed ECGs were available. For paroxetine and amitriptyline, the most frequently prescribed drugs in the Rotterdam Study cohort[35], we additionally analyzed a possible dose-response relationship.

In a subsample analysis, we adjusted all previously mentioned analyses for the CES-D score, in the subgroup for which CES-D scores were available. With this adjustment, we aimed to determine if the association between the antidepressants and HRV is mediated by depressive symptoms.

In the second set of analyses, we compared the change in HRV of those who started or stopped using TCAs or SSRIs by the time of the second visits with the HRV of non-users on two consecutive visits. Because the HRV variables were log-transformed and because $log(a) - log(b) = log(a/b)$, the difference between two log-transformed measurements is presented as the fold-change in HRV of the back-transformed measurement.

We used IBM SPSS Statistics version 21.0 (IBM Corp., Somers, NY, USA) for all analyses. Two-sided p-values below 0.05 were considered statistically significant.

Results

Study characteristics

In total, 27,833 ECGs were recorded. We excluded 3,004 ECGs (10.8%) because cardiac pathologies had been detected. An additional 1,072 ECGs (3.9%) were excluded because they had less than five normal heartbeats. 110 ECGs were excluded because they were recorded during use of antidepressants other than TCAs and SSRIs. In the final selection, 23,647 ECGs from 11,729 participants were used. Baseline characteristics of the final study population are shown in Table 1. In short, 59 percent of the total study population was woman, and the mean age was 64.6 years with a standard deviation (SD) of 9.5 years.

Table 1 Baseline characteristics of the study population

Characteristic	Total study population N = 11,729
Number of ECGs	23,647
ECGs per participant, median (range)	2 (1 – 5)
Time between ECG recordings in years, median (IQR)	4.1 (2.4 – 5.0)
Age in years, mean (SD)	64.6 (9.5)
Women, N (%)	6,896 (58.8)
Body-mass index in kg/m^2, mean (SD)	27.0 (4.1)
CES-D score, median (IQR)	3 (1 – 8)
RR interval in milliseconds (SD)	881 (141)
Heart rate in beats per minute, mean (SD)	70 (11)
Beta-blocker use, N (%)	1,470 (12.5)
Verapamil use, N (%)	67 (0.6)
Diltiazem use, N (%)	134 (1.1)
Current smoking, N (%)	2,458 (21.0)
Coronary heart disease, N (%)	677 (5.8)
Heart failure, N (%)	179 (1.5)
Hypertension, N (%)	6,060 (51.7)
Diabetes mellitus, N (%)	845 (7.2)

Abbreviations: CES-D, Center of Epidemiological Studies Depression Scale; ECG, electrocardiogram; IQR, interquartile range; SD standard deviation; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR intervals.

Antidepressant use and HRV

The lowest Akaike's information criterion was achieved with the 'first-order autoregressive covariance structure with heterogeneous variances'. This covariance structure was therefore used in all analyses. Table 2 presents the geometric estimated means of SDNN and RMSSD for ECGs made during non-use of antidepressants and during use of TCAs and use of SSRIs. Of all 23,647 included ECGs, 296 were recorded during TCA use and 380 ECGs were recorded during SSRI use.

Table 2 Heart-rate variability recorded during non-use and use of antidepressants

			SDNN (ms)	RMSSD (ms)
	ECGs	Median PDD / DDD	$Mean_{adj}$ in ms (95% CI)	$Mean_{adj}$ in ms (95%CI)
Non-use of antidepressants (ref)	22,971	NA	18.3 (17.0 – 19.7)	19.8 (18.4 – 21.4)
TCA use	296	0.33	15.6 (14.0 – 17.4) ‡	16.7 (15.0 – 18.7) ‡
– Imipramine	16	0.50	15.9 (11.3 – 22.5)	19.3 (13.6 – 27.4)
– Clomipramine	27	0.50	10.9 (8.3 – 14.4) ‡	11.4 (8.6 – 15.0) ‡
– Amitriptyline	185	0.33	16.3 (14.4 – 18.5)	17.3 (15.2 – 19.7) *
– Nortriptyline	13	0.67	10.3 (6.8 – 15.5) †	11.3 (7.4 – 17.2) †
– Maprotiline	48	0.50	15.5 (12.5 – 19.3)	17.0 (13.6 – 21.1)
SSRI use	380	1.00	15.4 (14.0 – 17.0) ‡	17.1 (15.4 – 19.0) ‡
– Fluoxetine	35	1.00	12.5 (9.8 – 15.8) †	14.1 (11.1 – 18.0) †
– Citalopram	28	1.00	16.8 (13.0 – 21.8)	18.0 (13.9 – 23.3)
– Paroxetine	237	1.00	15.2 (13.5 – 17.0) ‡	16.9 (15.0 – 19.0) †
– Sertraline	37	1.00	15.8 (12.4 – 20.0)	16.8 (13.2 – 21.5)
– Fluvoxamine	39	1.00	17.6 (14.0 – 22.2)	19.6 (15.5 – 24.7)

Abbreviations: ref, reference; CI, confidence interval; DDD, defined daily dosage; ECGs, number of electrocardiograms; $Mean_{adj}$: geometric mean adjusted for covariables; ms: milliseconds; NA, not applicable; PDD, prescribed daily dosage; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR-interval; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant. Only individual antidepressants prescribed during more than 10 ECG recordings were analyzed. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem.
* p-value <0.05; † p-value < 0.01; ‡ p-value < 0.001 compared with non-use of antidepressants.

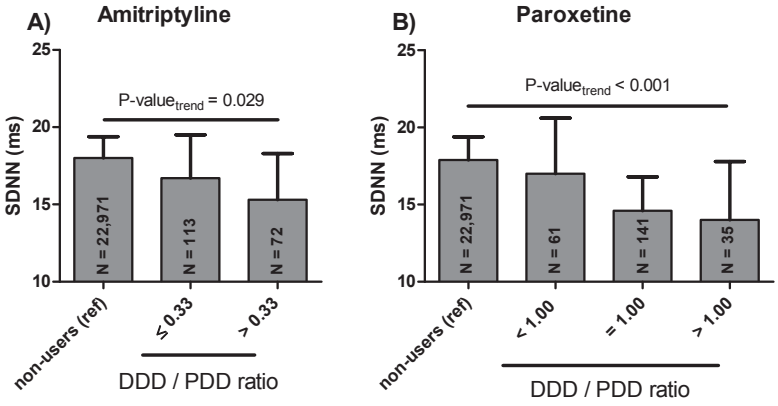

Figure 1 **Association of amitriptyline and paroxetine dosages with heart-rate variability**

Abbreviations: DDD, defined daily dosage; ms, milliseconds; PDD, prescribed daily dosage; SDNN, standard deviation of normal-to-normal RR intervals. The bars represent the mean SDNN, the whiskers are the upper limit of the 95% confidence interval. P-value$_{trend}$ indicates the level of statistical significance across the strata. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem.

Compared with ECGs recorded during non-use of antidepressants, ECGs recorded during TCA use had a significant (p-values < 0.05) lower SDNN (18.3 ms for non-use, and 15.6 ms for TCAs) and RMSSD (19.8 ms for non-use and 16.7 ms for TCAs). ECGs recorded during SSRI use also showed a significantly lower SDNN (18.3 ms for non-use and 15.4 ms for SSRI use) and a lower RMSSD (19.8 ms for non-use and 17.1 ms for SSRI use) than ECGs recorded during non-use. Of the individual antidepressants, ECGs recorded during use of clomipramine (27 ECGs), amitriptyline (185 ECGs), nortriptyline (13 ECGs), fluoxetine (35 ECGs) and paroxetine (237 ECGs) showed significantly lower HRV measures than ECGs recorded during non-use of antidepressants. A dose-response relationship was analyzed for amitriptyline and paroxetine, which were the most frequently prescribed antidepressants. A higher prescribed dosage of both amitriptyline (Figure 1A) and paroxetine (Figure 1B) was associated with a statistically significant trend toward a lower SDNN. Results were similar for RMSSD (results not shown).

Similar results were observed when adjusted for all considered covariables in this study (results not shown).

Analyses for possible mediation by depressive symptoms

A total of 14,693 ECGs from 9,194 participants were recorded during visit rounds when information of depressive symptoms was available. Of those ECGs, 180 were recorded during use of TCAs and 318 ECGSs were recorded during use of SSRIs. In this subgroup, the associations between TCAs, SSRIs and the HRV measures were similar as observed in the total cohort. Additional adjustment for CES-D score did not materially change these results (Supplementary Tables 1 and 2).

Longitudinal analysis of antidepressant use and HRV

Table 3 shows the ECGs made on two consecutive visits, and the estimated mean fold-change in SDNN and RMSSD between two visits. The results show that HRV is reduced when TCA use is started, and that HRV is increased when TCA use is stopped. For SSRIs the same pattern is observed, but with a smaller effect size and no statistically significant difference with consistent non-users.

Table 3 Antidepressant use and fold-change in heart-rate variability between two consecutive visits

			$Mean_{adj}$ fold-change (95%CI)	
Use on first visit	Use on second visit	ECGs	SDNN	RMSSD
None	None	11,103	1.06 (0.96 – 1.18)	1.08 (0.97 – 1.20)
None	TCA	82	0.76 (0.62 – 0.94) †	0.75 (0.61 – 0.93) †
None	SSRI	94	0.90 (0.74 – 1.10)	0.98 (0.80 – 1.20)
TCA	None	65	1.49 (1.18 – 1.87) †	1.64 (130 – 2.08) †
SSRI	None	62	1.10 (0.87 – 1.39)	1.10 (0.87 – 1.40)

Abbreviations: CI: confidence interval; ECGs : number of electrocardiograms; $Mean_{adj}$: geometric mean adjusted for covariables; RMSSD, root mean square of successive differences; SDNN, standard deviation of normal-to-normal RR intervals; SSRI; selective serotonin reuptake inhibitor; TCA: tricyclic antidepressant. Analyses were adjusted for age, sex, RR interval, use of beta-blockers, verapamil and diltiazem. † p-value < 0.001 compared with non-use of antidepressants during both ECGs.

Discussion

In this population-based study in older adults, use of TCAs and SSRIs was associated with a lower HRV. In addition, the individual antidepressants clomipramine, amitriptyline, nortriptyline, fluoxetine and paroxetine were significantly associated with a lower HRV and a dose-response relationship of amitriptyline and paroxetine with HRV was observed. In the longitudinal analysis, the start of TCA use, and not SSRI use, was associated with significant changes in HRV. Additional adjustment for CES-D score did not materially change the results.

To our knowledge, only one study to date has analyzed the effect of antidepressant use on HRV in the general older population[12]. This study reported a lower HRV in users of TCAs and SSRIs, which is in line with the results of our study. However, we additionally investigated the effect of individual antidepressants on HRV: all previous studies only considered antidepressant classes. We observed a lower HRV for all individual TCAs, but the difference with non-use of antidepressants was largest for clomipramine and nortriptyline. Imipramine and maprotiline showed much smaller differences in HRV compared with non-use, while the prescribed daily dosages were similar. For amitriptyline, the smaller effect on HRV can be explained by the fact that it was prescribed at a lower median daily dosage than the other TCAs. This is supported by the fact that we found a significant trend towards a lower HRV with higher amitriptyline dosages. For individual SSRIs, the results were difficult to interpret. Users of fluoxetine had a notably lower HRV than users of the other SSRIs while the median daily dosage of all individual SSRIs was the same. Although HRV was somewhat lower during the use of all individual SSRIs, the difference for these individual SSRIs was not statistically significant, which could be due to a low number of users. Therefore, we were not able to exclude the possibility that HRV is dependent on the use of only certain SSRIs. Nevertheless, we observed a significant dose-response relationship for paroxetine, which was not statistically significant in the overall analysis. For this reason, these analyses should be repeated in other studies.

Additional adjustment for depressive score did not materially change the results. This finding contradicts the meta-analysis[17], but is in line with a different and larger cohort study[16]. That cohort study suggested that the association between depression and HRV was predominantly driven by the use of antidepressants.

The previously mentioned population-based study[12] had only one measurement per participant and could not assess longitudinal changes over time. One study did assess longitudinal changes in HRV with respect to starting and stopping of TCA treatment and SSRI treatment[18]. They found that starting TCA treatment or starting SSRI treatment was associated with a reduction in HRV. However, starting

SSRI treatment was associated with less reduction in HRV than starting TCAs[18]. In our study, we observed no significant decrease in HRV after starting SSRIs, and no significant increase after stopping SSRIs. The inconsistent results for SSRIs for the cross-sectional and longitudinal analyses might suggest that other, unexamined, factors than the drug itself lead to a lower HRV, but additional research is required to support this suggestion.

The relatively low HRV in users of TCAs might have clinical consequences for an individual patient, as a relatively low HRV has been associated with an increased risk of all-cause mortality[4-9], cardiac mortality[8, 10], and sudden cardiac death[11]. TCAs have also been associated with an increased risk of cardiac mortality[36]. Whether this increased risk is due to a low HRV should be determined in subsequent studies.

This study has a number of strengths and limitations. Major strengths were the availability of detailed pharmacy-dispensing data and the availability of multiple ECG recordings for most participants. Also, we used MEANS, which calculates the RR interval and heart-rate variability systematically and automatically. This enhances precision of the measurements and prevents bias in ECG assessment[24, 26]. Information bias for drug use was limited as pharmacy-dispensing data was collected prospectively and irrespective of disease state. The analysis using the calculated within-person changes of HRV between two consecutive visit rounds is less subjected to confounding than our cross-sectional analysis and the results are complementary. Finally, we added a variable for depressive symptoms which did not change the results. There are also some limitations to address. First, some of the individual antidepressants were dispensed in low numbers. Second, the median time interval between two ECG recordings was 4.1 years, which limits the interpretation of a change in HRV between visits, as other confounding factors may occur in the long time period. Finally, we used standard 10-second ECG recordings, while the major studies in the literature are based on 5-minute or longer ECGs. However, in two previous studies, HRV derived from 10-second ECGs was still predictive for cardiovascular disease and mortality[9, 37], and the results of the first set of analyses in this paper are in line with that of previous studies[12, 18]. Besides this, 10-second ECGs are cheaper and more patient friendly.

In conclusion, the results indicate that TCAs are associated with a lower HRV in a general middle-aged and elderly population, a condition associated with an increased risk of mortality. For SSRIs this is less clear, although we observed an association between SSRIs and HRV and a dose-response relationship of paroxetine with HRV.

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CHAPTER 5

ANTIDEPRESSANT USE AND RISK OF MYOCARDIAL INFARCTION AND CEREBRAL MICROBLEEDS

CHAPTER 5.1

USE OF ANTIDEPRESSANTS AND THE RISK OF MYOCARDIAL INFARCTION IN MIDDLE-AGED AND OLDER ADULTS: A MATCHED CASE-CONTROL STUDY

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Purpose: Antidepressants, specifically selective serotonin reuptake inhibiting antidepressants (SSRIs), decrease platelet activation and aggregation in *in-vitro* experiments, and could therefore decrease the risk of myocardial infarction (MI). However, prior studies addressing this hypothesis showed contradictory results. Our purpose was to investigate the association between use of any antidepressant drug and incident MI among middle-aged and older adults.

Methods: We embedded a case-control study in the prospective Rotterdam Study (1991 – 2011). Controls were matched to MI cases based on sex and age at the same calendar date. The relative risk of MI during current use of an antidepressant was analyzed with conditional logistic regression with never-use of antidepressant drugs as the reference category.

Results: A total of 744 out of a cohort of 9,499 study participants developed MI during follow-up. After statistical adjustment for traditional cardiovascular risk factors, current use of any antidepressant was associated with a lower risk of MI (OR: 0.71; 95% CI: 0.51 – 0.98) compared to never-use of any antidepressant. SSRI use showed the lowest relative risk (OR: 0.65; 95% CI: 0.41 – 1.02), albeit marginally not statistically significant.

Conclusions: Current use of antidepressants was associated with a lower risk of MI. Of the different classes, use of SSRIs showed to the lowest risk of MI.

Introduction

Findings from *in-vitro* experiments have led to the hypothesis that the use of antidepressant drugs, and specifically use of selective serotonin reuptake inhibitors (SSRIs), might decrease the risk of myocardial infarction (MI). The inhibition of the serotonin reuptake transporter on blood platelets by SSRIs decreases platelet activation and aggregation in *in-vitro* experiments[1, 2]. Similarly, antidepressants other than SSRIs might decrease platelet activation and aggregation by antagonizing the serotonin receptor and/or inhibiting also serotonin reuptake, although with lower affinity than SSRIs[3, 4].

Studies addressing the hypothesis of a lower risk of MI during use of SSRIs showed inconsistent results. Some studies reported a statistically significantly lower risk of MI in users of SSRIs[5, 6], while others showed no decreased risk[7-10], or an increased risk[11, 12]. Depression is associated with an increased risk of MI, and may therefore complicate the interpretation of the study results[13-18]. Only one randomized clinical trial comparing cardiovascular event rates in antidepressants and placebo has been published[9]. In that study a 30 percent lower but non-significant risk of MI was observed in users of sertraline compared with users of placebo treatment. TCAs, which decrease platelet activation and aggregation to some extent[3, 4], were associated with higher risk of MI[10, 11].

Thus, despite evidence of decreased blood platelet aggregation in *in-vitro* experiments with antidepressants, more studies are required to shed light on this association. Studies using time-varying exposure of antidepressants as well as controlling for depression might provide further insights. Within this study, we aimed to investigate the association between use of any antidepressant and risk of MI in the general middle-aged and older population.

Methods

Study setting

The current study was conducted in the prospective population-based Rotterdam Study, designed to investigate risk factors for age-related diseases. From 1990 to 1993, all inhabitants aged 55 years and older from the Ommoord district located in Rotterdam, the Netherlands, were invited to participate in RS-I. In total, 7,983 individuals agreed to participate (response rate 78%). In 2000, all inhabitants of Ommoord aged 55 years and older were asked to participate in an extension of the original cohort when they were not previously invited. In total, 3,011 individuals agreed to participate (response rate 67%). Follow-up examinations were conducted

every 3-4 years after baseline. Both cohorts are continuously monitored for the occurrence of major morbidity and mortality through linkage with the records from the general practitioner. A more detailed description of the Rotterdam Study is published elsewhere[19, 20]. The Rotterdam Study has been approved by the medical ethics committee according to the "Wet Bevolkingsonderzoek: ERGO" (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands and written informed consent was obtained from all study participants.

Study population and design

For the present study, we included all participants from the Rotterdam Study cohorts free of MI at baseline. Within the prospective cohort, cases of MI were matched to all eligible participants in the cohort without MI with the same sex, and a similar age (±1 year) at the event date. For every matched set, the exposure status to antidepressants and covariables in each case and its corresponding controls was assessed on the event date as described below. Cases were censored at the event date, whereas controls were allowed to develop MI at a later date during the course of follow-up.

Antidepressant drug exposure

More than 95% of the participants have their drug prescriptions filled at one of the seven regional pharmacies. From January 1st, 1991 onwards, complete dispensing data is available on a day-to-day basis, which includes the Anatomical Therapeutical Chemical (ATC) code of the drug[21], the dispensing date, the total number of drug units per prescription, the prescribed daily number of units, and the product name of the drug.

A dispensing episode was calculated by dividing the total number of filled tablets/capsules by the daily prescribed number. Antidepressant use (based on ATC code: N06A) was additionally subdivided into TCAs (ATC code = N06AA), SSRIs (ATC code = N06AB) and other antidepressants (ATC code = N06AX). Participants were current antidepressant users if the event date fell within a dispensing episode. Participants were considered past users, if they previously filled an antidepressant, TCA or SSRI dispensing, but were not current users. Participants who did not use any antidepressant drug during the study period were defined as never-users.

