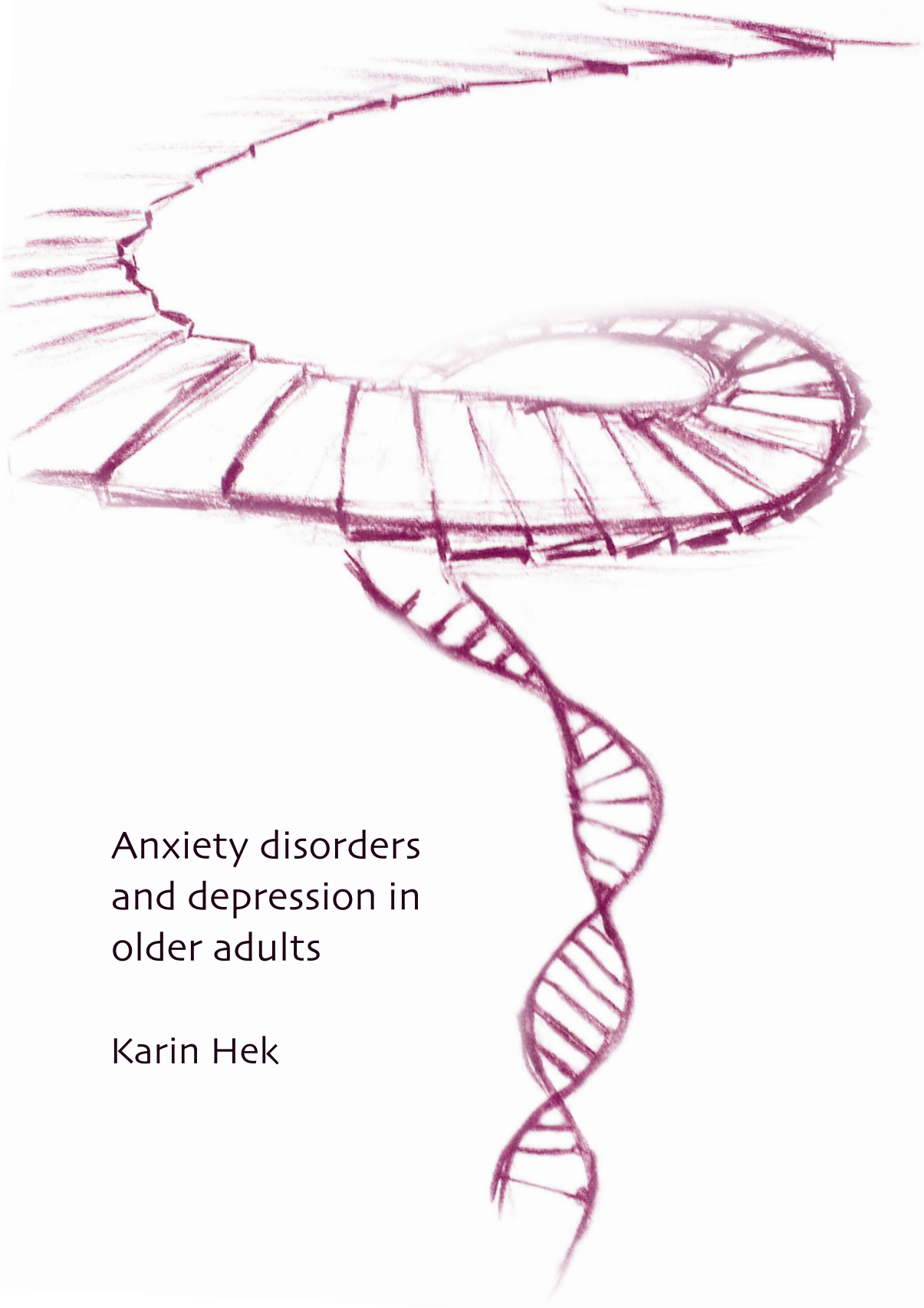

Anxiety disorders and depression in older adults

Karin Hek

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The work described in this thesis was conducted at the Epidemiological and Social Psychiatric Research institute, Department of Psychiatry and the Department of Epidemiology of the Erasmus MC, Rotterdam, the Netherlands.

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Publications and manuscripts in this thesis

Chapter 2

K Hek, H Tiemeier, RS Newson, HJ Luijendijk, A Hofman, CL Mulder. Anxiety disorders and comorbid depression in community-dwelling older adults. International Journal of Methods in Psychiatric Research 2011; 20(3): 157-168.

Chapter 3

K Hek, H Tiemeier, N Aarts, BHCh Stricker, A Hofman, AI Wierdsma, CL Mulder. Mental health service use of community-dwelling older adults with anxiety disorders. In preparation.

Chapter 4

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Chapter 5

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Chapter 6

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Chapter 8

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Chapter 9

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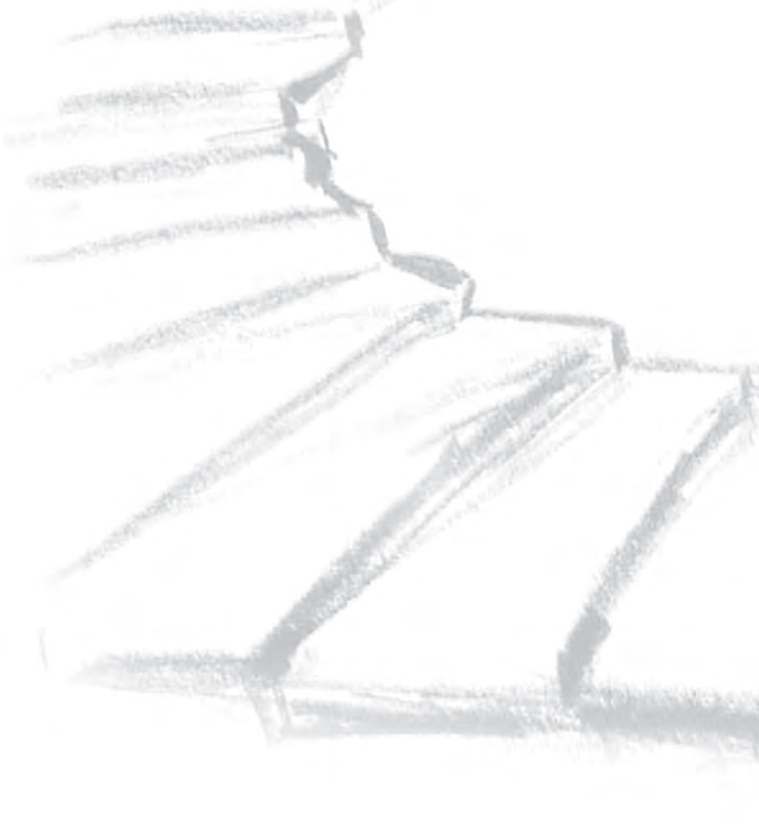

Chapter 1

General introduction

Introduction

Most people feel anxious or depressed at times. These feelings are normal reactions to stressors like getting laid off, getting divorced or even defending your thesis. However, some people experience persisting feelings of anxiousness and depression that impair their daily functioning. These people may have an anxiety disorder or a depressive disorder.

Anxiety disorder is a generic term covering different types of abnormal, pathological fear and anxiety. The anxiety disorders addressed in this thesis are generalized anxiety disorder (GAD), specific phobia, social phobia, panic disorder and agoraphobia. Generalized anxiety disorder is characterized by non-specific excessive fear and worry. Phobia is excessive fear for a stimulus or situation. People with a phobia generally attempt to avoid the source of their anxiety. Those with social phobia avoid social situations, those with agoraphobia avoid places where escape is difficult or embarrassing or where help is unavailable, and those with a specific phobia avoid other specific stimuli or situations, such as heights or the dentist. Panic disorder is the least common of these anxiety disorders and is characterized by brief attacks of extreme fear. Depressive disorders are characterized by persistent low mood or loss of interest or pleasure in activities that were formerly enjoyed. Other symptoms of depression are weight change, insomnia or hypersomnia, psychomotor changes, fatigue or loss of energy, feelings of worthlessness or guilt, concentration problems, or suicidal ideation.

Both anxiety and depression are highly prevalent mental disorders. Almost one-fifth of all people will have experienced a depressive disorder during their life and one-third of all people an anxiety disorder (1). Both disorders are associated with considerable distress, functional impairment and increased mortality and can occur throughout the entire life span (2-3). At old age anxiety and depression are relatively chronic (4). Research into mental disorders in old age is lacking. This thesis focuses on anxiety and depression in late life to further our understanding of these common and chronic disorders.