Validation of the study outcomes

MI was adjudicated based on a combination of symptoms, ECG measurements, and enzyme markers indicative of the presence of MI, as described in more detail elsewhere [22]. All potential MI cases were independently adjudicated by two research

physicians. A medical specialist, whose judgment was considered final, reviewed all potential cases.

All-cause mortality was based on information from the Central Register of Population of the municipality of Rotterdam and collaborating general practitioners.

Covariables

The following covariables were considered as potential confounders: body mass index (BMI), systolic and diastolic blood pressure, highest reached level of education, High Density Lipoprotein (HDL) and total cholesterol levels, treated diabetes mellitus, smoking status, history of heart failure, history of venous thromboembolism, presence of depression and/or anxiety, use of blood-pressure lowering drugs, beta-blockers, cholesterol lowering agents, anxiolytics, hypnotics and antipsychotics. For covariables that were assessed during the examination rounds (e.g., BMI and blood pressure), the assessment closest prior to the index date was considered. BMI was calculated by dividing the weight (in kg) by the height (in meters squared). Blood pressure was measured twice, in a sitting position at the upper right arm. The average blood pressure was used in the analyses. Four categories of education were defined (basic = primary education, low = lower vocational, lower and intermediate general, medium = intermediate vocational, higher general, high = higher vocational and university), similar to the UNESCO classification and has previously been described for the Rotterdam Study[23, 24]. HDL and total cholesterol were measured in serum using the CHOD-PAP method (Monotest Cholesterol kit, Boehringer Mannheim Diagnostica) and an automated enzymatic procedure (Hitachi analyzer, Roche Diagnostics, Washington DC), depending on the examination round. Because of the two methods, HDL and total cholesterol transformed in round-specific Z-scores. Treated diabetes mellitus was defined as the use of glucose lowering agents (ATC code: A10). The following drugs were considered based on pharmacy records: antithrombotic agents, statins, the number of concomitantly dispensed blood-pressure lowering drugs, antipsychotics, anxiolytics and hypnotics. Current smoking was assessed during the interviews at every examination round. Heart failure was assessed based on typical signs and symptoms confirmed by objective cardiac dysfunction[22]. History of venous thromboembolism was defined by diagnoses (ICPC codes: K93 and K94) and notes made by a general practitioner or medical specialist. A specialist was consulted if two independent research physicians did not meet consensus on a potential case of venous thromboembolism. The diagnoses of depression and anxiety were defined on the basis of patient records of the general practitioner, which is described in more detail elsewhere[25]. In short, possible cases of depression and anxiety were scanned from the general practitioner's records. Available information included information

of observations by the general practitioner, as well as diagnoses made by the general practitioner and psychiatrist. Two independent research associates validated all potential cases. A psychiatrist was consulted when no consensus was reached on a potential case.

Statistical analyses

The association between current antidepressant drug use and incident MI was studied using conditional logistic regression analyses with never-users of antidepressants as the reference population. In addition, we also compared the risk of MI in current users of antidepressants with past users of antidepressants. These analyses were repeated per antidepressant drug class (TCA, SSRI). Because we had small numbers of other antidepressant users, we did not evaluate those separately. We present results for all models unadjusted (model 1) and multivariably adjusted (models 2 and 3). Model 2 was adjusted for all covariables that were considered potential confounding factors. Model 3 is additionally adjusted for covariables that were considered intermediate factor, based on previous research[26-29]. These factors were: BMI, total cholesterol, HDL cholesterol, use of statins and diabetes mellitus. Multiple imputations were used (5 times) to include participants with missing covariables (6.5% was missing maximally).

A number of additional analyses were conducted. First, we assessed the association with the number of concomitantly prescribed cardiovascular drugs (ATC codes: "B01A", "C02", "C03", "C07", "C08", "C09", "C10AA"), and studied the percentage of antidepressant use within these strata. In addition, we restricted our population to cases and controls that used at most one of these cardiovascular drugs, and thus excluding those with the highest cardiovascular risk, and repeated the analyses of the risk of MI. And second, we studied the association between current use of antidepressants and mortality (compared with non-use and past use of antidepressants). The dataset for the analysis on all-cause mortality was similar constructed as for the analysis on MI, as is described above. Results from these analyses provide arguments whether results in the overall analysis were subjected by residual confounding and/or confounding by indication.

We used IBM SPSS Statistics (version 21.0, IBM Corp., Somers, NY, USA) for all analyses.

Results

Characteristics of the study population

A total of 744 MI cases were successfully matched to controls from the total cohort of 9,499 participants (Table 1). Participants who developed an MI during follow-up were, on average, 69.7 (standard deviation [SD]: 8.1) years at baseline, and 44.8% was female. The total cohort had a mean age of 69.4 (SD: 8.7) years at baseline, and 60.9% was female.

Antidepressant use and risk of MI

Of the 744 MI cases, 19 were current users and 93 were past users of antidepressants (Table 2). Compared to never users of antidepressants, current use of any antidepressant was associated with a lower risk of MI (OR: 0.71; 95% CI: 0.51 – 0.98) after adjustment for confounding factors (Model 2). These results remained similar when adjusted for intermediate factors (Model 3). We observed no association between past use of antidepressants and the risk of MI after adjustment for confounding factors (Model 2; OR: 1.17; 95% CI: 0.95 – 1.45).

With past use of antidepressants as a reference group, current antidepressant use was associated with a lower risk of MI (Model 2; OR: 0.57; 95%CI: 0.32 – 0.99), which remained similar when additionally adjusted for intermediate factors (Model 3).

SSRIs, TCAs and risk of MI

Compared with never use of SSRIs, current use of SSRIs was associated with a lower risk of MI, although marginally not statistically significant (OR: 0.65; 95%CI: 0.41 – 1.02) (Table 3). Past use of SSRIs was associated with a higher risk of MI (OR: 1.42; 95%CI: 1.06 – 1.49) compared with never use of SSRIs. A similar point estimate of current SSRI use was observed when compared with past use of SSRIs, although not statistically significant (OR: 0.58; 95% CI: 0.23 – 1.49). These results did not materially differ after additional statistical adjustment for intermediate factors (results not shown).

Compared with never use, as well as with past use of TCAs, current use of TCAs was not associated with a lower risk or MI (Table 3), although the point estimate of current use compared with past use was lower.

Table 1 Baseline characteristics of the study population

	Cases of Myocardial Infarction (N = 744)	Cohort (N = 9,499)
Age in years, mean (SD)	69.7 (8.1)	69.4 (8.7)
Female, N (%)	333 (44.8)	5787 (60.9)
Body mass index in kg/m^2, mean (SD)	26.8 (3.4)	26.7 (3.8)
Current smoking, N (%)	194 (26.1)	1908 (20.1)
Education, N (%)		
Basic	145 (19.5)	1,870 (19.7)
Low	295 (39.5)	4,156 (43,8)
Medium	215 (28.9)	2,465 (26.0)
High	90 (12.1)	1,008 (10.6)
Systolic blood pressure in mmHg, mean (SD)	146 (21)	132 (21)
Diastolic blood pressure in mmHg, mean (SD)	77 (11)	77 (11)
Total cholesterol in mmol/L, mean (SD)	6.7 (1.2)	6.4 (1.2)
HDL cholesterol in mmol/L, mean (SD)	1.2 (0.3)	1.4 (0.4)
History of venous thromboembolism, N (%)	2 (0.3)	13 (0.1)
History of heart failure, N (%)	22 (3.0)	239 (2.5)
Depression, N (%)	4 (0.5)	86 (1.0)
Anxiety, N (%)	2 (0.3)	53 (0.6)
Glucose lowering agents, N (%)	73 (9.8)	476 (5.0)
Antithrombotic agents, N (%)	86 (11.6)	1035 (10.9)
Blood pressure lowering agents, N (%)	172 (23.1)	2098 (22.1)
Beta blockers, N (%)	142 (19.1)	1246 (13.1)
Lipid-lowering agents, N (%)	40 (5.4)	524 (5.5)
Antipsychotics, N (%)	5 (0.7)	87 (0.9)
Anxiolytics, N (%)	31 (4.2)	453 (4.8)
Hypnotics, N (%)	47 (6.3)	540 (5.7)

Abbreviations: N, number of participants. SD, standard deviation; HDL, high-density lipoprotein.

Table 2 Association between antidepressant use and myocardial infarction

	Percentage*	Events	Model 1 OR (95% CI)	Model 2 OR (95% CI)	Model 3 OR (95% CI)
Analysis 1					
Never use	85.0	632	1 (reference)	1 (reference)	1 (reference)
Current anti-depressant use	3.8	19	0.77 (0.56 – 1.06)	0.71 (0.51 – 0.98)	0.71 (0.51 – 0.98)
Past anti-depressant use	11.2	93	1.18 (0.95 – 1.46)	1.17 (0.95 – 1.45)	1.17 (0.94 – 1.44)
Analysis 2					
Past-use	11.2	93	1 (reference)	1 (reference)	1 (reference)
Current anti-depressant use	3.8	19	0.72 (0.42 – 1.23)	0.57 (0.32 – 0.99)	0.56 (0.32 – 0.99)

Abbreviations: 95% CI, 95% confidence interval; OR, Odds Ratio.
Model 1) Matched on age and sex, further unadjusted.
Model 2) Matched on age and sex, and adjusted for: history of deep venous thrombosis, history of heart failure, systolic and diastolic blood pressure, highest obtained level of education, total cholesterol, high-density lipoprotein cholesterol, smoking, blood-pressure lowering agents, antithrombotic agents, antipsychotic agents, anxiolytics, hypnotics, depression and anxiety.
Model 3) Model 2 and additionally adjusted for the intermediate factors: body mass index, HDL cholesterol, total cholesterol, statins and diabetes mellitus.
* As we studied the associations with time-varying exposure analysis, controls contributed more than once in the computations before they were censored or became a case. For this reason, exposure is reported as a percentage.

Table 3 **Association between individual antidepressant drug classes and incident myocardial infarction**

	Use of SSRIs			Use of TCAs		
	Percentage*	Events	OR (95% CI)	Percentage*	Events	OR (95% CI)
Analysis 1						
Nonuse	92.4	681	1 (reference)	90.7	672	1 (reference)
Current use	1.8	8	0.65 (0.41 – 1.02)	1.6	10	0.80 (0.52 – 1.24)
Past use	5.8	55	1.42 (1.06 – 1.90)	7.7	62	1.04 (0.79 – 1.38)
Analysis 2†						
Past use	5.8	50	1 (reference)	7.7	54	1 (reference)
Current use	1.8	7	0.58 (0.23 – 1.49)	1.6	10	0.60 (0.26 – 1.41)

Abbreviations: 95% CI, 95% confidence interval; OR, Odds Ratio; SSRIs, Selective Serotonin Reuptake Inhibitors; TCAs, tricyclic antidepressants. Matched on age, and sex, and adjusted for: History of deep venous thrombosis, history of heart failure, systolic and diastolic blood pressure, maximum level of education, total cholesterol, HDL cholesterol, smoking, blood-pressure lowering agents, antithrombotic agents, antipsychotic agents, anxiolytics, hypnotics, depression and anxiety and current use of the other antidepressant drug classes (including other antidepressants).

* As we studied the associations with time-varying exposure analysis, controls contributed more than once in the computation of the odds ratios before they were censored of became a case. For this reason, exposure is reported as a percentage.

† For the analysis on SSRIs, 1 case currently using had no matched controls and 5 cases who were past user had no matched controls. For the analysis on TCAs, 8 past users could not be matched. These were therefore not included in the analyses.

Additional analyses

Participants who used multiple cardiovascular drugs had a higher risk of MI (Figure 1). Furthermore, they used antidepressants more frequently than participants who used no cardiovascular drug at the index date. After exclusion of participants using multiple cardiovascular drugs at the index date, results for the association between current use of antidepressants and MI remained similar (OR: 0.63; 95% CI: 0.38 – 1.06) after adjustment for confounding factors.

Current use of antidepressants was associated with a higher risk of all-cause mortality than nonusers of antidepressants (OR: 1.22; 95% CI: 1.08 – 1.38). This was also observed in current users of SSRIs (OR: 1.32; 95% CI: 1.10 – 1.58). However, no difference in risk of all-cause mortality was observed between current and past users of any antidepressants and SSRIs specifically. Furthermore, current and past use of TCAs were not associated with the risk of all-cause mortality.

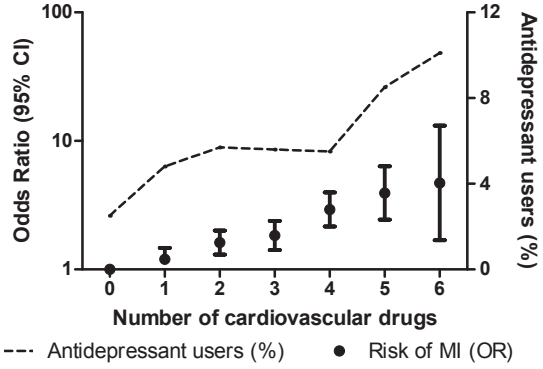

Figure 1 **Association between concomitantly used cardiovascular drugs and incident myocardial infarction, and the use of antidepressants.**

Abbreviations: CI, confidence interval; MI, myocardial infarction; OR, odds ratio. Analyses adjusted for age, sex and body mass index.

Discussion

Within the present case-control study, nested in the prospective Rotterdam Study cohort, current use of antidepressants was associated with a lower risk of MI. Of the different antidepressant drug groups, SSRI use was associated with the lowest risk of MI, although marginally not statistically significant and the number of cases using SSRIs was low.

These findings were supported by some of our extra analyses. First, past use of SSRIs was associated with a non-significantly higher risk of MI which suggests that in people with the same indication, discontinuation of SSRIs is followed by disappearance of a protective effect. Because current use of antidepressants was not associated with an increased risk of all-cause mortality, the association was not explained by confounding due to competing risk. Second, results remained similar when current use of antidepressants was compared with past use of antidepressants. Past users of antidepressants were assumed to be more comparable to current users of antidepressants with respect to confounding factors than never users. However, the population of past users was considerably smaller than the group of never users. And third, results remained similar after exclusion of participants with multiple concomitantly dispensed cardiovascular drugs, indicative of the participants with the highest cardiovascular risk.

Our findings are also supported by a number of other epidemiological studies. Our observation was similar to a previously published case-control study on the risk of MI in users of SSRIs[5]. Furthermore, a lower risk of MI was observed in users of

antidepressants with a high affinity to the serotonin reuptake transporter, and thus may indicate a specific effect of serotonin inhibition[6]. However, none of the other published studies reported a lower risk of MI in users of SSRIs[7-9], or even observed a higher risk[11, 12]. Differences in study design and availability of information on covariables (specifically depression[13-18]) might explain these contradictory results published in the literature on this topic.

In our study population, we did not observe a decreased risk of MI in users of TCAs. Previous studies showed that the use of TCAs was associated with an increased risk of MI[6, 10, 11]. However, when compared with past use of TCAs, a lower point estimate was observed, although not statistically significant.

Besides data from some of the conducted epidemiological studies, also data on the relation between serotonin and MI support our findings. The serotonin receptor 2A (5-HT_{2A}) and the serotonin transporter are both expressed at the surface of blood platelets and facilitate the activation and aggregation of blood platelets as well as coronary vasoconstriction[30, 31]. In patients with depression it has been shown that sensitivity of the 5-HT_{2A} receptor is increased and expression of the serotonin transporter decreased[4]. This was thought to be one of the explanations why patients with depression are at a greater risk of MI[4]. Also, a high serotonin concentration in serum was associated with a higher risk of MI[32]. Furthermore, use of SSRIs was associated with a decreased serotonin and platelet concentration in whole blood, and with a lower platelet activation[33, 34].

For case-control and cohort studies, results might be subject to residual confounding. To our knowledge, only one randomized clinical trial investigating the association between sertraline and risk of MI has been conducted as a secondary study outcome. Within that study, a 30% lower risk of MI was observed in the sertraline-treated patients after a 24 weeks treatment period compared with patients on placebo treatment[9]. However, this difference was not statistically significant, probably because of the low number of MI cases. Both groups had a similar relief in depressive symptoms. Thus, a difference in depression during follow-up did not explain the lower risk of MI in the sertraline-treated patients[13-18]. Together with the results of our study, these are additional arguments in favor of a protective effect of antidepressants, in particular SSRIs, on the risk of MI.

Antidepressants, and specifically SSRIs, have been associated with an increased BMI[26], elevated serum low density lipoprotein cholesterol levels[27, 28], and a higher risk of diabetes mellitus[29], which have all been associated with a higher MI risk. However, although additional adjustment for these factors did not materially change the observed associations, it is unlikely that antidepressants decrease the risk of MI more than statins do in the first-line prevention of cardiovascular diseases[35]. In addition, it is worth noting that the use of antidepressants, and especially the SSRIs,

has also been associated with an increased risk of bleeding, presumably through the effects of antidepressants on blood platelets[36].

This study has strengths and limitations. First, the available pharmacy dispensing records allowed us to study drug use at the date of MI. We were able to study antidepressant drug use in a time-dependent manner and are able to clearly define episodes of antidepressant drug use. Second, MI adjudication was done using standardized definitions using hospital discharge letters and records of the general practitioner[22]. Next, our study was limited by the small number of participants with an MI who were current users of antidepressants. Moreover, we could not ascertain whether the participants were actually taking the drugs, or only picked up their dispensing without initiation of treatment. Also, due to the observational nature of the data, the results may be subject to residual confounding.

In conclusion, current use of antidepressants was associated with a lower risk of incident MI. Of the antidepressant drug groups, use of SSRIs showed the lowest risk of MI. However, more studies are required to confirm our results.

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CHAPTER 5.2

INHIBITION OF SEROTONIN REUPTAKE BY ANTIDEPRESSANTS AND CEREBRAL MICROBLEEDS IN THE GENERAL POPULATION

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Background and purpose: Serotonin reuptake inhibiting antidepressants decrease platelet aggregation. This may cause an increased risk of intracerebral hemorrhage. However, the risk of subclinical microbleeds, which are highly prevalent in middle-aged and elderly people, is unknown. We studied whether serotonin reuptake inhibiting antidepressants increase the frequency of cerebral microbleeds and secondarily whether they lower the presence of ischemic vascular damage.

Methods: Within the population-based Rotterdam Study, information on antidepressant use was obtained from continuously monitored pharmacy records. Brain MRI was available in 4,945 participants (55% women, mean age 64 years) between 2005-2011. We categorized antidepressants based on affinity for the serotonin transporter: high, intermediate or low. Microbleeds (presence and location) and ischemic lesions (lacunes, white matter lesions) were rated on MRI. Logistic and linear regression, adjusted for age, sex, depressive symptoms and cardiovascular risk were used to study the association of antidepressants with microbleeds, and ischemic vascular lesions.

Results: Antidepressant use with strong serotonin reuptake inhibition was not associated with microbleed presence (odds ratio compared to non-use: 1.03, confidence interval:0.75;1.39) irrespective of microbleed location in the brain. Exclusion of antithrombotic users or persons with cortical infarcts did not change our results. Furthermore, serotonin reuptake inhibition was not related to ischemic vascular brain damage.

Conclusions: In the general population, use of serotonin reuptake inhibiting antidepressants is not related to presence of cerebral microbleeds. This strengthens the idea that the platelet inhibitor effects of antidepressant drugs with affinity for serotonin are minimal, and further supports the safety of SSRIs for non-gastrointestinal bleedings.

Introduction

The use of antidepressant medication in the general population has increased considerably in the last decades, in particular the use of selective serotonin reuptake inhibitors (SSRIs)[1, 2]. This increase in SSRI use may be explained by a broadened indication of SSRI, a different adverse effect profile and a lower toxicity compared with classic tricyclic antidepressants (TCAs)[3-5].