Epidemiology of anxiety and depression

Anxiety and depression are complex disorders that are caused by both genetic and non-genetic factors. The study of the occurrence and the determinants, i.e. the epidemiology, of anxiety disorders and depressive disorders, helps unravel their etiology. Epidemiological studies aim to describe the prevalence and incidence of disease and to examine factors that influence the occurrence of disease (e.g., comorbidity, health care use, physiological determinants, genetic variants). Such studies can ultimately guide recognition, prevention and treatment of the disorder under study. This thesis examines the epidemiology of anxiety disorders and depressive disorders in older adults. In

particular, comorbidity, health care use, cortisol and atherosclerosis and genetic factors are studied in relation to anxiety and, or depression.

Comorbidity and health care use

Comorbidity is the co-existence of a disease with another disorder. Anxiety and depression are highly comorbid disorders. The extent of depression comorbidity in older people with an anxiety disorder ranges between 12 and 65% (5-6). Comorbidity varies with e.g., types of disorders, disorder definition, and diagnostic instrument (7-8).

Two etiological models help explain the high comorbidity of anxiety and depression. First, anxiety and depression may share genetic and non-genetic risk factors. Indeed, there is considerable overlap between risk factors for anxiety and depression (e.g., psychiatric history (9) and genetic factors (10)). However, disease-specific risk factors have also been identified; for example, having an ill partner increases the risk for anxiety disorders, while recent widowhood is associated with depression (11).

Second, a high comorbidity would also be observed if one disorder is a risk factor for the other. Anxiety disorders other than GAD are generally thought to have an onset early in life during childhood and adolescence, whereas the onset of depression is mostly during adulthood (12-13). Indeed several longitudinal studies reported that often anxiety precedes depression (14-15). However, the reverse has also been observed (16-17). In addition, whether anxiety and depression arise from the same diathesis or are risk factors for each other may differ per subtype of anxiety disorder.

People with both anxiety and depression experience more disability and distress than people with a singular disorder (18). This is also reflected in mental health care use: a study amongst Canadian older adults reported that approximately 20% of people with an anxiety disorder used mental health services, compared to 40% of those with a mood disorder and more than 75% of the people with comorbid anxiety and mood disorder (19). Mental health care use in those with anxiety or depression is low, although treatment is available, with variable success.

Further study on the comorbidity of anxiety and depression would help elucidate the relation between these disorders to inform disease recognition and disease treatment. In addition, further knowledge on the comorbidity between anxiety and depression will guide phenotype decisions in research on anxiety and depression. Study of the different levels of (mental) health care use by people with anxiety and depression would shed light on the barriers to health care use. Such studies should separate the different subtypes of particularly anxiety disorders that may behave differently.

Physiological factors: cortisol and atherosclerosis

The hypothalamic-pituitary-adrenal axis (HPA axis) is one of the body's main systems

controlling the stress response and has been associated with anxiety and depression (20-21). It involves a complex set of interactions between the hypothalamus and the pituitary gland in the brain and the adrenal glands on top of the kidneys. The hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates adrenocorticotropic hormone (ACTH) secretion from the pituitary gland. ACTH in turn acts on the adrenal cortex which produces the end product cortisol. Cortisol then suppresses CRH and ACTH secretion from the hypothalamus and the pituitary gland in a negative feedback cycle. In addition, cortisol has many other functions like increasing blood sugar and suppressing the immune system. Cortisol is secreted in a distinct daily pattern whereby cortisol levels rise rapidly after awakening (the cortisol awakening response or CAR) and decrease slowly thereafter. Dysregulations of the CAR and total cortisol secretion over the day have been found associated with anxiety disorders and depression (20-21). Activation of the HPA axis leads to temporarily increased blood glucose, heart rate, blood pressure, arousal and vigilance. These symptoms are related to sensations experienced by people with certain anxiety disorders. However, evidence for the association between anxiety and cortisol is inconsistent. Further study on this relation particularly in older adults is required, because age-dependent HPA-axis dysregulation may increase the vulnerability of older adults to anxiety and depression (22) and studies in older adults are scarce. The association between depression and cortisol has been more widely studied. Major depression has been found associated with a higher CAR (20).

Depression in older adults is suggested to be preceded by cerebrovascular disease (23-24). Cerebrovascular damage particularly in frontal-subcorticol circuits may disrupt neurotransmitter systems involved in mood regulation thereby causing depression (25). The vascular depression hypothesis is supported by the high comorbidity of depression and vascular risk factors and by the high incidence of depression after stroke. However, studies that showed an association between depression and vascular factors have been largely cross-sectional. From these studies the temporal nature of the association could not be inferred. In addition, depression following overt vascular events such as stroke is sometimes suggested to be psychological rather than biological. Longitudinal studies on the relation between depression and vascular factors in older adults are needed to verify the vascular depression hypothesis.