Yet, despite a more favorable adverse effect profile, the use of SSRIs is not entirely risk free.[6-9] SSRIs block the reuptake of serotonin by platelets and decrease serotonin platelet concentration, which may lead to impaired aggregation and prolonged bleeding times[10-14]. SSRIs have therefore extensively been studied in relation to intracerebral hemorrhages[15-22], and a recent meta-analysis of controlled observational studies showed an increased risk of intracerebral hemorrhages in SSRI users compared to non-users[23]. In addition, via the same pathophysiological pathway of reducing platelet aggregation, antidepressants with a high inhibition for serotonin reuptake may also reduce the risk of ischemic stroke, although to date this hypothesis is scarcely supported by literature[15, 16, 21, 24, 25].

Apart from major cerebrovascular events, it has not yet been investigated whether SSRIs or strong inhibitors of serotonin reuptake are associated with subclinical cerebrovascular lesions, and more particularly with subclinical bleedings. Cerebral microbleeds have increasingly been recognized on magnetic resonance imaging (MRI) in stroke patients, and mostly in association with larger intracerebral hemorrhages[26-28]. Yet, microbleeds are also highly prevalent in the general population, and microbleeds may similarly represent bleeding-prone vessels in these people. Support for this is provided in our previous studies in which we showed an association between antiplatelet drugs use and the presence of cerebral microbleeds in the general population[29, 30].

Given the association of microbleeds with symptomatic bleeds and antiplatelet drug use we hypothesized that people who use antidepressants with a great inhibition of serotonin reuptake may have a higher prevalence of cerebral microbleeds than non-users, and users of antidepressant with a low serotonin affinity. Moreover, we secondarily investigated whether the use of these drugs is associated with the presence of ischemic vascular damage on MRI, in particular a lower frequency of lacunes of presumed vascular origin[31] and lower white matter lesions (WML) volume.

5.2

Methods

Participants

The Rotterdam Study is a prospective population-based cohort study, within Ommoord, a suburb in Rotterdam, the Netherlands. The study comprises 14,926 participants, and investigates the prevalence, incidence of, and risk factors for diseases in an aging population[32]. The study started in 1990 and after baseline examination, follow-up assessments were conducted every 4-5 years including interviews and an extensive set of examinations. From 2005 onwards, brain MRI was embedded within the core protocol of the Rotterdam Study to investigate age-related brain changes on imaging[33]. The institutional review board approved the study. Between 2005 and 2011, 5,735 participants visiting the study center in that period were eligible to undergo a brain MRI. After informed consent was signed, a total of 5,074 non-demented people were scanned. After excluding participants in whom MRI was not completed (N=72) and scans with low quality (N=57), data on 4,945 participants were available for analyses.

Assessment of antidepressant drug use

We determined antidepressant drug use prior to brain MRI based on fully computerized pharmacy records from the 7 pharmacies in the Ommoord district. More than 99% of the participants have their drug prescriptions filled at these regional pharmacies. Medication records were continuously monitored from January 1st 1991 onwards. Records included the date of prescribing, the total amount of drug units per prescription, the prescribed daily number of units, the product name of the drugs and the anatomical therapeutic chemical (ATC) code. The duration of treatment was calculated by counting the number of prescription days. The average prescribed daily dose was expressed in standardized defined daily doses (DDD) calculated by summing up the total number of prescribed DDDs from all prescriptions divided by the total duration.

We classified antidepressants based on their degree of serotonin reuptake inhibition. The classification is based on the dissociation constant (K_d) for the serotonin transporter. A lower dissociation constant reflects a higher affinity for the serotonin transporter and therefore a higher inhibition of serotonin reuptake. Based on previous literature, we categorized antidepressants into high (paroxetine, clomipramine, sertraline, duloxetine, fluoxetine), intermediate (escitalopram, citalopram, imipramine, fluvoxamine, amitriptyline, venlafaxine), and low (desimipramine, opipramol, nortriptyline, doxepin, dosulepin, maprotiline, moclobemide, mianserin, trazodone, nefazodone, mirtazapine) degrees of serotonin reuptake inhibition[17, 34-38].

People who used multiple antidepressants from the different groups were excluded from the main analyses (n=268), to secure a pure exposure. These users were considered switchers and were analyzed in subsequent analyses.

Brain MRI and assessments of MRI markers

Brain MRI scans were performed on a 1.5-Tesla MRI scanner (GE Healthcare Milwaukee, WI).[33] Our multisequence MRI protocol included the following scans: T1-weighted, proton-density weighted, T2-weighted, and fluid-attenuated inversion recovery (FLAIR)[33]. For microbleed detection we used a custom-made accelerated three-dimensional T2*-weighted gradient-recalled echo sequence with high spatial resolution and long echo-time to enhance the detection of microbleeds[39]. Microbleeds were defined as focal areas of low signal intensity on T2*-weighted imaging. Their presence, location, and numbers were scored by 1 of 5 trained research-physicians, with good intraobserver and interobserver agreement[40]. We categorized microbleeds based on their presumed underlying etiology into lobar microbleeds (presumably reflective of cerebral amyloid angiopathy [CAA]), and deep or infratentorial microbleeds (presumably reflective of hypertensive arteriopathy)[39]. Lacunes and cortical infarcts were rated on FLAIR, proton-density-weighted and T1-weighted sequences by the same raters of microbleeds. Lacunes were defined as focal lesions between ≥3mm and <15mm in size[40]. Infarcts showing involvement of grey matter were classified as cortical infarcts. Brain tissue was segmented into grey matter, white-matter, and cerebrospinal fluid, using validated automated post-processing steps that include conventional k-nearest-neighbour brain tissue classifier extended with WML segmentation[41, 42].

Assessment of covariables

We addressed potential confounders by characterizing depressive symptoms, cardiovascular risk factors and cardiovascular medication use in our study population. Antidepressant drugs are mainly prescribed for depressive disorders. Depression has a bidirectional association with cardiovascular disease, and cardiovascular disease is related to the presence of microbleeds[43, 44].

Presence of depressive symptoms was evaluated using the Center for Epidemiological Studies Depression Scale (CESD)[45]. A score of 16 or higher was indicative of participants with clinically relevant depressive symptoms. A very high sensitivity for major depression for this score was reported in older adults in the Netherlands[46].

Participants' cardiovascular risk was assessed during the center visit preceding MRI, using interview, laboratory, and physical examinations[47]. This included presence of diabetes mellitus, smoking status (ever versus never), serum total

cholesterol levels, serum high-density lipoprotein (HDL) cholesterol levels, and systolic and diastolic blood pressure. Finally, use of lipid lowering drugs (C10), antihypertensive drugs (C02, C03, C07, C08, and C09), and antithrombotic drugs (B01AA, B01AB, B01AC, and B01AX) was assessed from pharmacy records during follow-up before MRI.

Statistical analysis

We analyzed the association between use of antidepressants, their degree of serotonin reuptake inhibition (high, intermediate, low) with the presence of cerebral microbleeds (present versus absent) using multiple logistic regression, taking non-users as reference category. Analyses were repeated for microbleeds at different locations in the brain, namely strictly lobar regions and deep or infratentorial regions (with or without lobar microbleeds). Furthermore, we repeated all analyses using low and intermediate serotonin reuptake inhibition antidepressant users as reference category. Switchers were excluded from the main analyses and the subsequent analyses were repeated including switchers.

All analyses were adjusted for age and sex. We additionally adjusted for presence of depressive symptoms, diabetes mellitus, smoking, total and HDL cholesterol, systolic and diastolic blood pressure, use of lipid-lowering medication, antihypertensive medication, and antithrombotic agents. Sensitivity analyses were performed with exclusion of MRI-defined cortical infarcts or exclusion of antithrombotic drug users. Moreover, analyses were stratified for sex, the exposure was dichotomized based on the duration of treatment (cut-off was 90 days) and interaction tests with antithrombotic drug users were performed. The average prescribed daily dose, expressed in standardized DDD, was also studied dichotomized on 1.00 DDD as the cut-off to look at an effect of dose.

Furthermore, we studied the association between the degree of serotonin reuptake inhibition of antidepressants and the presence of lacunes and WML volume with, respectively, multiple logistic and linear regression. People with cortical infarcts were excluded from these analyses. Analyses were adjusted for the same factors as described above. Analyses of WML volume were additionally adjusted for intracranial volume. WML was log-transformed due to the skewed distribution.

We considered a p-value <0.05 as statistically significant, and analyses were performed with a commercially available software program (IBM SPSS Statistics for Windows, Version 21.0).

Results

Characteristics of the study population are presented in Table 1. Mean age was 64.0 years (SD 11.0) and 2724 (55.1%) were female. A total of 930 (18.8%) persons had a history of antidepressant use before MRI, and 311 (6.2%) had exclusively used antidepressants with a high degree of serotonin reuptake inhibition, 304 (6.1%) of an intermediate, and 47 (1.0%) antidepressants of a low degree. Among users, 268 (5.4%) switched between the different antidepressant drug categories. In the total study population, 957 (19.4%) had microbleeds, of whom 629 had strictly lobar and 328 deep or infratentorial microbleeds. In the group of antidepressant drug users (n=930), 18.9% had microbleeds, which did not significantly differ from the 19.5% in the population of non-users. Of all participants in our study, lacunes were present in 370 (7.5%), and median WML volume was 3.0 ml.

Compared to non-use, the use of antidepressants with a high serotonin reuptake inhibitory potential was not associated with cerebral microbleed presence (age, sex-adjusted odds ratio [OR]: 1.03; 95% CI 0.75;1.39). In addition, no association was found for low (OR: 0.76; 95% CI 0.36;1.62) or intermediate (OR: 1.04; 95% CI 0.77;1.39) serotonin affinity antidepressants. Compared to non-use, the use of antidepressant medication with either high, intermediate, or low affinity for serotonin was neither related to lobar, nor to deep or infratentorial microbleeds (Table 2). Additionally, no association between antidepressants use and microbleeds was found for people who switched between different antidepressant drugs (OR: 0.95; 95% CI 0.68;1.33). Additional adjustments for cardiovascular risk factors, cardiovascular medication and depressive symptoms did not change any of the results significantly (Table 2). Excluding participants with MRI-defined cortical infarcts (n=158), and excluding ever antithrombotic drug users (n=1,326) also did not materially change our results (data not shown). Moreover, the exposure split by duration and average prescribed daily dose of antidepressant drug treatment and stratification by gender did not significantly change our results (data not shown). Effect modification of antidepressant drug exposure by antithrombotic drugs was not present (p=0.96).

We did not find a higher frequency of cerebral microbleeds, irrespective of their location in the brain, when comparing the high affinity group with the combined intermediate and low affinity group (OR: 1.03; 95% CI: 0.68;1.56) (Table 3).

Finally, we did not find a lower frequency of lacunes (OR: 1.14; 95% CI 0.67;1.94) nor a smaller WML volume (mean difference of WML volume: 0.06; 95% CI -0.03;0.15) for use of antidepressants with a high serotonin reuptake inhibition potential compared to non-use, neither did we find a relation when investigating the use of low and intermediate degree of serotonin reuptake inhibition (Table 4).

Table 1 Baseline characteristics of the study population

	N=4945
Age, years	64.0 (11.0)
Females	2724 (55.1)
Depressive symptoms	417 (8.6)
Diabetes mellitus	433 (8.9)
Smoking	3436 (69.8)
Antidepressant drug users	
High degree of inhibition*	311 (6.2)
Intermediate degree of inhibition*	304 (6.1)
Low degree of inhibition*	47 (1.0)
Switchers	268 (5.4)
Presence of cerebral microbleeds	957 (19.4)
Strictly lobar	629 (13.6)
Deep or infratentorial	328 (7.6)
White matter lesion volume, mL	3.0 (1.6 – 6.5)
Lacunes	370 (7.5)
Cortical infarcts	165 (3.3)
Total cholesterol, mmol/L	5.5 (1.1)
High-density lipoprotein cholesterol, mmol/L	1.4 (0.4)
Systolic blood pressure, mmHg	138.9 (21.2)
Diastolic blood pressure, mmHg	82.2 (10.9)
History of lipid lowering drug use	1185 (24.2)
History of antihypertensive drug use	1696 (34.6)
History of antithrombotic drug use	1415 (28.6)

Values represent mean (standard deviation) or number (percentage). White matter lesion volume is represented as median (interquartile range).
* Degree of serotonin reuptake inhibition: High=paroxetine, clomipramine, sertraline, duloxetine, fluoxetine. Intermediate = escitalopram, citalopram, impramine, fluvoxamine, amitriptyline, venlafaxine. Low = desimipramine, opipramol, nortriptyline, doxepin, dosulepin, maprotiline, moclobemide, mianserin, trazodone, nefazodone, mirtazapine.

Table 2 Degree of serotonin reuptake inhibition for antidepressant drugs and the presence of cerebral microbleeds.

Degree of serotonin reuptake inhibition	Any microbleeds		Deep or infratentorial microbleeds		Strictly lobar microbleeds	
	n / N	Odds ratio (95% CI)	n / N	Odds ratio (95% CI)	n / N	Odds ratio (95% CI)
Model 1						
Non-use	781 / 4015	1.00 (Reference)	270 / 3504	1.00 (Reference)	511 / 3745	1.00 (Reference)
Low	9 / 47	0.76 (0.36;1.62)	2 / 40	0.51 (0.12;2.15)	7/ 45	0.92 (0.40;2.10)
Intermediate	65 / 304	1.04 (0.77;1.39)	23 / 262	1.07 (0.67;1.70)	42 / 281	1.04 (0.73;1.47)
High	53 / 311	1.03 (0.75;1.39)	20 / 278	1.17 (0.72;1.90)	33 / 291	0.93 (0.64;1.36)
Model 2						
Non-use	741 / 3850	1.00 (Reference)	257 / 3366	1.00 (Reference)	484 / 3593	1.00 (Reference)
Low	8 / 45	0.70 (0.32;1.57)	2 / 39	0.50 (0.11;2.15)	6 / 43	0.80 (0.33;1.96)
Intermediate	60 / 291	0.96 (0.70;1.30)	21 / 252	0.94 (0.58;1.54)	39 / 270	0.98 (0.68;1.41)
High	51 / 298	0.97 (0.70;1.35)	19 / 266	1.10 (0.66;1.84)	32 / 279	0.88 (0.59;1.30)

Abbreviations: n= number of cases; N= total population within the exposure category. Values represent odds ratios for microbleeds in relation to antidepressant drugs with low, intermediate, and high affinity for serotonin. Non-users are the reference population for all analyses presented in Table 2. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, depression, diabetes mellitus, smoking, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, ever use of lipid-lowering drugs, antihypertensive drugs, and antithrombotic drugs. Number of cases/total population deviate from model 1, as we performed a complete caseset analysis.

Table 3 **Degree of serotonin reuptake inhibition for antidepressant drugs and the presence of cerebral microbleeds within drug users**

Degree of serotonin reuptake inhibition	Any microbleeds		Deep or infratentorial microbleeds		Strictly lobar microbleeds	
	n / N	Odds ratio (95% CI)	n / N	Odds ratio (95% CI)	n / N	Odds ratio (95% CI)
Low/intermediate	74 / 351	1.00 (Reference)	25 / 302	1.00 (Reference)	49 / 326	1.00 (Reference)
Model 1						
High	53 / 311	1.03 (0.68;1.56)	20 / 278	1.14 (0.60;2.16)	33 / 291	0.93 (0.56;1.52)
Model 2						
High	51 / 298	1.02 (0.66;1.58)	19 / 266	1.16 (0.59;2.31)	32 / 279	0.91 (0.54;1.52)

Abbreviations: n= number of cases; N= total population within the exposure category. Values represent odds ratios for microbleeds in relation to antidepressant drugs with high affinity for serotonin. Users of low and intermediate degree of serotonin reuptake inhibition antidepressants are the reference population for the presented analyses in Table 3. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, depression, diabetes, smoking, total and high-density lipoprotein cholesterol, systolic and diastolic blood pressure, ever use of lipid-lowering drugs, antihypertensive drugs, and antithrombotic drugs. Number of cases/total population deviate from model 1, as we performed a complete caseset analysis.

Table 4 **Degree of serotonin reuptake inhibition for antidepressant drugs and the presence of lacunes and white matter lesion volume.**

Degree of serotonin reuptake inhibition	Lacunes		White matter lesion volume	
	n / N	Odds ratio (95% CI)	N	Difference in mean (95% CI)
Model 1				
Non-use	260 / 3888	1.00 (Reference)	3881	0.00 (Reference)
Low	3 / 45	0.82 (0.25;2.74)	45	-0.05 (-0.28;0.18)
Intermediate	23 / 288	1.20 (0.76;1.90)	288	0.08 (-0.02;0.17)
High	16 / 298	1.14 (0.67;1.94)	298	0.06 (-0.03;0.15)
Model 2				
Non-use	247 / 3728	1.00 (Reference)	3722	0.00 (Reference)
Low	3 / 43	0.89 (0.26;3.02)	43	-0.04 (-0.27;0.19)
Intermediate	22 / 275	1.13 (0.70;1.82)	275	0.06 (-0.03;0.16)
High	15 / 285	1.05 (0.60;1.86)	285	0.06 (-0.03;0.15)

Abbreviations: n= number of cases; N= total population within the exposure category. Lacunes: values represent odds ratios for lacunes in antidepressant drug users with low, medium, and high affinity for serotonin compared to non-users. White matter lesions volume: values represent differences in mean log transformed white matter lesion volumes (ml) in antidepressant drug users with low, medium, and high affinity for serotonin compared to non-users. Model 1: adjusted for age and sex. Model 2: adjusted for age, sex, depression, diabetes, smoking, total and HDL cholesterol, systolic and diastolic blood pressure, ever use of lipid-lowering drugs, antihypertensive drugs, and antithrombotic drugs. Number of cases/total population deviate from model 1, as we performed a complete caseset analysis. White matter lesion volume analyses were additionally adjusted for intracranial volume.

Discussion

In the general population, we did not find an association between antidepressant drug use with a greater inhibition of serotonin reuptake and the presence of cerebral microbleeds. In addition, the degree of serotonin reuptake inhibition was not associated with presence of lacunes or WML volume.

Microbleeds are thought to precede the onset of large symptomatic hemorrhages, and may thus reflect a clinically relevant preclinical imaging marker, although evidence from longitudinal studies is still limited.[26, 48, 49] The novelty of our study lays in the fact that we investigated the use of SSRIs in relation to subclinical hemorrhagic brain lesions in the general population, in contrast to clinical studies investigating symptomatic hemorrhage. We did not observe an association between degree of serotonin reuptake inhibition and the presence of microbleeds. This is in line with

the majority of previous studies on SSRIs and symptomatic brain hemorrhages[15, 18, 20], although a recent meta-analysis did find an increased risk of brain hemorrhages in SSRI users (OR cohort studies: 1.68; 95% CI 1.04;2.51)[23].As a methodological consideration; heterogeneity in sample size, quality of the individual studies and different approaches to handle the influence of confounding may have influenced the validity of the meta-analysis to a certain degree[50].

SSRIs might increase the risk of clinical or subclinical bleedings via the following main biological mechanism. Damage to endothelial layers leads to activation of hemostatic mechanisms, and platelets adhere to damaged vessel walls. Intracellular serotonin is subsequently released into the blood stream and promotes clot formation and vasoconstriction at the site of injury. SSRIs inhibit the reuptake of serotonin by platelets from the blood, reduce intracellular serotonin concentrations, thereby decrease platelet aggregation and increase the risk of bleeding[12, 13]. Moreover, a second mechanism proposes that some SSRIs may inhibit cytochrome 450 (CYP) enzymes such as CYP 1A2, 2D6, 3A4 and 2C9. This may increase the bleeding risk by inhibition of the metabolism of certain drugs that have anticoagulant properties such as NSAIDS and antithrombotic drugs[13, 14].

Nonetheless, for both mechanisms we could argue that diminishing intraplatelet serotonin levels only affects hemostasis to a limited extent, and thus that remaining platelet function is sufficient to halt significant bleeding. Depletion of serotonin levels in platelets may well be compensated for by other adequately working hemostatic mechanisms. This would partly explain why SSRI use was more consistently associated with extra-cranial bleeds, in particular gastro-intestinal bleedings. Here, SSRI use increases serotonin levels and stimulates the production of gastric acid, which increases the risk of gastrointestinal bleedings. Bleeding complications may therefore be induced by a third mechanism which does not necessarily involve platelet dysfunction[14].

No association was found for antidepressants with an affinity for serotonin with microbleeds in either lobar or deep or infratentorial regions of the brain. Although microbleeds at both locations are representative of bleeding-prone vessels, their etiologies differ. Microbleeds in lobar regions are more likely to result from blood leaking from destructed vessel walls containing amyloid, whereas deep or infratentorial microbleeds most likely represent hemosiderin deposits as a consequence of hypertensive arteriopathy[28, 40, 43, 51]. Our findings suggest that regardless of the underlying pathology, the decrease of intracellular serotonin platelets caused by antidepressants with a strong serotonin reuptake potential is insufficient to increase the frequency of small, asymptomatic bleedings.

Finally, we did not find a protective effect of antidepressant drugs, with a high affinity for the serotonin transporter, on ischemic vascular brain lesions. This is in

line with findings from previous studies in patients with ischemic stroke[15, 21], and strengthens the idea that the platelet inhibitor effects of antidepressant drug with affinity for serotonin are minimal. Two previous studies showed an increased risk of ischemic stroke in current SSRI users[16, 24]. This increased risk could be explained by a different biological mechanism which postulates that serotonin induces vasoconstriction of large vessel, and may lead to thromboembolic ischemic stroke in the presence of atherosclerosis.[52] However, in our study, we focused on silent ischemic vascular lesions, involving the small cerebral arteries, which are typically not caused by thromboembolic events.

Strengths of our study are the large sample size, population-based character of our study, and the prospectively gathered electronic pharmacy records which we used to determine antidepressant drug use. Based on a 19.5% prevalence of microbleeds in unexposed subjects, with a two-sided significance of 0.05, we had sufficient power (80%) to detect an odds ratio of 1.22 or greater. Less strong associations may not have been detected in our study, although based on the recent meta-analysis on SSRIs and symptomatic brain hemorrhages we would expect an estimate of at least this magnitude for subclinical bleedings[23]. Some limitations of our study need to be considered. The cross-sectional design of our study limits our conclusions on a causal pathway. MRI does not provide information on the timing of when cerebral microbleeds occurred, as cerebral microbleeds remain visible in the brain for an undefined period. Therefore, there is a possibility that cerebral microbleeds occurred before antidepressant use was initiated. This may have led to an underestimation of the true association presented due to non-differential misclassification of SSRI users, and further longitudinal investigations are warranted. Furthermore, confounding by indication and contra-indication poses a problem in our observational study. Depression, the most important indication to prescribe antidepressants, has a bidirectional association with cardiovascular disease, and cardiovascular diseases are associated with an increased number of microbleeds. Moreover, TCAs are relatively contra-indicated for patients with cardiovascular disease. We minimized these forms of confounding by adjusting for presence of depressive symptoms, cardiovascular risk factors, and cardiovascular medication. Also, we reclassified the antidepressant drugs based on their their affinity to the serotonin reuptake transporter. Although we aimed to address all potential confounders in our study, residual confounding cannot be ruled out and may have affected our results to an extent that associations may have been overestimated.

In conclusion, this study adds important information to the previous reports on antidepressant drug use and bleeding risk. We report that, in the general population, the use of antidepressant drugs that inhibit serotonin reuptake is not related to

the presence of cerebral microbleeds. This further supports the safety of these antidepressants for non-gastrointestinal bleedings. Since these results are cross-sectional, further longitudinal research regarding antidepressant drug use and the risk of microbleeds in relation to major intracerebral hemorrhage is of high interest.

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CHAPTER 6

GENETIC ASSOCIATION STUDIES

CHAPTER 6.1

ASSOCIATION BETWEEN GENETIC VARIATION IN THE ABCB1 GENE AND SWITCHING, DISCONTINUATION, AND DOSAGE OF ANTIDEPRESSANT THERAPY: RESULTS FROM THE ROTTERDAM STUDY

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The objective of this study was to investigate whether polymorphisms in the *ABCB1* gene were associated with switching, with discontinuation of antidepressants within 45 days after starting therapy, and/or with dose change in a large prospective population-based cohort study. Between April 1, 1991, and December 31, 2007, there were 1257 incident users of antidepressants with known *ABCB1* genotypes (1236C>T, 2677G>T/A, 3435C>T) in the population-based Rotterdam Study. Logistic regression models were used to estimate the genotype and haplotype effect on the risk of switching and discontinuation. In addition, the association between the haplotypes and the prescribed drug dosage was assessed per drug class. The separate polymorphisms in the *ABCB1* gene were associated with increased risks of switching and discontinuation but reached only statistical significance for the association between the 3435C>T polymorphism and switching. In a model adjusted for age and sex, homozygous carriers of the T-T-T haplotype had an increased risk of switching (odds ratio, 4.22; 95% confidence interval, 1.30-13.7; P = 0.017) and discontinuation (odds ratio, 1.47; 95% confidence interval, 0.98-2.22; P = 0.063). Explained variance was 10.4% for switching and 2.5% for discontinuation. In contrast, no association was observed between the T-T-T haplotype and the prescribed dosage. In summary, this study showed that genetic variation in the *ABCB1* gene might play a role in the risk of switching and discontinuation of antidepressant therapy but the clinical relevance is limited.

Introduction

P-glycoprotein (P-gp) operates as an adenosine triphosphate-dependent efflux pump that is encoded by the adenosine triphosphate-binding cassette B1 (*ABCB1*) or multidrug resistance 1 (*MDR1*) gene, which is located on chromosome 7q21. In pharmacologic studies, P-gp was shown to influence the pharmacokinetics of numerous antidepressants, for example, citalopram, amitriptyline, and venlafaxine[1, 2].

Single nucleotide polymorphisms (SNPs) in the *ABCB1* gene, the most well-studied SNPs being 3435C>T, 2677G>T/A, and 1236C>T, have been associated with an altered expression level, functionality, and specificity for substrates, although in replication studies these findings were not convincingly confirmed[3-7]. In relation to antidepressant drugs, transport across the blood-brain barrier seemed to be affected by these polymorphisms[1]. One small study (n = 68) showed that the major haplotype (1236C, 2677G, and 3435C) was associated with a lower rate of remission in patients with major depression treated with paroxetine[8]. In addition, the major alleles of these polymorphisms have been individually associated with both the therapeutic response and the risk of adverse drug reactions, but replication studies often lacked sufficient sample sizes to replicate these findings[9-11].

For the current study, we aimed to assess whether genetic variation in the *ABCB1* gene was associated with a higher risk of switching and discontinuation. Because intolerance to a drug can also result in a lowering of the daily dose during therapy, we also assessed whether subjects with variant haplotypes were prescribed lower dosages than carriers of the homozygous C-G-C haplotype.

Methods

Setting of the Rotterdam Study

The Rotterdam Study is a prospective population-based cohort study that investigates the incidence of and risk factors for several age-related diseases and conditions. In total, 7983 individuals (78% response rate) were included between 1990 and 1993. All participants were 55 years or older and lived in the Ommoord district in Rotterdam, the Netherlands. Follow-up examinations (interview and health examinations) were conducted every few years starting at the moment of inclusion. Between the follow-up examinations, participants are continuously monitored on morbidity and mortality via the records of the general practitioner and on the use of medications via the 7 regional pharmacies. Data on medication use were available from 1991 onward and include the Anatomical Therapeutical Chemical (ATC) code, the dispensing date, the total amount of drug units per prescription, the prescribed

daily number of units, and the product name of the drug. A more detailed description of the Rotterdam Study is published elsewhere[12]. This study was approved by the Medical Ethical Committee of the Erasmus Medical Center, and written informed consent was obtained from all participants.

Study Population

For the current study, we included all participants receiving their first antidepressant medication (ATC code = N06) between April 1, 1991, and December 31, 2007, and for whom data on the ABCB1 1236C>T (rs1128503), 677G>T/A (rs2032582), and 3435C>T (rs1045642) polymorphisms were available. More than 99% of the participants had white ancestry. Participants using monoamine oxidase A inhibitors and participants who were prescribed 2 different antidepressant drugs at the same time were excluded from the study population. To ensure that participants were incident users, participants who received antidepressant drugs within the first 3 months of 1991 were excluded because we did not have pharmacy data before January 1, 1991. Subjects were followed up until one of the outcomes, death, or the end of the study period (December 31, 2007).

Outcome Definition

Switching was defined as a switch to any other antidepressant drug (irrespective of drug class) within 45 days after start of treatment. Discontinuation was defined as no further prescriptions of any antidepressant drug within 45 days after start of therapy. A period of 45 days was used because drug effectiveness is usually assessed for the first time after 6 weeks of treatment. Dosages are presented as the ratio between the prescribed daily dosage and the defined daily dosage[13]. For these analyses, we used only the first 10 prescriptions from an individual, as titration to the optimal tolerable and effective dosage would then be completed.

Genotyping

DNA was isolated from all participants from whom blood was drawn. Genotyping of the 1236C>T, 2677G>T/A, and 3435C>T polymorphisms in the *ABCB1* gene, the *4 variant in the *CYP2D6* gene, and the *1B variant of the *CYP3A4* gene was performed using Taqman allelic discrimination assays on the ABI Prism 7900 HT Sequence detection system (Applied Biosystems, Foster City, CA). Corresponding haplotypes within the *ABCB1* gene were estimated using the estimation maximization algorithm in the Haplostat 1.3.0 package for R 2.5.0 using haplo.em[14]. Estimated haplotypes with a posterior probability lower than 0.95 were excluded from the analyses.

Covariables

Considered covariables in the analyses were age, sex, body mass index (BMI), serum creatinine levels, the initial antidepressant drug dosage, drug class (tricyclic antidepressants [TCA], selective serotonin reuptake inhibitors [SSRI], and others), comorbidities (heart failure, stroke, myocardial infarction, cancer, chronic obstructive pulmonary disorder, and type 2 diabetes), and the *CYP2D6*4* and *CYP3A4*1B* genotype. Age was calculated at the moment of the start of antidepressant therapy. Body mass index and serum creatinine levels were assessed during the first visit to the study center. Body mass index was calculated by dividing the weight in kilograms by the height in meters squared.

Statistical Analyses

The associations between *ABCB1* polymorphisms and haplotypes and switching and discontinuation were modeled using logistic regression models adjusted for covariables. For all analyses, the major allele or the C-G-C haplotype (major haplotype) was taken as the reference. Odds ratios are presented as the effect of a variant genotype or haplotype (heterozygous and homozygous for the minor variant) on switching and discontinuation. All analyses were adjusted for age and sex. Other potential covariables were only included in the multivariable model if the point estimate changed by more than 10%. Analyses were additionally stratified for P-gp substrate specificity. P-glycoprotein substrates were amitriptyline, citalopram, venlafaxine, paroxetine, sertraline, doxepin, and nortriptyline[1]. Analyses on prescribed dosages of the first 10 prescriptions were performed separately for TCAs and SSRIs using repeated-measurement analyses. In addition, a possible interaction between the haplotype and the consecutive prescription number was evaluated.

Statistical analyses were performed using SPSS (version 20.0; IBM, Chicago, IL).P values below 0.05 were considered statistically significant.

Results

Of 1257 incident antidepressant users in the Rotterdam Study, 1148 had a posterior probability of the estimated haplotype higher than 0.95. Baseline characteristics of the study population are presented in Table 1. The study population had a mean age of 75 years, and 68.4% of the participants were women. The 3 polymorphisms used in this study were in Hardy-Weinberg equilibrium ($P > 0.1$), and allele frequencies were comparable with other white populations[8, 9]. From the study population, 50% was initially prescribed a TCA (of which 77.6% was for amitriptyline), 40.9% was

Table 1 Baseline characteristics of the study population

	N = 1,257
Demographics	
Age (years), mean (SD)	75.1 (8.7)
Females, no.(%)	860 (68.4)
BMI (kg/m^2), mean (SD)	26.8 (3.9)
Serum creatinine (μmol/L), mean (SD)	82.0 (19.5)
Co-morbidities, no.(%)	
Heart failure	112 (8.9)
Stroke	107 (8.5)
Myocardial infarction	91 (7.2)
Cancer	115 (9.1)
Chronic obstructive pulmonary disorder	130 (10.3)
Type 2 diabetes	178 (14.2)
Polypharmacy, no.(%)	550 (43.8)
CYP2D6 *4/*4, no.(%)	81 (6.4)
CYP3A4 wt/*1B or *1B/*1B[1], no.(%)	80 (6.4)
Initial choice antidepressant, no.(%)	
TCAs	629 (50.0)
SSRIs	514 (40.9)
Others	114 (9.1)

Abbreviations: TCAs = Tricyclic antidepressants; SSRIs = Selective Serotonin Reuptake Inhibitors. BMI = Body Mass Index. Data on body mass index was missing for 362 (28.8%) and data on creatinine levels was missing for 375 participants (29.8%). 1) Only 2 participants were homozygous for the *1B variant.

initially prescribed an SSRI (of which 46.9% was for paroxetine), and 9.1% was initially prescribed other antidepressant drugs.

Associations between *ABCB1* alleles and switching and discontinuation are presented in Table 2. For the 3 genotyped SNPs, we observed per SNP a trend toward an increased risk of switching and discontinuation of antidepressant drugs. Homozygous variant carriers had a 1.8 (1236C>T) to 3.3 (3435C>T) increased risk of switching compared with homozygous major allele carriers. Increased risks were more prominent in the analyses on switching as compared with the analyses on discontinuation. The explained variance did not exceed 6.7% for switching and 1.5% for discontinuation.

Table 2 **Association between the minor genotypes of the *ABCB1* gene and switching and discontinuation**

				Wt/var		Var/var		
		Cases (N)	Controls (N)	OR (95% CI)	P-Value	OR (95% CI)	P-Value	R^2
1236C>T	Switching	49	637	1.02 (0.50 – 2.08)	0.95	1.86 (0.86 – 4.01)	0.11	0.049
	Discontinuation	530	637	1.42 (1.09 – 1.85)	0.010	1.25 (0.90 – 1.75)	0.19	0.015
2677G>T[1]	Switching	46	617	1.14 (0.54 – 2.41)	0.73	2.22 (1.00 – 4.95)	0.050	0.061
	Discontinuation	506	617	1.38 (1.05 – 1.82)	0.019	1.23 (0.88 – 1.74)	0.23	0.017
3435C>T	Switching	42	621	1.73 (0.62 – 4.80)	0.29	3.31 (1.21 – 9.07)	0.020	0.067
	Discontinuation	524	621	1.14 (0.85 – 1.53)	0.39	1.13 (0.81 – 1.56)	0.47	0.007

Analyses were performed with the homozygous major allele carriers as a reference. Odds ratios indicate the genotype effect of the variant allele. Analyses were adjusted for age and gender. 1) 'A' variants were not taken into account, due to a low allele frequency.

T-T-T variant haplotype carriers (heterozygous and homozygous) had increased risks to switch or discontinue from antidepressant therapy (Table 3). Risk estimates were most prominent with switching as an outcome (4.22 vs 1.47, respectively, in case of the analyses on the homozygous haplotype carriers). Point estimates were not materially different for P-gp and non-P-gp substrates. Explained variance did not exceed 12.9% for switching and 3.1 for discontinuation, when also considering P-gp substrate specificity.

We did not observe a statistically significant effect of the T-T-T haplotype on the prescribed dosage of TCAs or SSRIs (Figure 1). No statistically significant interaction was observed between prescription number and haplotype. These results did not materially change when we stratified for P-gp substrate specificity (results not shown).

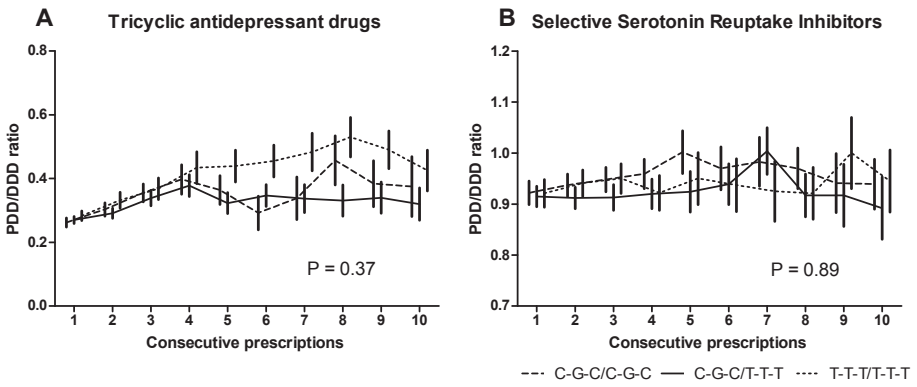

Figure 1 **Association of *ABCB1* haplotype and dose change.**

Presented are the association between the *ABCB1* haplotypes and the ratio between the prescribed daily dosages and the defined daily dosages of the first 10 consecutive prescriptions for TCA drugs (A) and SSRIs (B) separately. Data are presented as the mean with the SEM. Analyses are adjusted for age and sex. Consecutive dosages were not different for TCAs and SSRIs between the haplotypes (P = 0.37 and P = 0.89, respectively).

Table 3 **Association between the T-T-T haplotype of the *ABCB1* gene and switching and discontinuation**

				C-G-C / T-T-T		T-T-T / T-T-T		
		Cases (N)	Controls (N)	OR (95% CI)	P-Value	OR (95% CI)	P-Value	R^2
All	Switching	41	598	1.70 (0.52 – 5.59)	0.38	4.22 (1.30 – 13.7)	0.017	0.104
	Discontinuation	509	598	1.53 (1.08 – 2.16)	0.018	1.47 (0.98 – 2.22)	0.063	0.025
P-gp substrate	Switching	17	398	1.34 (0.24 – 7.53)	0.74	3.21 (0.56 – 18.5)	0.19	0.072
	Discontinuation	346	398	1.45 (0.94 – 2.23)	0.093	1.57 (0.95 – 2.59)	0.083	0.028
Non-P-gp substrate	Switching	24	200	2.16 (0.41 – 11.4)	0.37	4.93 (0.96 – 25.3)	0.056	0.129
	Discontinuation	163	200	1.67 (0.92 – 3.04)	0.094	1.32 (0.65 – 2.69)	0.44	0.031

Analyses were performed with the homozygous C-G-C haplotype carriers as a reference. Odds ratios indicate the haplotype effect of the variant haplotype. Analyses were adjusted for age and gender.

6.1

Discussion

The data presented in this study showed that genetic variation in the *ABCB1* gene was associated with an increased risk of switching and discontinuation but not with prescribed dosages of antidepressant therapy. However, the explained variance is still, although fairly high for an individual SNP, too low to predict whether patients on antidepressant therapy will switch or discontinue from antidepressant therapy. The observed increased risk of switching and discontinuation from antidepressant drugs in carriers of the T-T-T haplotype is in line with previous research on this topic. For example, the 2677G>T polymorphism was associated with an increased risk of suicidal ideation in depressed outpatients treated with antidepressant drugs, possibly by higher levels of serotonin. Homozygous 3435C>T carriership was associated with an increased risk of nortriptyline-induced postural hypotension in patients with depression[15, 16], and lower heart rate[17]. Next to intolerance to antidepressant drugs, genetic variation

was also shown to be associated with effectiveness. For example, rs2032583, which is located only 57 base pairs away from the 2677G>T polymorphism, is associated with a faster remission from depression compared with the major allele carriers[11]. The 1236T and 3435T variants were overrepresented in depressive patients showing a clinical response[18], and the major haplotype (C-G-C) was associated with a poorer response to antidepressant drugs[8]. An increased drug response and an increased risk of drug intolerance might be caused by an increased bioavailability of antidepressant drugs in the brain of those with a T-T-T haplotype. Although laboratory experiment results are conflicting[3-7], results from clinical studies suggest that these polymorphisms cause a higher bioavailability of antidepressant drugs in the brain.

For the T-T-T haplotype analyses, the explained variance was approximately 10% in case of switching and approximately 3% in case of discontinuation. These percentages indicate that there are several other still unknown genetic and nongenetic factors. No difference was observed between antidepressant P-gp substrates and non-P-gp substrates, for which we have 2 possible reasons. First, a lack of power because 2 of the 3 most frequently prescribed drugs are P-gp substrates (amitriptyline and paroxetine), which made the group of non-P-gp substrates small. Second, drugs now included in the non-P-gp substrate group might be misclassified because of a lack of literature. Further biochemical research on the interaction between antidepressant drugs and P-gp might clarify whether drugs now in the nonsubstrate group are in fact substrates of P-gp.

A limitation of the current study is that the outcome measures switching and discontinuation are subjective and might be the result of other considerations than

intolerance to antidepressants. However, these other reasons for the outcomes of this study are, as far as we are aware, not associated with the genotypes within the *ABCB1* gene, suggesting that any misclassification is nondifferential. Nondifferential misclassification is likely to result in point estimates closer to the zero hypothesis suggesting that the true effect of the *ABCB1* genotypes on switching and discontinuation is higher unless misclassification is minimal. The use of other (nonantidepressant) drugs that are competitive ligands of P-gp were not considered in this study because they are probably effect modifiers instead of confounders. Moreover, concomitant use of such drugs is probably independent of haplotype status and therefore nondifferential. The most important types of bias, notably selection bias, information bias, and confounding, are unlikely explanations for our observations. Participants of the Rotterdam Study were not selected based on the *ABCB1* genotype and neither on health condition. Local pharmacies provided us with an electronically standardized list of all prescribed medications from nearly all participants of the Rotterdam Study without knowledge of the study hypothesis or genetic status. Furthermore, we considered a large number of potential confounders.

The current study showed that genetic variation in the *ABCB1* gene is associated with increased risk of switching and discontinuation. The current data might therefore provide insight into the possible role of the *ABCB1* gene in intolerance to antidepressant drugs. Because the explained variance by the *ABCB1* haplotypes did not exceed 10.3%, however, an additional search for other markers (both genetic and nongenetic) is needed to provide more information to predict which patients are more likely to display intolerance to antidepressant drugs.

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CHAPTER 6.2

IDENTIFYING GENETIC LOCI ASSOCIATED WITH ANTIDEPRESSANT DRUG RESPONSE WITH DRUG-GENE INTERACTION MODELS IN A POPULATION-BASED STUDY

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It has been difficult to identify genes affecting drug response to Selective Serotonin Reuptake Inhibitors (SSRIs). We used multiple cross-sectional assessments of depressive symptoms in a population-based study to identify potential genetic interactions with SSRIs as a model to study genetic variants associated with SSRI response. This study, embedded in the prospective Rotterdam Study, included all successfully genotyped participants with data on depressive symptoms (CES-D scores). We used repeated measurement models to test multiplicative interaction between genetic variants and use of Selective Serotonin Reuptake Inhibitors (SSRIs) on repeated CESD scores. Besides a genome-wide analysis, we also performed an analysis which was restricted to genes related to the serotonergic signaling pathway. A total of 273 out of 14,937 assessments of depressive symptoms in 6,443 participants, use of an SSRI was recorded. After correction for multiple testing, no plausible loci were identified in the genome-wide analysis. However, among the top 10 independent loci with the lowest p-values, findings within two genes (*FSHR* and *HMGB4*) might be of interest. Among 26 genes related to the serotonergic signaling pathway, the rs6108160 polymorphism in the *PLCB1* gene reached statistical significance after Bonferroni correction (p-value = 8.1e-5). Also, the widely replicated 102C>T polymorphism in the *HTR2A* gene showed a statistically significant drug-gene interaction with SSRI use. Therefore, the present study suggests that drug-gene interaction models on (repeated) cross-sectional assessments of depressive symptoms in a population-based study can identify potential loci that may influence SSRI response.

Introduction

Failure of antidepressant therapy is common in the treatment of depression [1], and is genetically inherited[2-4]. Most genetic variants associated with antidepressant response were identified within candidate gene studies, in which a predefined hypothesis was tested[5, 6]. Beside genetic variation in Cytochrome P450 metabolizing enzymes (e.g. CYP2D6) and transporter proteins (e.g. P-glycoprotein), which affects the pharmacokinetics of antidepressants, some genes encoding proteins that influence the pharmacodynamics of antidepressants also showed associations with the antidepressant drug response[6]. However, the heterogeneity between the studies in a meta-analysis was substantial, which complicates the interpretation of the research findings[6]. Indeed, some of the findings were, in more recent studies, not replicated or only observed in subtypes of depression, such as melancholic or psychotic depression[7, 8]. In addition, Genome-Wide Association Studies (GWAS) have not been able to identify statistically significant associations between Single Nucleotide Polymorphisms (SNPs) and response to antidepressants[9-12].

Most studies used a treated-only design, in which patients with depression were followed over time from start of antidepressant therapy until relief of the depressive symptoms or the end of the study period. However, the use of this design has limitations, as it does not allow distinction between the drug response and the natural course of the disease[13]. In fact, loci observed within such studies might be associated with depressive symptoms rather than with the response to antidepressants[14]. The uncertainty in the interpretation can be reduced when untreated participants are included in the analyses[14].

Prospective population-based studies might have data available that is suitable for research on antidepressant drug response[15]. We hypothesized that if an association between a SNP and depressive symptoms differs between participants treated with Selective Serotonin Reuptake Inhibitors (SSRIs) and untreated participants, the SNP is possibly associated with drug response to SSRIs. Therefore, we studied whether genetic loci of potential interest for SSRI drug response can be identified in prospective population-based studies using drug-gene interaction models of repeated cross-sectional assessments of depressive symptoms.

Materials and Methods

Setting of the Rotterdam Study

The current study was embedded in the prospective Rotterdam Study which aims to investigate the incidence of and risk factors for several age-related diseases.

A more detailed description of the design and rationale of the study was published elsewhere[16, 17]. From 1990 to 1993, all inhabitants aged 55 years and older from a district (Ommoord) located in Rotterdam, the Netherlands, were asked to participate in the original cohort (denoted hereafter as RS-I). In total, 7,983 individuals agreed to participate (response rate 78%). An extension of the original cohort was initiated in 2000 (denoted hereafter as RS-II). Within this subcohort, all inhabitants from Ommoord aged 55 years and older, and not already participating in RS-I, were asked to participate in RS-II. In total, 3,011 individuals agreed to participate (response rate 67%). Follow-up examinations were conducted approximately every 4-5 years after baseline. The Rotterdam Study has been approved by the medical ethics committee according to the "Wet Bevolkingsonderzoek ERGO" (Population Study Act Rotterdam Study), executed by the Ministry of Health, Welfare and Sports of the Netherlands and written informed consent was obtained from all study participants.

Study population

We included all participants from RS-I and RS-II who were successfully genotyped and completed at least one questionnaire about their current depressive symptoms (collecting started in the second follow-up round, 1997). Assessments of depressive symptoms at which participants were using tricyclic antidepressants or other antidepressants were excluded from the study.

Drug exposure

More than 99% of the participants have their drug prescriptions filled at seven fully computerized regional pharmacies. The available data included the Anatomical Therapeutical Chemical (ATC) code, the dispensing date, the total amount of drug units per prescription, the prescribed daily number of units, and the product name of the drug. SSRI drug exposure (ATC code: N06AB) was defined as 'current' if the center visit date fell within a prescription episode. The duration of the episode was calculated by dividing the total number of filled tablets/capsules/suspensions by the daily-prescribed number. Participants without a SSRI drug prescription at a center visit were considered as non-users. Ever users of antidepressants were defined based on the presence of at least one SSRI prescription between start of follow-up and December 31, 2011.

Genotyping

Genotyping of the polymorphisms in RS-I was performed with the Infinium II HumanHap 550K Genotyping BeadChip® version 3 (Illumina, San Diego, CA, USA). For RS-II, genotyping was performed with the Infinium II HumanHap 550K + 610K Quad Genotyping GenomeStudio® (Illumina, San Diego, CA, USA). Polymorphisms

were genotyped according to the instructions of the manufacturer. Quality controls and results of the genotyping are published elsewhere[18]. The number of polymorphisms was increased by imputing surrounding SNPs using the Caucasian Hapmap population (release 22) with MACH software version 1.0.15 (RS-I) and version 1.0.16 (RS-II)[19]. Additive genetic models were used. SNPs located on the X chromosome were not considered for analyses.

Assessment of depressive symptoms

Current depressive symptoms were screened with a Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D). The questionnaire resulted in a score, ranging between 0 and 60, with higher scores indicative of more depressed feelings[20, 21]. The CES-D score was not normally distributed and therefore log transformed (ln(CES-D+1)).

Statistical Analyses

The CES-D questionnaire was completed at multiple center visits during follow-up. Therefore, all analyses adjusted for the within-person correlation of repeated measures. Specifically, we used Generalized Estimating Equations (GEE)[22]. However, the robust standard error estimates in GEE have an inflated type I error when the number of participants is small[23]. In our interaction analyses, many SSRI-SNP strata were small, so type I error was higher than the specified significance level. To reduce type I error, we modified the reference distribution to a *t*-distribution with degrees of freedom approximated via Satterthwaite's methods[24, 25]. Analyses were adjusted for age and sex.

For SNPs with a low minor allele frequency, the number of participants using SSRIs was too small to observe valid results, even with our correction to the *t*-distribution. Therefore, we filtered out the SNPs with the lowest allele frequencies and imputation quality, i.e. those with values less than 10 in the following calculation: two times the estimated number of independent visits at which participants were using SSRIs[26] times the SNP imputation quality times the minor allele frequency. A more detailed description is published elsewhere[27]. A weighted *Z*-statistic-based meta-analysis with genomic control was performed using METAL to combine the results from RS-I and RS-II[28]. All SNPs with a p-value < 5e-5 were pruned using the clumping function in PLINK to obtain independent SNPs. We grouped the SNPs within 500 kb of the index SNP that have r^2 >0.2 and had a p-value below 0.05[29]. We considered a SNP-SSRI interaction to be statistically significant if the p-value was less than 5e-8. Of the top 10 independent SNPs with the lowest p-values, beta estimates were calculated using inverse variance meta-analysis based on the beta estimates calculated in the two separate cohorts.

To evaluate and minimize the effect of confounding by indication, we conducted two secondary analyses. First, p-values for main effect (association between the SNP and CES-D scores) were calculated among untreated participants. Second, we repeated all analyses restricting the reference population to participants who ever used an SSRI during follow-up.

Interpretation of the data can be done as follows: Positive point estimates of the regression coefficient for drug-gene interaction indicate greater differences in CES-D scores between SSRI-treated and non-treated participants in carriers of the effect allele. The higher CES-D scores in carriers of the effect allele might therefore be indicative of poorer antidepressant drug response. Similarly, negative point estimates are indicative of an enhanced antidepressant drug response.

Pathway analysis and SNP look-up

Following the genome-wide analyses, we additionally examined the results of SNPs from 28 genes that are related to the serotonergic signaling pathway[30]. SNPs were included if they were located on the gene or within 10kb in either direction. Two genes (*HTR2C* and *MAOA*) are located on the X chromosome, and were excluded from our analyses, leaving 26 genes for consideration. Locations, as obtained from HapMap[19], are presented in the supplementary table 1. In total, 3,654 genotyped and imputed polymorphisms located in or around the genes associated with the serotonin pathway were present in our imputed dataset and were used for analyses. Because of the linkage between SNPs closely located on the genome, we did Bonferroni correction for multiple testing for independent SNPs ($R^2 < 0.4$) using PLINK[29]. The threshold for statistical significance in this subset of SNPs was p = 1.2e-4 based on approximately 400 independent SNPs. Also, we visualized the mean CES-D score across genotypes of the 102C>T (rs6313) polymorphism in the *HTR2A* gene, which has been repeatedly associated with antidepressant drug response[6, 31-33]. We additionally looked at the loci that were previously observed to be suggestively associated with antidepressant response in previous GWAS analyses in the *UBE3C* (rs6966038), *BMP7* (rs6127921), *RORA* (rs809736), *CDH17* (rs6989467), *EPHB1* (rs1502174) and *UST* (rs1126757) genes[9-11]. Furthermore, we looked at the rs6265 polymorphism in the *BDNF* gene (Val66Met), which has previously been observed in a meta-analysis on antidepressant drug response[6].

Results

Characteristics of the study population

In total, 4 316 participants from RS-I and 2,127 participants from RS-II were included, who had a combined total of 14 937 CES-D assessments (table 1). During 151 visits of RS-II and during 122 visits of RS-II, participants were using an SSRI. Both cohorts had more females than males (58.5% and 54.3%, respectively). The median CES-D score was higher for participants who were prescribed a SSRI (median score of 9 or 10, depending on the cohort) compared with non-use participants (median score of 3).

Table 1 Baseline characteristics of the study population

	RS-I (N = 4,316)	RS-II (N = 2,127)
SSRI exposure[1], N	151	122
Age (years), mean (SD)	71.9 (7.7)	64.8 (7.9)
Females, N (%)	2,523 (58.5)	1 153 (54.3)
CES-D score, median (IQR)		
– SSRI exposed	10 (3 – 18)	9 (3 – 18)
– SSRI unexposed	3 (1 – 9)	3 (0 – 7)

Abbreviations: N, number of participants; SSRI, Selective Serotonine Reuptake Inhibitor; RS-I, Rotterdam Study 1; RS-II, Rotterdam Study 2; CES-D, Center of Epidemiological Studies of Depression Scale; IQR, Interquartal range. [1]Total number of CES-D assessments at which participants were using an SSRI. Adjusted for the correlation between visits, these exposed visits were from a total of 175 independent participants (94 from RS-I and 81 from RS-II).

Genome Wide Association Study

Cohort-specific and meta-analytic QQ-plots of the analysis in all and ever-users are presented in supplementary figures 1 and 2, respectively. The corresponding –log(p-value) plot of SNPs present in both cohorts is presented in figure 1.

Of the 10 independent SNPs with the lowest p-values in the genome-wide analysis (table 2), one SNP (rs10514475 located in the *MAF* gene) reached genome-wide significance (p-value = 2.13e-8). However, heterogeneity was high and the drug-gene interaction did not reach statistical significance in the restricted sub-analysis in which only RS-I contributed. All other SNPs displayed in table 2 had p-values below 1e-5, but none of these reached genome-wide significance. For these, heterogeneity and p-value for the association with CES-D score were mostly non-significant. Also, in most cases, these SNPs reached nominal statistical significance when the reference population was restricted to ever-SSRI-users. Regional plots of the meta-analysis from these loci are presented in supplementary figure 3.

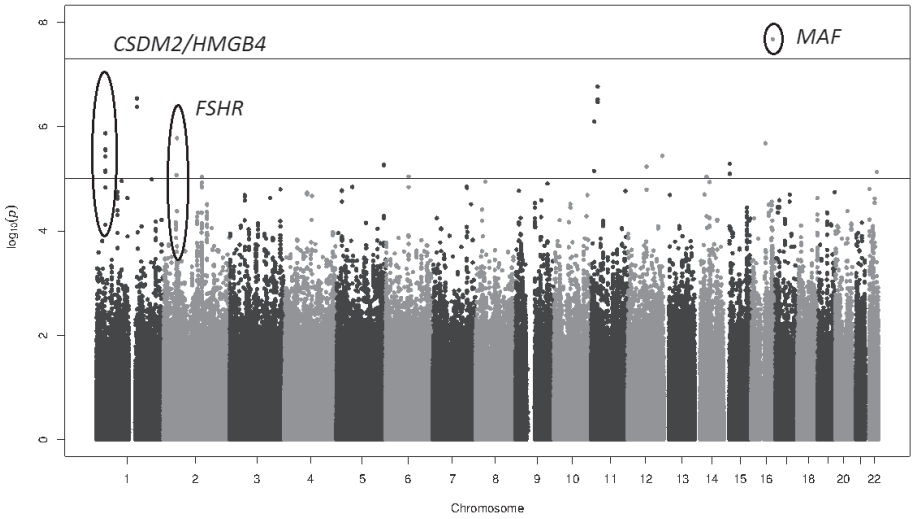

Figure 1 **Manhattan plot of the genome-wide meta-analysis**

Manhattan plots show the chromosomal position of SNPs in comparison to the (-log) p-value. Only SNPs present in both study cohorts are displayed. Three loci with special interest on chromosomes 1, 2 and 16 are circled. The lower horizontal line indicates uncorrected statistical significance (p-value < 1e-5). The upper horizontal line indicates Bonferroni-corrected genome-wide significance (p-value < 5e-8).

A summary of the known biological functions of the genes in table 2 is given in supplementary table 2.

Serotonergic signaling related genes

The 10 independent SNPs with the lowest p-values from genes that are part of the serotonergic signaling pathway are presented in table 3. Within these genes we found one locus, rs6108160 in the *PCLB1* gene, showing a significant interaction with SSRI use on depressive symptoms (p-value = 8.08e-5). The direction of the effect allele of the rs6108160 was positive, had low heterogeneity among the studies (p-value = 0.38) and still reached nominal statistical significance when the reference population was restricted to ever-users (p-value = 1.1e-3). The other independent loci, close to statistical significance, were located in the *HTR2A*, *ADCY2* and *GNAS* genes. In all cases, heterogeneity was small and the interaction remained statistically significant when the analysis was restricted to ever-SSRI-users.

A graphical visualization of the mean depressive symptoms across 102C>T genotype, which was in linkage with other SNPs in the *HTR2A* gene presented in

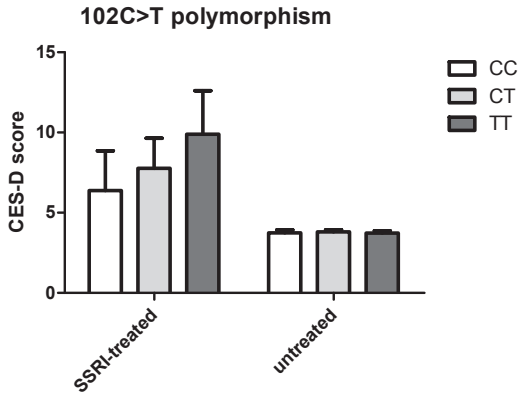

Figure 2 **Mean CES-D scores across 102C>T genotypes, stratified by treatment group**

Data presented as the geometric mean and the 95% confidence interval.

table 3, are presented in figure 2. Within SSRI-treated participants, carriers of the CC allele had lower CESD scores compared to CT and TT allele carriers. No association was observed within untreated participants.

Previously observed suggestive GWAS hits and meta-analyses

Analyses of SNPs previously observed to have suggestive associations with antidepressant drug response are presented in supplementary table 3. None of the seven previously reported SNPs (located in the *UBE3C, BMP7, RORA, CDH17, EPHB1, UST* and *BDNF* genes) reached nominal statistical significance (all p-values > 0.17) in the combined sample of RS-I and RS-II.

Discussion

We investigated whether the use of drug-gene interaction models on repeated cross-sectional CES-D assessments can identify genetic loci of potential interest in antidepressant drug response in population-based studies. Our findings can be divided into three parts. First, the genome-wide association analysis, we identified a significant finding in the *MAF* gene, but there are reasons to question the validity of this finding because of great heterogeneity. Second, in genes related to the serotonergic pathway, genetic variation in the *PLCB1* gene and the 102C>T polymorphism in the *HTR2A* gene showed statistically significant associations with depressive symptoms that differed between treated and untreated participants. And

Table 2 **Top 10 independent loci from the Genome-wide analysis**

SNP	Chr	Position	Gene	No. of supporting SNPs[1]	Effect allele	MAF	Beta (SE)[2]	P-value	P-value for main effect	Heterogeneity P-value	P-value in ever users
rs10514475	16	78549193	*MAF*	5	A	0.06	1.11 (0.12)	2.13e-8	6.9e-3	2.71e-11	5.1e-1[3]
rs11028175	11	24766016	*LUZP2*	12	A	0.07	0.87 (0.14)	1.72e-7	0.67	6.74e-1	4.9e-4[3]
rs3790515	1	150052996	*RORC*	14	T	0.08	0.79 (0.13)	2.89e-7	0.81	4.44e-2	2.6e-5
rs954123	11	12450877	*PARVA*	1	A	0.43	-0.52 (0.09)	8.09e-7	0.38	1.44e-1	2.4e-5
rs483069	1	34197078	*CSMD2 / HMGB4*	9	A	0.32	-0.46 (0.09)	1.35e-6	0.04	5.29e-1	4.0e-7
rs17835319	2	49495060	*FSHR*	40	A	0.12	0.74 (0.14)	1.67e-6	0.01	1.92e-1	5.5e-5
rs3743772	16	52094924	*AKTIP*	20	A	0.07	-0.64 (0.10)	2.09e-6	0.53	5.54e-7	9.2e-1[3]
rs12812736	12	126640965	*intergenic*	4	T	0.14	-0.55 (0.12)	3.66e-6	0.94	1.80e-2	2.2e-5
rs824192	15	21553420	*NDN*	3	T	0.41	-0.43 (0.09)	5.22e-6	0.12	5.14e-1	2.0e-4
rs794728	5	173805592	*MSX2*	13	T	0.48	-0.42 (0.08)	5.43e-6	0.74	5.20e-1	2.6e-4

Abbreviations: SNP, Single Nucleotide Polymorphism; Chr, Chromosome; MAF, Minor Allele Frequency; *MAF* (gene), v-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian); *LUZP2*, leucine zipper protein 2; *RORC*, RAR-related orphan recepto C; parvin, alpha; *HMGB4*, high mobility group box 4; *CSMD2*, CUB and Sushi multiple domains 2; *FSHR*, follicle stimulating hormone receptor; *AKTIP*, AKT interacting protein; *NDN*, necdin; *MSX2*, msh homeobox 2. [1] Number of SNPs in linkage disequilibrium with the top SNP (r^2>0.2) within 500 KB, with a p-value less than 0.05. [2] Beta estimates represent the additive interaction effect size of the effect allele in treated participants compared to untreated participants. [3] Genetic variant did not pass the quality control in Rotterdam Study 2 for the ever user analysis.

Table 3 **Top 10 independent loci from genes related to the serotonergic signaling pathway**

SNP	Chr	Position	Gene	No. of supporting SNPs[1]	Effect allele	MAF	Beta (SE)[2]	P-value	P-value for main effect	Heterogeneity P-value	P-value in ever users
rs6108160	20	8520225	*PLCB1*	9	A	0.14	0.46 (0.11)	8.08e-5	0.72	3.84e-1	1.1e-3
rs16995121	20	8632506	*PLCB1*	2	A	0.10	-0.55 (0.14)	3.60e-4	0.32	1.92e-2	2.0e-3
rs9567743	13	46338801	*HTR2A*	28	T	0.23	-0.38 (0.11)	8.05e-4	1.00	6.74e-1	4.2e-4
rs17288723	13	46355694	*HTR2A*	3	T	0.13	-0.41 (0.13)	1.71e-3	0.72	3.84e-1	2.3e-3
rs16994436	20	8067554	*PLCB1*	22	T	0.18	-0.45 (0.14)	1.83e-3	0.19	5.97e-1	3.9e-3
rs4333285	5	7550783	*ADCY2*	13	A	0.33	-0.30 (0.10)	2.16e-3	0.92	9.18e-1	1.4e-2
rs17826395	5	7556056	*ADCY2*	31	A	0.22	0.32 (0.10)	2.31e-3	0.41	0.54e-1	1.4e-3
rs6056037	20	8649385	*PLCB1*	0	T	0.48	-0.29 (0.10)	2.75e-3	0.18	9.97e-1	2.4e-2
rs965808	20	56841821	*GNAS*	7	A	0.22	0.32 (0.12)	6.64e-3	0.93	7.57e-1	7.0e-3
rs6039148	20	8273488	*PLCB1*	17	T	0.15	-0.33 (0.12)	7.31e-3	0.20	4.47e-1	5.3e-3

Abbreviations: SNP, Single Nucleotide Polymorphism; Chr, Chromosome; MAF, Minor Allele Frequency; *PCLB1*, phospholipase C, beta 1; *HTR2A*, 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled; *ADCY2*, edenylate cyclase 2; *GNAS*, GNAS complex locus.

[1] Number of SNPs in linkage disequilibrium with the top SNP (r^2>0.2) within 500 KB, with a p-value less than 0.05.

[2] Beta estimate represents the additive effect size of the effect allele in treated participants compared to untreated participants.

third, none of the previously published suggestive findings in GWAS were replicated in our study.

For most of the suggestive loci in the genome-wide analysis, no evidence of any relation with depression or antidepressant drug response was found in the literature. However, some indications of a relationship were found for the SNPs in the *FSHR* and *CMD2/HMGB4* genes. In zebrafish, expression of the *FSHR* gene was decreased upon fluoxetine stimulation[34]. In mice and humans, expression of the *HMGB4* gene was altered when taking escitalopram[35]. However, a role of variations in these two genes in antidepressant drug response has yet to be confirmed[35].

The locus in the *PLCB1* gene that was statistically significant in the analysis of the serotonergic signaling pathway has not previously been associated with antidepressant drug response. However, a publicly available patent on a pharmacogenetic testing kit for the prediction of the success rate of antidepressant drug treatment includes the *PLCB1* gene that has a large number of SNPs statistically significantly associated with antidepressant drug response[36]. The specific loci associated with antidepressant drug response, the direction of effects and the effect sizes were not presented. The positive direction of the interaction effect estimate indicates that the CES-D score is higher in SSRI-treated than in untreated study participants, which means that this variant might be associated with an impaired treatment response. In addition, two independent SNPs in the *HTR2A* gene had interaction effects that were close to statistical significance. In the literature, this gene, which encodes one of the serotonin receptors, was repeatedly associated with antidepressant drug response and the direction of effect of the 102C>T polymorphism was similar to what has been described previously[6, 31-33]. Despite these positive findings, we did not confirm other variants in the *TPH1*, *HTR1A* and *SLC6A4* genes, which were found previously in a meta-analysis[6]. Other studies also had mixed success in confirming these results. The SNP in the *THP1* gene was not confirmed in later studies[37]. Additionally, SNPs in the *THP1* gene were observed to affect antidepressant treatment response only in certain subtypes of depression[7]. Also for the *HTR1A* receptor, a subsequent meta-analysis did not show a significant effect on antidepressant response[8]. For the *SLC6A4* gene the widely replicated 5-HTTPLR polymorphism is a variant tandem repeat which is not measured on GWAS platforms, and thus not studied within our study population.

The loci that were close to statistical significance in the individual published GWAS studies on antidepressant drug response, were not identified in the other published GWAS studies as well as in the meta-analysis of the three individual GWAS studies[9-12]. Reasons for this might include small sample size and the lack of a reference population. However, it also confirms that reporting loci close to statistical significance, without a biological basis, might be a finding by chance

rather than a true association with antidepressant drug response[13]. They were not replicated in our study population either. In our study population, we did not find a nominal statistical significant drug-gene interaction with the rs6265 genetic variant in the *BDNF* gene, previously consistently observed to associate with antidepressant response[6]. However, other meta-analyses showed that this variant was predominantly associated with antidepressant response in Asian populations, and not in Caucasian populations as ours[38, 39].

This study has a couple of strengths and limitations which should be addressed. The use of drug-gene interaction analyses allowed us to distinguish genes associated with depression from genes associated with antidepressant drug response[14], and it might also reduce potential confounding by the natural course of the depression[13]. Further, the use of repeated cross-sectional CES-D assessments increases the statistical power without increasing the number of unique participants[40]. Potential confounding by indication was addressed in two ways: first, the reference population comprised participants who were not treated, but still had a CES-D score indicative of a clinical depression. And second, when the reference population was restricted to those who ever used an SSRI during follow-up, similar results were obtained for most SNPs. The identified SNPs are therefore likely not associated with the use of SSRIs (and thus indirectly with depression). For this reason, this suggests that the use of a reference population comprising all untreated participants is valid in the search to identify loci associated with antidepressant response. Nevertheless, we cannot fully exclude the possibility that a genetic variant is associated with placebo response instead of the drug response due to the use of the cross-sectional assessments. Therefore, genetic variants observed in studies as ours should be replicated in studies with more frequent assessments of depressive symptoms. The effect of recall and information bias is negligible as drug use was obtained from pharmacy dispensing records. A limitation of our study is the relatively low sample size to detect genome-wide significant hits in loci that are plausible to affect antidepressant response. Performing such an analysis requires a larger sample size to increase the statistical power to identify loci associated with antidepressant drug response at a genome-wide level[41]. When we restricted our study to a hypothesis-driven approach (restriction to the serotonergic pathway[30]), loci in the *PCLB1* and *HTR2A* were identified. The benefit of restricting the analysis to a single pathway is that it is already partly hypothesis driven and has a higher Bonferroni corrected significance threshold than the genome-wide analysis. Furthermore, our study population was older compared with other studies investigating the genetic basis of antidepressant response, which limits the comparability between studies[9-12]. In addition, the older age might modify antidepressants response by multiple mechanisms, which introduces more random error in the study outcome, which makes it more difficult to reach statistical significance.

In summary, the recognition of loci in biologically plausible genes associated with antidepressant response indicates that the methods proposed in this study can be used to identify potential loci of interest that are associated with antidepressant drug response. Population-based studies with cross-sectional assessments of depressive symptoms and drug exposure can contribute to the search for genes involved in the response to antidepressants.

Supplementary material

Supplementary material published online (http://www.journalofpsychiatricresearch.com/article/S0022-3956(15)00006-0/addons).

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CHAPTER 7

GENERAL DISCUSSION

In this thesis, we aimed to investigate the utilization (**Chapter 2**) and unintended effects (**Chapters 3, 4, and 5**) of antidepressant drugs in older adults and elderly. We additionally aimed to study the role of common genetic variation in antidepressant response and adverse drug reactions (**Chapter 6**).

In this chapter, we will discuss the main findings of the different conducted studies. Furthermore, some methodological considerations to investigate antidepressant-related research questions in cohort studies will be discussed. Finally, we will provide some recommendations for future research.

Main findings

Utilization of antidepressant drugs in Dutch primary care

In a dynamic population-based cohort study, embedded in a general practitioner's health care database in the Netherlands, we studied prevalence and incidence of antidepressant drugs between 1996 and 2012. We observed that the prevalence of prescribing of antidepressants increased during this period, in particular of selective serotonin reuptake inhibitors (SSRIs). However, the incidence of prescribing of antidepressants, and specifically of SSRIs, decreased from 2000 onwards. These opposing trends may seem counterintuitive but are most likely explained by an increased duration and increased recurrence of antidepressant drug use in more recent years, as previously observed in other populations[1, 2]. In addition, since 2003 the multidisciplinary guidelines for the treatment of depression are more restrictive with respect to pharmacological treatment of depression, and might thus have contributed to the decreased incidence of prescribing of antidepressants, in particular of SSRIs[3]. On the other hand, antidepressants were also increasingly prescribed for off-label indications, which could have made the decrease in incidence less explicit[4, 5]. Nevertheless, similar results were observed in other populations[2, 6, 7].

We additionally observed that in the period from 1996 to 2012, antidepressants, and specifically tricyclic antidepressants (TCAs), were less frequently prescribed as the first drug for the treatment of depression, but increasingly for sleep disorders and neuropathic pain. Furthermore, the preference of SSRIs for the treatment of depression, as defined in the Dutch multidisciplinary guidelines of 2003, is reflected by the highest percentage of SSRIs being prescribed for depression in our study population[3].

Adverse drug reactions related to the use of antidepressant drugs

There are many unintended effects which have been attributed to the use of antidepressants, but some have not been conclusively documented. Furthermore,

premarketing studies investigating intended and unintended effects of antidepressants often exclude elderly. Given that antidepressant use is highest in this population, a relatively large population is at risk to develop adverse drug reactions. Moreover, this population is already at higher risk as a result of a relatively slow elimination of the drug from the body or increased susceptibility. In the following paragraphs, the conducted studies described in **chapters 3, 4 and 5** of this thesis will be discussed and placed into the context of drug safety of antidepressants.

Antidepressant use, effects on body weight, and unintended endocrinologic effects

The neurotransmitter serotonin plays a pivotal role in depression and other psychiatric disorders, and inhibition of the reuptake of serotonin by SSRIs is the starting point of their therapeutic effect. Also, serotonin affects energy metabolism and weight control[8]. Targeting serotonergic signaling with SSRIs is therefore hypothesized to affect body weight and outcomes of energy metabolism (e.g., lipid metabolism, glucose-insulin metabolism).

In **chapter 3.1**, we reported that the use of SSRIs was associated with an increased BMI. This association was probably a serotonin-specific effect as a similar result was observed for use of antidepressants with a high binding affinity to the serotonin reuptake transporter[9, 10]. Previous studies were predominantly conducted using a treated-only study design[11-14], in which depressive patients who started antidepressant treatment were followed over time. However, the observed weight gain in those studies could be the result of the drug or be the result of a relief of depressive symptoms. Because users of antidepressants were compared with non-users of antidepressants (who also had depressive symptoms) in our study, and we statistically adjusted for depressive symptoms in separate analyses, we provided evidence in favor of SSRI-induced weight gain independent of depression. Additionally, we observed that SSRI-induced weight gain was only present in females. An SSRI-induced weigh gain in females was observed in one other study, but the authors interpreted this result with caution as data was self-reported, and might have been biased[15]. Interestingly, some studies suggested that serotonergic signaling is different between males and females[16, 17]. However, whether this underlies the differences in weight gain by serotonin-specific antidepressants between males and females is unclear.

In **chapter 3.2**, we observed that, independent of BMI, use of antidepressants with a high affinity to the serotonin reuptake transporter was associated with a higher LDL cholesterol in serum. The effect size of a 0.2 mmol/L higher level of LDL cholesterol was similar as what was observed previously in healthy volunteers[18, 19]. Secondly, we observed that in participants with the CC genotype of the 102C>T

polymorphism in the 5-hydroxytryptamine (serotonin) receptor 2A (*HTR2A*) gene, the difference in serum LDL cholesterol between users of antidepressants with high affinity to the serotonin transporter and non-users of antidepressants was 0.47 mmol/L. As the effect size was dependent on the genotype, this result suggests effect modification by the 102C>T polymorphism. This polymorphism has previously been associated with metabolic abnormalities and antidepressant drug response[20-25]. Both observations in this study add to the evidence of a drug-induced increase in LDL cholesterol level. However, although biologically relevant, the clinical relevance might be limited considering the modest increase.

In **chapter 3.3**, we reported that use of SSRIs was associated with a lower level of insulin, a lower insulin secretion, and a higher peripheral insulin sensitivity in a non-diabetic population. However, no association was observed between SSRI use and glucose, which was similar to findings from an earlier study[26]. A lower degree of insulin secretion was hypothesized, as serotonin has been shown to affect insulin secretion in *in-vitro* experiments[27]. Furthermore, the lower level of insulin was observed in some other human studies[28, 29]. Perhaps more surprising was the lower peripheral insulin resistance in users of SSRIs, as previous studies have shown that SSRIs are associated with a higher risk of type 2 diabetes mellitus[26, 30, 31]. Possibly, the higher peripheral insulin sensitivity (lower peripheral insulin resistance) was a peripheral reaction to the lower secretion of insulin by the pancreas to preserve glucose-insulin homeostasis. In addition, use of SSRIs for more than 90 consecutive days was associated with a higher incidence of starting insulin treatment in a cohort of diabetic patients using oral glucose-lowering agents, a condition which has previously been associated with a higher risk of mortality[32, 33].

In none of the studies in this chapter, an association was observed with use of TCAs although this may be the result of lower numbers. Nevertheless, it seems that unintended endocrinological effects are predominantly the result from effects of drugs acting on the serotonergic autonomous nervous system.

Antidepressant use and adverse effects on electrophysiology

A prolonged heart rate-corrected QT interval (QTc) has been associated with an increased risk of sudden cardiac death[34, 35]. Many antidepressants have been associated with a prolonged QTc[36]. However, there are reasons to question the validity of these findings. In **chapter 4.1**, we reported that TCAs are associated with a prolonged QTc when corrected for heart rate with the formula by Bazett[37]. However, no prolonged QTc was observed when we corrected for heart rate with the formula by Fridericia[38] or by a formula based on regression coefficients. Of the individual TCAs, only maprotiline was associated with a longer QTc when corrected with Fridericia, although effect size was smaller than when corrected with Bazett. The

overestimation of QTc with Bazett's formula in patients with a higher heart rate may explain this difference[39, 40]. Because of anticholinergic properties of TCAs[41], which increase the heart rate, the QTc was consequently overestimated when corrected for heart rate with Bazett's formula. Other drugs that are described to have both anticholinergic and QTc-prolonging properties might have to be reinvestigated as a consequence of this finding[36, 42]. For example, several antipsychotics that probably induce QTc prolongation, also exert anticholinergic properties[41]; the QTc prolongation of these drugs might have been overestimated as well. For this reason, the American Food and Drug Administration (FDA) recommends the use of Fridericia's formula instead of Bazett's formula, which has been used most frequently in the clinic[39, 40]. In **chapter 4.2,** we investigated the association between SSRIs and QTc prolongation. In this study, we already used only the QTc corrected with Fridericia. We observed that of the individual SSRIs only citalopram was associated with a considerably longer QTc than non-use of antidepressants. Furthermore, in participants aged 60 years and older who were prescribed citalopram with a maximum daily dosage of 20 mg, an increase in QTc was observed despite recommended restrictions of the FDA. From these two studies we could conclude that for the prescribed antidepressants in our study population, we only found evidence of QTc prolongation for citalopram and to a lesser extent also for the TCA maprotiline.

A different measure obtained by electrocardiogram is heart rate variability. In **chapter 4.3**, we described the observation that TCAs and SSRIs were associated with a lower variability in heart rate than non-users of antidepressants, a condition indicative of an imbalance between the sympathetic and parasympathetic autonomous nervous system[42-44]. To date, only one population-based cohort study was conducted which reported similar results as ours[45]. However, in a longitudinal analysis we observed only that start of TCA treatment was associated with a reduction in heart rate variability between two consecutive examination rounds. This suggests that other factors than the drug itself might underlie the observed association between SSRIs and lower heart rate variability, whereas for TCAs the lower HRV is more likely a drug effect.

Antidepressant use and myocardial infarction and cerebral microbleeds

Serotonin promotes the activation and aggregation of blood platelets[46, 47]. A decreased activation and aggregation of blood platelets has been observed when SSRIs were tested in *in-vitro* experiments[48, 49]. This has led to the hypothesis that the use of antidepressants, and in particular SSRIs, increase the risk of bleeding, and ultimately affects the risk of outcomes such as cerebral microbleeds. As platelet

activation and aggregation also play a role in the onset of myocardial infarction, antidepressants have also been hypothesized to reduce its risk.

In line with this, we report in **chapter 5.1**, that antidepressant use was associated with an approximately 30 percent lower risk of myocardial infarction. Of the different antidepressant drug classes, SSRI use was associated with the lowest risk of myocardial infarction, although marginally not statistically significant. However, the effect size was similar to previously reported studies[50, 51]. Furthermore, one randomized controlled trial reported a 30% decreased risk in users of sertraline compared with users of placebo treatment, although this was not statistically significant due to a limited number of cases[52]. Other studies that did not observe an association might have been affected by depression, which has been associated with an increased risk of myocardial infarction[53-58]. Nevertheless, our study provides additional evidence of a protective effect of antidepressants, and in particular SSRIs, on the risk of myocardial infarction. In **chapter 5.2**, we did not observe an association between the use of antidepressants with a high binding affinity to the serotonin reuptake transport and an increased prevalence of cerebral microbleeds and white matter lesions. Nevertheless, our study had sufficient statistical power to detect a 22% higher prevalence of cerebral microbleeds or white matter lesions, which was the estimate previously observed for the association between SSRIs and brain hemorrhages[59]. The cross-sectional design of our study and the disability to detect microbleeds at the moment they occur might explain the discrepancy between the two studies described in this chapter. A better approximation of the risk of microbleeds when using antidepressants with high affinity to the serotonin reuptake transporter might be achieved using longitudinal studies in a population in which individuals with microbleeds or white matter lesions at baseline are excluded.

Contribution of genetics to the response and safety of antidepressant drugs

The ability to respond to antidepressant treatment has previously been shown to be partly genetically determined[60-62]. However, only few genetic regions have been identified to be associated with antidepressant drug response and adverse drug reactions[24].

In **chapter 6.1**, we reported that common genetic variation in the *ABCB1* gene (notably 1236C>T, 2677G>T, 3435C>T), which encodes P-glycoprotein, was associated with a higher risk of early switching and early discontinuation of antidepressant treatment, which were proxy indicators of the occurrence of an adverse drug reaction. In our statistical models, explained variance did not exceed 10.4% in case of switching and 2.5% in case of discontinuation. Explained variance of discontinuation is probably lower because there may be other reasons for stopping

than adverse drug reactions. Nevertheless, the observed associations were in line with previously published studies[63, 64].

In **chapter 6.2**, we applied drug-gene interaction models on cross-sectional assessments of depressive symptoms in a population-based study as a method to identify genetic regions associated with antidepressant drug response. We hypothesized that when the association between a genetic variant and depressive symptoms is different for treated and untreated participants, this variant might be associated with antidepressant drug response. Nevertheless, in general, our study did not identify genetic regions that showed statistically significant interaction with SSRI use on depressive symptoms, which is at least partly explained by the limited sample size. Collaborating with other population-based studies in the future will increase the statistical power and increase the probability of identifying genetic regions that are statistically significant[65]. However, our primary intention was to focus on the question whether drug-gene interaction models can be used to study antidepressant drug response. Several genetic regions close to statistical significance might be of interest for future studies. Of those, the observed variants in the *HMGB4* and *FSHR* genes might have the greatest potential. In lower organisms, expression of these genes was previously observed to be influenced by SSRIs[66, 67]. However, their function in antidepressant treatment response is not known and requires more research[66]. Genetic variation in the *PLCB1* gene which is related to the serotonergic signaling pathway, encoding a postsynaptic signaling protein, interacted with SSRI treatment on depressive symptoms. This gene has been described in a patent on a pharmacogenetic testing kit to predict antidepressant drug response[68]. However, no studies were published that have investigated the association between common genetic variation in the *PLCB1* gene and antidepressant response. The applicability of the drug-gene interaction model to study antidepressant drug response was further supported by the observation that genetic variation in the *HTR2A* gene, a well-known gene associated with antidepressant drug response[24], was replicated in our study population. Nevertheless, it should be noted that obtained results from studies as ours are more difficult to interpret and not useful for prediction purposes, and should only be used to come to new hypotheses for future research.

Still, little is known about the genetic basis of antidepressant drug response. To predict which patients are more likely to respond to antidepressant drug treatment than others, more effort is required. Nevertheless, it should be noted that genetic variants observed for other outcomes predict only a few percent of the population variation. With a few exceptions such as variation in the important drug-metabolizing isoenzyme cytochrome P450 *CYP2D6,* future findings will more likely increase our biological understanding than improve prediction of antidepressant drug response and adverse drug reactions.

Methodological considerations

Research on outcomes related to the use of antidepressant drugs is challenging for a number of reasons. Below, we described some methodological considerations.

The prospective population-based cohort study

With the exception of chapter 2, all studies described in this thesis have been conducted in the prospective Rotterdam Study cohort[69, 70]. Participants in this cohort were recruited irrespective of disease history. The only criteria were an age above 45 years old and living in the Ommoord district in Rotterdam, the Netherlands. The greatest advantage of this study was that almost 80 percent of the invited inhabitants participated and that information was prospectively collected, thereby limiting potential selection and information bias. Such a population-based study is fundamentally different from studies conducted in health care databases, which contain only information gathered for healthcare purposes. Although the fact that health care databases may be extremely large in sample size makes them suitable for studying rare adverse drug reactions, the quality of the data is dependent on the general practitioner or medical specialist who registers these data. Therefore, information on potential confounding factors in studies conducted in such databases are more likely to be incomplete or biased than the information on potential confounding factors in prospective population-based studies, such as the Rotterdam Study.

However, an important limitation of an unselected population-based study is that there is often a limited number of users of a specific drug (class). Especially for questions related to individual antidepressant drugs, sample sizes might often be too limited to observe a statistically significantly association with a moderate effect size.

Randomized controlled trials are often seen as the "golden standard" to access drug-related research questions. However, prospective population-based cohort studies might provide valid results when well-designed[71]. Nevertheless, causality between exposure and outcome remains difficult to ascertain because of the observational nature of the data from both the prospective cohort as well as the healthcare database. In genetic epidemiology, Mendelian Randomization analyses have been demonstrated to be an effective tool to investigate whether observed associations in cohort studies were causal or the result of confounding or reverse causality[72, 73]. To what extent Mendelian Randomization analyses can contribute to investigate effect modification by genetic variation on associations between exposure to a drug and an unintended drug effect is currently unclear. In **chapter 3.2**, we studied effect modification of the association between antidepressant drug use

and LDL cholesterol levels by the 102C>T polymorphism. As this polymorphism has been previously associated with antidepressant drug response[24], this polymorphism might add evidence for a causal relationship between antidepressant drug exposure and certain study outcomes.

Confounding by depression

Depression is an important confounding factor for assessing associations between antidepressant use and a given outcome. For instance, for questions related to TCA use, confounding by depression might be an issue, even though we have shown that TCAs were often prescribed for other indications than depression (**chapter 2**). In almost all studies described in this thesis, we adjusted for the presence of depressive symptoms. In **chapter 3.1**, we additionally stratified the analysis by depression on the basis of the depressive symptoms screening questionnaire. As the associations were not materially different between participants with or without depression, the observed associations are more likely due to the drug rather than a result of confounding by depression.

The use of a reference group

Most of the unintended antidepressant drug effects described in this thesis were investigated using a treated-only design in previous studies, in particular the studies on weight gain and unintended endocrinological effects. Especially these potentially unintended effects are difficult to study with a treated-only design as the natural course of the depression might interfere with the observed associations. For studies on the genetic role in antidepressant response, simulation studies showed that an overall-population design, which we used in our studies, was superior to studies in which only treated patients were included with respect to the number of false-positive observed associations[74].

We mostly compared the exposure of interest with non-use of any antidepressant. However, in some studies we used TCAs (**chapter 3.3**) or past antidepressant use (**chapter 5.1**) as a reference. This comparison was assumed to be superior compared with non-use of antidepressants as reference, as the groups are more similar with respect to the indication of use. In such situations, confounding by indication is likely smaller than when compared with non-use of antidepressants. Unfortunately, we had often a too low number of users to conduct such analyses in all studies. In these cases, adjustment for the presence of depressive symptoms was assumed to be most optimal.

Future perspectives

The results presented in this thesis provide additional insights into the pharmacoepidemiology of antidepressant drug use in older adults. Nevertheless, more research is required to fully understand the unintended effects of antidepressants in the general older population. A few examples of potential future research directions are described below.

In general, the results from our studies on the unintended effects of antidepressants drugs at a population level show risks of small magnitude and should be interpreted as of limited clinical significance. However, for subgroups of patients, some of the findings could be more relevant. In **chapter 3.1** and **3.2**, we already showed that some associations were in particular observed in specific patient populations, such as females and carriers of a specific genotype. Of these, females might be of special interest for future research in relation to unintended effects of antidepressant drug use. In previous studies, females had a more successful response to SSRI treatment than males[75-78]. For this reason, it is reasonable to hypothesize that the risk of other unintended effects than an increased BMI, as described in **chapter 3.1**, is also different between females and males. Unfortunately, most of our studies were too small in size to study effect modification by sex. Collaborating with other prospective cohort studies as well as large health care databases is therefore required. Nevertheless, other patients characteristics might modify the risk of unintended antidepressant drug effects as well (e.g., kidney function and percentage of body fat), which should be examined as well.

The search for genetic regions associated with antidepressants drug response should continue to improve our knowledge of the biological mechanisms underlying effectiveness and adverse reactions of antidepressants. In **chapter 6.2**, we already reported some potentially interesting genetic regions to be investigated further in other studies. Future studies should not only replicate these findings, but should also continue to investigate whether some genetic regions only associate with antidepressant drug response under certain environmental conditions[79].

If we want to promote personalized medicine of antidepressants drugs, both effect- and risk stratification (identifying subpopulations of patients responding better or worse than others, and patients at particular risk) will be pivotal. However, even if that intention remains with only limited future success, it is still possible to obtain a considerable gain in biological knowledge on antidepressant response and adverse effects.

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CHAPTER 8

APPENDICES

ENGLISH SUMMARY

Over the last decades, antidepressant drug use increased in Western countries, including in the Netherlands. Older adults using antidepressant drugs are of special interest, as they often have a slower metabolism of drugs, impaired renal function, more comorbidities, and more concomitantly used medications. These factors increase the risk of adverse drug reactions, such as vomiting, dizziness and sexual dysfunction. However, multiple potential adverse drug reactions are not yet well-defined and require more research. Limitations in study design as well as in the definition of the study outcome are reasons why potential adverse drug reactions are still not conclusively reported. Furthermore, premarketing studies investigating intended and unintended effects of antidepressants are rarely performed in elderly. In this thesis, the aim was to investigate antidepressant drug use in the Dutch population, and to investigate unintended effects related to antidepressant drug use in an older population. Furthermore, the aim was to identify genetic regions associated with adverse drug reactions and with response to antidepressants.

In **chapter 2**, a study was conducted to investigate antidepressant drug prescribing between 1996 and 2012 in a Dutch general practitioners health cate database. In this study, we observed that prevalent prescribing of antidepressants, and specifically selective serotonin reuptake inhibitors (SSRIs), increased during this period. However, specifically the incidence of prescribing of SSRIs decreased from 2000 onwards. These opposing trends were most likely due to an increased duration of use and an increased prevalence of recurrent episodes during the study period. Although prevalent prescribing was higher among elderly than younger patients, the increase in prevalence and decrease in incidence during the study period were similar in different age groups (e.g., 20 – 40 years and >80 years of age). In addition, incident antidepressant prescriptions, and in particular incident prescriptions of tricyclic antidepressants (TCAs), were less frequently prescribed for depression and more frequently prescribed for indications such as neuropathic pain and sleep disorders in the more recent years of the study period.

In **chapter 3**, three studies were conducted to investigate the association between antidepressant drug use and body weight and endocrinological effects

in the population-based Rotterdam Study cohort. Based on previous studies, we hypothesized that antidepressants specifically targeting serotonergic signaling affect these outcomes. In **chapter 3.1**, the association between antidepressant drug use and body weight was addressed. We reported that the use of SSRIs as well as antidepressants with high binding affinity to the serotonin reuptake transporter, independently from depressive symptoms, was associated with a higher body weight. Additionally, the increase in body weight was only observed in females, and not in males. This study adds evidence of a drug-related increase in body weight. Nevertheless, more research is required to elucidate the biological mechanism why only females showed increase in body weight when using SSRIs or when using antidepressants with high binding affinity to the serotonin transporter. In **chapter 3.2**, a study was conducted to assess the association between antidepressants, categorized by serotonin transporter binding affinity, and serum lipid levels. We described that, independently of body weight, use of antidepressants with a high binding affinity to the serotonin transporter was associated with a 0.2 mmol/L higher level of serum low density lipoprotein (LDL) cholesterol. No association was observed between antidepressants with high binding affinity and other studied serum lipid levels (high density lipoprotein, total cholesterol and triglycerides). The largest difference in serum LDL cholesterol level between users of the high-affinity antidepressants and non-users of antidepressants was observed in homozygous carriers of the C allele of the 102C>T polymorphism in the *HTR2A* gene, which is indicative for effect modification by the 102C>T polymorphism. As the C allele in this polymorphism has previously been associated with increased risk of dyslipidemia, increased boy weight, and enhanced antidepressant drug response, these findings may add evidence of an drug effect on serum LDL cholesterol levels. In **chapter 3.3**, a study was conducted to investigate the association between use of SSRIs and glucose-insulin homeostasis. In our study population, use of SSRIs was associated with a lower level of circulating insulin, a lower degree of peripheral insulin resistance, and a lower level of pancreatic insulin secretion, but not with a higher level of glucose. Furthermore, the use of SSRIs was associated with a higher risk to become insulin dependent in diabetic patients. The expression of the serotonin transporter on pancreatic beta cells might be the underlying mechanism of SSRIs affecting insulin secretion. However, more research is required to fully understand the biological mechanism.

In **chapter 4**, three studies were conducted to investigate the effects of antidepressants on measures of electrophysiology. In the first two studies, we studied the association of antidepressants and prolongation of the heart-rate corrected QT interval; in the last study the effect of antidepressants on heart rate variability was studied. In **chapter 4.1**, we reassessed the association between

the use of tricyclic antidepressants (TCAs) and prolongation of the QT interval. The rationale of this study was that the heart-rate corrected QT interval (QTc) calculated with Bazett's formula is overestimated in users of anticholinergic drugs, which also include the TCAs. In our study population, we observed that the use of TCAs was associated with a longer QTc as calculated with Bazett's formula. However, when other correction methods were used or heart rate was included as an additional covariable in the statistical model, no association between use of TCAs and QTc was observed. Thus, QTc-prolonging properties of individual TCA drugs are the result of the formula of Bazett instead of a real effect on QT. We assessed the association between individual SSRIs and QTc prolongation in **chapter 4.2**. Of the individual SSRIs, only citalopram use was associated with an longer QTc in our study. Even though regulatory authorities restricted the use of citalopram to 20 mg in patients 60 years and older, participants aged 60 years and older in our study using citalopram dosage with a daily dose of more than 20 mg had a longer QTc. Although our study had a limited number of citalopram users, this study may rise questions about the safety of citalopram in high-risk patients populations (e.g., elderly). In **chapter 4.3**, we reported that the use of TCAs as well as SSRIs were associated with a lower variability of the heart rate. A lower heart-rate variability indicates an imbalance between the parasympathetic and sympathetic autonomous nervous system, a condition associated with increased risk of cardiovascular mortality. However, in a longitudinal analysis, only start of TCA treatment was associated with a reduction of the heart-rate variability. Similar, stop of TCA treatment was associated with an increased the heart-rate variability. Therefore, the association between SSRIs and lower heart rate variability might be due to other factors than the use of the SSRI. For this reason, this study only adds evidence of a drug effect of TCAs on heart-rate variability.

In **chapter 5**, we investigated the association between antidepressant drug use and myocardial infarction and cerebral microbleeds. The rationale of these studies was that antidepressants that specifically target serotonergic signaling decrease blood platelet activation and aggregation. In **chapter 5.1**, we observed in a case-control study, which was embedded in the Rotterdam Study cohort, that use of antidepressants was associated with an approximate 30% lower risk of myocardial infarction. Of the different antidepressant drug classes, SSRIs had the lowest effect size, although this was marginally not statistically significant. In **chapter 5.2**, we described that, in a cross-sectional study, use of antidepressants with a high binding affinity to the serotonin reuptake transporter, which strongly inhibits the reuptake of serotonin, was not associated with a higher prevalence of cerebral microbleeds and white matter lesions. Limitations of the cross-sectional design

might be reasons explaining the lack of significant results. Therefore, additional research is warranted, including longitudinal studies.

In **chapter 6**, we conducted two studies to investigate the role of genetics in outcomes related to antidepressant treatment. In **chapter 6.1**, we studied the association between common genetic variation in the P-glycoprotein (encoded by the *ABCB1* gene) and early switching and discontinuation of antidepressant treatment. These outcomes were assumed to be indicative of an antidepressant adverse drug reaction. Previously, variants in this gene have been associated with antidepressant drug response as well as antidepressant adverse drug reactions. In a study comprising all incident antidepressant users of the Rotterdam Study cohort, variant carriers of the *ABCB1* gene were at higher risk to switch or discontinue antidepressant treatment within 45 days. In **chapter 6.2**, we investigated whether the association between common genetic variation and antidepressant drug response can be investigated in population-based cohort studies using drug-gene interaction models. The rationale of this study was that genetic variants that show a different association with depressive symptoms between treated and untreated participants are genetic variants that might be associated with the pharmacological response to antidepressants. In our study, multiple genetic variants might be of interest for follow-up studies, although none was statistically significant after correction for multiple testing. The *HMGB4* and *FSHR* genes, in which genetic variants were identified that were close to statistical significance, have been previously associated to be differentially expressed when fluoxetine was administered to lower model organisms. Therefore, these two genes might be of interest for further studies. Furthermore, of genes encoding for proteins related to the serotonergic signaling pathway, the *PLCB1* gene reached statistical significance. Furthermore, the *HTR2A* gene was replicated, which is a well-known gene involved in antidepressant drug response. These results indicate that, in addition to conventional methods, the use of drug-gene interaction models can help to identify genes involved in antidepressant drug response. However, future studies should be conducted in larger study populations or conducted with a predefined hypothesis.

In the general discussion (**chapter 7**), the results of the different studies were discussed in the light of the current existing literature. Furthermore, methodological considerations and future directions for research in this field were discussed.

NEDERLANDSE SAMENVATTING

Gedurende de laatste tientallen jaren is het antidepressiva gebruik in Westerse landen gestegen, zo ook in Nederland. Ouderen die antidepressiva gebruiken zijn een interessante groep, omdat zij over het algemeen een trager geneesmiddel metabolisme, een lagere nierfunctie en meerdere comorbiditeiten hebben en vaak meerdere geneesmiddelen tegelijkertijd gebruiken. Deze factoren dragen bij aan een verhoogd risico op bijwerkingen, zoals misselijkheid, duizeligheid of een verlaagd libido. Er zijn echter meerdere bijwerkingen die nog niet in detail zijn beschreven en waarnaar meer onderzoek nodig is. Tekortkomingen in studieopzet en verschillen in de definitie van de studie uitkomst kunnen redenen zijn geweest dat potentiële bijwerkingen niet eenduidig zijn geobserveerd. Daarbij worden studies voordat het geneesmiddel op de markt wordt gebracht zelden uitgevoerd in ouderen wegens de vaak aanwezige comorbiditeiten en de daarbij behorende polyfarmacie. Het doel van dit proefschrift was om antidepressiva gebruik in de Nederlandse populatie te bestuderen en om bijwerkingen te onderzoeken die gerelateerd zijn aan het gebruik van antidepressiva in een oudere populatie. Daarnaast hebben we tevens onderzocht welke genetische regio's geassocieerd zijn met bijwerkingen en response van antidepressiva.

In **hoofdstuk 2** hebben we een studie uitgevoerd naar het voorschrijven van antidepressiva tussen 1996 en 2012 in een Nederlandse huisartsendatabase. In deze studie vonden we dat het prevalent voorschrijven van antidepressiva gedurende deze periode steeg. Echter daalde het incident voorschrijven van antidepressiva vanaf 2000. Beide observaties werden voornamelijk gevonden voor de Selectieve Serotonine Heropname Remmers (SSRIs). Deze tegenovergestelde trends worden mogelijk verklaard door een toename van de duur van gebruik of door een toename in het herstarten van gebruik van antidepressiva gedurende de studie periode. Alhoewel het prevalent voorschrijven hoger was in oudere dan in jongere patiënten, de toename van prevalent voorschrijven en de afname in incident voorschrijven gedurende de studie periode was vergelijkbaar voor de verschillende leeftijdscategorieën (bijvoorbeeld: 20-40 jaar of >80 jaar). Incident voorgeschreven antidepressiva, en voornamelijk incident voorgeschreven tricyclische antidepressiva

(TCAs), werden minder vaak voorgeschreven voor depressie en vaker voorgeschreven voor indicaties als neuropatische pijn en slaapstoornissen in recentere jaren van de studie periode.

In **hoofdstuk 3** zijn 3 studies uitgevoerd naar de associatie tussen het gebruik van antidepressiva en lichaamsgewicht en endocriene effecten in de Rotterdam Studie. Gebaseerd op voorgaand onderzoek zouden antidepressiva die specifiek werken op de serotonine signalering effect kunnen hebben op deze uitkomsten. In **hoofdstuk 3.1** hebben we de relatie onderzocht tussen het gebruik van antidepressiva en lichaamsgewicht. We beschrijven hierin dat het gebruik van SSRIs en antidepressiva met een hoge bindingsaffiniteit op de serotonine heropname transporter, onafhankelijk van depressieve symptomen, waren geassocieerd met een hoger lichaamsgewicht. Daarnaast vonden we dat de toename in lichaamsgewicht enkel in vrouwen en niet in mannen. Deze studie voegt daarom verder bewijs toe over een geneesmiddel-gerelateerde toename in lichaamsgewicht. Er is alleen wel verder onderzoek nodig om het biologische mechanisme te ontrafelen waarom vrouwen wel en mannen niet in lichaamsgewicht toenemen wanneer men SSRIs of antidepressiva met een hoge bindingsaffiniteit op de serotonine transporter gebruiken. In **hoofdstuk 3.2** hebben we een studie uitgevoerd naar de associatie tussen het gebruik van antidepressiva, gecategoriseerd op basis van de bindingsaffiniteit op de serotonine heropname transporter, en lipide levels in bloed. We beschreven dat, onafhankelijk van lichaamsgewicht, het gebruik van antidepressiva met een hoge bindingsaffiniteit op de serotonine heropname transporter was geassocieerd met een 0.2 mmol/L hoger LDL cholesterol in serum. Er werd geen associatie gevonden tussen deze groep van antidepressiva en de andere bestudeerde lipide concentraties (triglyceriden, HDL cholesterol en totaal cholesterol). Het grootste verschil tussen niet gebruikers van antidepressiva en gebruikers van antidepressiva met een hoge bindingsaffiniteit werd gevonden in homozygote dragers van het C allel van het 102C>T polymorfisme in het *HTR2A* gen, wat kan duiden op effect modificatie door het 102C>T polymorfisme. Uit voorgaande studies is gebleken dat het C allel van dit polymorfisme is geassocieerd met een verhoogd risico op dyslipidemie, een hoger lichaamsgewicht en een betere antidepressiva response. Deze bevindingen kunnen er daardoor extra op duiden dat een geneesmiddel effect de LDL cholesterolconcentraties kunnen verhogen. In **hoofdstuk 3.3** hebben we de associatie tussen het gebruik van SSRIs en glucose-insuline homeostase onderzocht. In onze studie populatie was het gebruik van SSRIs geassocieerd met een lager insulineconcentratie, een lagere insuline resistentie en een lagere insuline afgifte door de pancreas, maar niet met een lagere glucoseconcentratie in bloed. Daarnaast vonden we dat het gebruik van SSRIs was geassocieerd met een hoger risico op insuline afhankelijkheid in een cohort van diabetes patiënten. De expressie

van de serotonine transporter op beta cellen in de pancreas is mogelijk één van de onderliggende mechanismen hoe SSRIs de afgifte van insuline beïnvloeden. Er is echter wel meer onderzoek nodig om het biologisch mechanisme beter te kunnen begrijpen.

In **hoofdstuk 4** staan 3 studies beschreven naar het effect van antidepressiva op maten van elektrofysiologie in het hart. In de eerste 2 studies hebben we de associatie bestudeerd tussen het gebruik van antidepressiva en het hartslag gecorrigeerde QT interval (QTc). In de derde studie hebben we de associatie bestudeerd tussen het gebruik van antidepressiva en de variabiliteit in hartslag. In **hoofdstuk 4.1** hebben we de associatie tussen TCAs en verlenging van het QT interval onderzocht. De achterliggende gedachte van deze studie was dat voor anticholinerge middelen (waaronder de TCAs) de QTc wordt overschat wanneer men voor hartslag corrigeert met de formule van Bazett. In onze studie populatie vonden we dat het gebruik van TCAs was geassocieerd met een langer QTc wanneer deze gecorrigeerd was met de formule van Bazett. Echter, wanneer andere correctie methoden werden gebruikt of wanneer hartslag als een additionele covariaat werd toegevoegd aan het statistisch model dan was het gebruik van TCAs niet langer geassocieerd met een verlengd QTc. Hierdoor is het aannemelijk dat het QTc verlengende effect van individuele TCA medicijnen het resultaat is van de formule van Bazett en niet een echt effect is op het QT interval. We bestudeerden de associatie tussen individuele SSRIs en QTc verlenging in **hoofdstuk 4.2**. Van de individuele SSRIs was enkel het gebruik van citalopram geassocieerd met een langer QTc in onze studie. Alhoewel de autoriteiten aanraden de maximale dagelijkse dosering van citalopram te verlagen tot 20 mg in patiënten van 60 jaar en ouder, vonden we in onze studie dat een dagelijkse citalopram dosering van maximaal 20 mg nog steeds was geassocieerd met een langer QTc in deelnemers van 60 jaar en ouder. Ondanks dat onze studie een beperkt aantal gebruikers van citalopram had, kunnen deze resultaten wel leiden tot twijfel over de veiligheid in populaties die een hoger risico hebben op bijwerkingen (zoals ouderen). In **hoofdstuk 4.3** beschrijven we dat het gebruik van TCAs en SSRIs zijn geassocieerd met een lagere hartslagvariabiliteit. Een lagere variabiliteit van de hartslag betekent een disbalans tussen het parasympatisch en sympathisch autonoom zenuwstelsel, een conditie die geassocieerd is met een hoger risico op cardiovasculaire mortaliteit in voorgaande studies. Echter, in een longitudinale studie vonden we enkel dat het starten van behandeling met TCAs was geassocieerd met een verlaging van de hartslagvariabiliteit; het stoppen van TCA behandeling was weer geassocieerd met een verhoging van de hartslagvariabiliteit. De associatie tussen het gebruik van SSRIs en de lagere hartslagvariabiliteit zou mogelijk kunnen worden verklaard door andere factoren dan het gebruik van de

SSRI zelf. Om die rede voegt deze studie enkel bewijs toe dat de associatie tussen TCA en lagere hartslagvariabiliteit mogelijk een geneesmiddel-gerelateerd effect is.

In **hoofdstuk 5** hebben we de associatie onderzocht tussen het gebruik van antidepressiva en het krijgen van een hartinfarct en cerebrale microbloedingen. De rationale achter deze studies was dat antidepressiva die specifiek werken op de serotonine signalering de activatie en aggregatie van bloedplaatjes kunnen verminderen. In **hoofdstuk 5.1** vonden we in een case-control studie, welke was ingebed in de Rotterdam Studie, dat het gebruik van antidepressiva was geassocieerd met een ongeveer 30% lager risico op een hartinfarct. Van de verschillende antidepressiva klassen was het gebruik van SSRIs geassocieerd met het laagste risico op MI, alhoewel dit net niet statistisch significant was. In **hoofdstuk 5.2** beschrijven we, in een cross-sectionele studie, dat het gebruik van antidepressiva met een hoge bindingsaffiniteit op de serotonine transporter niet was geassocieerd met een hogere prevalentie van cerebrale microbloedingen en witte stof laesies. Tekortkomingen van de cross-sectionele studieopzet zou een mogelijke verklaring kunnen zijn dat we geen significant hogere prevalentie van deze bloedingen vonden in de gebruikers groep. Verder onderzoek is daardoor noodzakelijk, waaronder met longitudinale studies.

In **hoofdstuk 6** hebben we 2 studies uitgevoerd naar de rol van genetica in uitkomsten gerelateerd aan het gebruik van antidepressiva. In **hoofdstuk 6.1** hebben we de associatie bestudeerd tussen genetische variatie in het P-glycoproteïne (gecodeerd door het *ABCB1* gen) en vroegtijdig veranderen of stoppen van antidepressiva behandeling. We veronderstelde dat deze uitkomsten indicatief waren aan een antidepressiva bijwerking. In eerdere studies werden variaties in dit gen geassocieerd aan antidepressiva response en antidepressiva bijwerkingen. In een studie met alle incident gebruikers van antidepressiva van de Rotterdam Studie hadden dragers van variant allelen van het *ABCB1* gen een hoger risico op vroegtijdig (binnen 45 dagen na start behandeling) veranderen en stoppen van antidepressiva behandeling. In **hoofdstuk 6.2** hebben we onderzocht of de associatie tussen genetische varianten en antidepressiva respons kon worden onderzocht in populatie studies door middel van geneesmiddel-gen interactie modellen. De rationale van deze studie was dat een genetische variant waarvan de associatie met depressieve symptomen anders is in mensen die worden behandeld en niet worden behandeld mogelijk een genetische variant is die is geassocieerd met de farmacologische respons van antidepressiva (in deze studie het gebruik van SSRIs). In onze studie hebben we op deze manier verschillende genetische varianten gevonden die interessant zijn voor vervolgonderzoek, alhoewel er geen van hen statistisch significant was geassocieerd na correctie voor de hoeveelheid statistische toetsen. Het *HMGB4* en *FSHR* gen, waar genetische varianten werden

gevonden die bijna statistisch significant waren, zijn in eerdere studies geassocieerd met expressie concentraties in lagere organisme onder behandeling van fluoxetine. Om die reden zouden deze twee genen interessant kunnen zijn voor additioneel onderzoek. Daarnaast was van de genen die betrokken zijn bij de serotonine signalering, het *PLCB1* statistisch significant en werd het *HTR2A* gen gerepliceerd. Dit laatste gen is al vaker beschreven in relatie met antidepressiva respons. Deze resultaten suggereren dat, naast de conventionele methoden, het gebruik van geneesmiddel-gen interactie modellen kunnen helpen bij het identificeren van genen die betrokken zijn bij antidepressiva response. Echter zullen toekomstige studies moeten worden uitgevoerd met een grotere studiepopulatie of worden uitgevoerd met een hypothese die voorafgaand op het onderzoek wordt opgesteld.

In de discussie (**hoofdstuk 7**) worden de resultaten van de verschillende studies bediscussieerd en daarbij gebruik makend van de huidige literatuur. Daarnaast worden ook methodologische aspecten van onderzoek naar antidepressiva gebruik en toekomstige onderzoeksrichtingen bediscussieerd.

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2014 'Association between the VKORC1 -1639G>A polymorphism, mRNA expression levels, methylation and coumarin maintenance dosage' (oral), Scientific Spring Meeting, Dutch Society for Clinical Pharmacology and Biopharmacy, Leiden, the Netherlands

2014 'Tricyclic antidepressants prolong the Bazett's heart rate corrected QT interval, but do not cause repolarization disturbances' (poster), Scientific Spring Meeting, Dutch Society for Clinical Pharmacology and Biopharmacy, Leiden, the Netherlands

2014 'Identifying genetic loci associated with antidepressant drug response using drug-gene interaction models on repeated cross-sectional assessments of depressive symptoms in a population-based study' (oral), Netherlands Network of Precision Medicine, Leiden, the Netherlands

(Inter)national conferences and symposia:

2011	GENERO Symposium, Rotterdam, the Netherlands (November 15)
2012	28th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Barcelona, Spain (August 23 – 26 2012)
2012	Dutch Medicines Days, Figon, Lunteren, the Netherlands (October 1 – 2)
2013	Wetenschapsdagen Inwendige Geneeskunde 2013, Antwerp, Belgium (January 10 – 11)
2013	Netherlands Consortium of Healthy Ageing Congress, the Hague, the Netherlands (February 8 – 9)
2013	CHARGE Investigator Summer Meeting, Rotterdam, the Netherlands (June 12 – 14)
2013	29th International Conference on Pharmacoepidemiology & Therapeutic Risk Management, Montreal, Canada (August 26 – 28)
2013	Dutch Medicines Days, Figon, Ede, the Netherlands (September 30 – October 1)
2014	CHARGE Investigator Winter Meeting, Redondo Beach, CA, USA (January 22 – 24)
2014	Scientific Spring Meeting, Dutch Society for Clinical Pharmacology and Biopharmacy, Leiden, the Netherlands (April 11)
2014	Netherlands Network of Precision Medicine, Leiden, the Netherlands (May 13)

Teaching activities

2012 – 2013	Supervising second year medical students, course 'Ever thought of doing research', Rotterdam, the Netherlands
2012 – 2014	Supervising practicals, course 'Pharmaco-epidemiology and Drug Safety', Netherlands Institute for Health Sciences, Rotterdam, the Netherlands
2012 – 2013	Supervising project Kiki Cheung 'Association antidepressant use and suicide (attempts) in a population-based study'
2014	Supervising project Nevena Maljuric 'Use of Selective Serotonin Reuptake Inhibitors and the heart rate-corrected QT-interval: a population-based study in older adults'

Others:

- Peer review for Pharmacogenomics Journal
- Peer review for Experimental Gerontology

- Peer review for Annals of Internal Medicine
- Peer review for Journal of Clinical Psychopharmacology

LIST OF PUBLICATIONS

R Noordam, N Aarts, H Tiemeier, A Hofman, BH Stricker, LE Visser. Sex-specific association between antidepressant use and body weight in a population-based study in older adults. Journal of Clinical Psychiatry 2015. (in press)

NM Maljuric*, **R Noordam***, N Aarts, MN Niemeijer, ME van den Berg, A Hofman, JA Kors, BH Stricker, LE Visser. Use of selective serotonin reuptake inhibitors and the heart-rate corrected QT interval in a real-lfe setting: the population-based Rotterdam Study. British Journal Clinical Pharmacoly 2015 (in press). *contributed equally

K van Drielen, DA Gunn, **R Noordam**, CE Griffiths, RG Westendorp, AJ de Craen, D van Heemst. Disentangling the effects of circulating IGF-1, glucose, and cortisol on features of perceived age. *Age (Dordr)*. 2015 Jun;37(3):9771

R Noordam, ME van den Berg, MN Niemeijer, N Aarts, MJG Leening JW Deckers, A Hofman, PR Rijnbeek, M Eijgelsheim, JA Kors, BH Stricker, LE Visser. Assessing prolongation of the hart-rate corrected QT interval in users of tricyclic antidepressants: advice to use Fridericia rather than Bazett's correction. *Journal of Clinical Psychopharmacology*. 2015 (in press)

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R Noordam, N Aarts, KM Verhamme, MC Sturkenboom, BH Stricker, LE Visser. Prescription and indication trends of antidepressant drugs in the Netherlands between 1996 and 2012: a dynamic population-based study. *Eur J Clin Pharmacol*. 2015 Mar;71(3):369-75

K Cheung, N Aarts, **R Noordam**, JC van Blijderveen, MC Sturkenboom, R Ruiter, LE Visser, BH Stricker. Antidepressant use and the risk of suicide: A population-based cohort study. *J Affect Disord*. 2015;174:479-84

CM Sitlani, KM Rice, T Lumley, B McKnight, LA Cupples, CL Avery, **R Noordam**, BH Stricker, EA Whitsel, BM Psaty. Generalized estimating equations for genome-

wide association studies using longitudinal phenotype data. *Stat Med*. 2015 Jan 15;34(1):118-30

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Aarts N*, Akoudad S*, **Noordam R**, Hofman A, Ikram MA, Stricker BH, Visser LE, Vernooij MW. Inhibition of serotonin reuptake by antidepressant and cerebral microbleeds in the general population. *Stroke*. 2014 Jul;45(7):1951-7 *contributed equally

Noordam R, Aarts N, Hofman A, van Schaik RHN, Stricker BH, Visser LE. Association between genetic variation in the *ABCB1* gene and switching, discontinuation, and dosage of antidepressant therapy: results from the Rotterdam Study. *Journal of Clinical Psychopharmacology*. 2013 Aug;33(4):546-50

Noordam R, Gunn DA, Tomlin CC, Maier AB, Griffiths T, Catt SD, Ogden S, Slagboom PE, Westendorp RG, Griffiths CEM, van Heemst D. Serum insulin-like growth factor 1 and facial ageing: high levels associate with reduced skin wrinkling in a cross-sectional study. *British Journal of Dermatology*. 2013 Mar;168(3):533-8

Noordam R, Craen AJ, Pedram P, Maier AB, Mooijaart SP, van Pelt J, Feskens EJ, Streppel MT, Slagboom PE, Westendorp RG, Beekman M, van Heemst D. Levels of 25-hydroxyvitamin D in familial longevity: the Leiden Longevity Study. *Canadian Medical Association Journal*. 2012 Dec 11;184(18):E963-8

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Mooijaart SP, van Heemst D, **Noordam R**, Rozing MP, Wijsman CA, de Craen AJ, Westendorp RG, Beekman M, Slagboom PE. Polymorphisms associated with type 2 diabetes in familial longevity: The Leiden Longevity Study. *Aging (Albany NY).* 2011 Jan;3(1):55-62.

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Raymond

ABOUT THE AUTHOR

Raymond Noordam was born on May 16th 1988 in Westvoorne, the Netherlands. He attended Maerlant College (athenaeum-beta) in Brielle and graduated in 2006. In September 2006, he started his study Biomedical Sciences at Leiden University. In 2008, he was part of an exchange program (30 ECTS) with Karolinska Institutet in Stockholm, Sweden. During his study, he conducted multiple research projects at the departments of Gerontology and Geriatrics (supervision: Dr. Diana van Heemst and Dr. Anton JM de Craen) and Clinical Oncology (supervision: Prof.dr. Sjoerd J van den Burg and Dr. Moniek Heusinkveld) at the Leiden University Medical Center.

From September 2011 until September 2014 he worked at the departments of Internal Medicine (head: Prof.dr. Jan L.C.M. van Saase) and Epidemiology (head: Prof.dr. Albert Hofman) on his PhD research in pharmacoepidemiology. The studies presented in this thesis were conducted under the supervision of Prof.dr. Bruno H.Ch. Stricker (Pharmacoepidemiology) and dr. Loes E Visser (Internal Medicine).

As from October 2014 onwards, he was appointed as a postdoctoral researcher at the department of Gerontology and Geriatrics of the Leiden University Medical Center (head: Prof.dr. Anthonius J. Rabelink).