Genetic factors

From family studies we know that anxiety and depression aggregate in families and that family history is a risk factor for these disorders (26-27). Also twin studies provide evidence for an underlying heritable component for anxiety and depression (26-27). These studies suggest that genetic variants play a role in the etiology of anxiety and

depression and genetic studies can thus be used as a tool to gain further insight into the biology of these disorders. However, the long search for genetic variants associated with anxiety and depression has had few successes. Linkage studies have been performed and these yielded only few loci, suggesting the absence of disease loci with a large effect (28-29). In addition, hundreds of candidate genes have been investigated in association studies, but only few have been confirmed in meta-analyses (30). One of the most widely studied candidate genes for depression is SLC6A4 (serotonin receptor). The association of this gene with depression has been verified (30). However, the association of another well-studied candidate gene for depression, BDNF (brain-derived neurotrophic factor), has not been confirmed in meta-analysis (30).

More recently, genome-wide association studies (GWAS) have become a standard method for gene discovery. GWAS focuses on common genetic variants (typically single-nucleotide polymorphisms or SNPs) across the entire genome and studies the association of these SNPs with a trait or disease. The idea is simple; frequencies of variants across the genome are compared one by one among cases and controls. Variants that vary significantly between cases and controls are validated in independent samples. Validation or replication is essential to distinguish the true positives from false positive hits that are inevitably generated when performing approximately 1 million independent statistical tests. This hypothesis-free genome-wide approach has been very successful in detecting genetic variants associated with complex disorders like diabetes, and complex traits like hypertension (31-32). However, GWAS efforts to find new candidate genes for anxiety and depression so far have been unsuccessful (33-34). The candidate genes that have been identified with GWAS, like the PCLO gene (35), remain to be replicated. In addition, the abovementioned SLC6A4 was one of the few previously identified candidate genes for depression that has been replicated in GWAS (36).

The presence of genome-wide data allowed for the rapid evolution of a wealth of tools and methods for data analysis. Besides 'ordinary' GWAS, that aims to find an association between genetic variants and an outcome as described above, several other uses of the data were developed, that focus on different aspects of genetics. In this thesis we applied three other methods to study the genetic data, namely pathway analysis, risk score analysis and Mendelian randomization analysis. Pathway analysis tests whether variants in a certain pathway are overrepresented among cases compared to controls. Pathway analysis can be applied to GWAS results. Draw-back of this method is that it requires allocation of genes to pathways, whereas the advantage of GWAS is that it is a hypothesis-free approach that does not require knowledge of genes and even tests genetic variants that are located outside genes. Risk score analysis can be used to investigate the presence of a polygenic component, which suggests that the effects of many SNPs combined increase the risk for a disorder (37). Presence of a polygenic

component in the etiology of anxiety and depression could explain the lack of GWAS success for these disorders. In addition, this method can be used to study whether anxiety and depression share a genetic vulnerability. Last, Mendelian randomization studies use genetic variants in observational epidemiology to make causal inferences about non-genetic risk factors for disease (38). The study of the relation between obesity and depression, for example, is hampered by the effects of unmeasured confounders, like diet and past depression. Genetic variants are passed on randomly from parents to child and are therefore unconfounded. Genetic variants can be used as an instrument to test the relation between obesity and depression.

Analyzing the available genetic data with different methods helps to further unravel the etiology of anxiety and depression. However, an important bottleneck in the success of these studies is the phenotype. Both anxiety and depression are consensus-based diagnoses that comprise a combination of symptoms and vary between patients. Furthermore, depression and anxiety exist on a continuum of varying severity and duration. Major depression, for example, can be defined as a diagnostic entity applied to the extreme of the depression continuum (39). Therefore, the ability to detect genetic predictors might be improved by analyzing these disorders quantitatively (40).

Rotterdam Study

The studies in this thesis were based on data from the Rotterdam Study, a prospective population-based cohort study ongoing since 1990 in the city of Rotterdam in the Netherlands (41). The study was designed in response to the increase of the proportion of older adults in the population with the aim to assess risk factors for chronic diseases. As of 2008 this study includes 14,926 people aged 45 years or over. Every four years study participants undergo an extensive home interview and a physical examination at a research centre. Cross-sectional data for anxiety disorders and longitudinal data for depression were available for this thesis.

Outline of this thesis

This thesis focuses on different aspects of anxiety disorders and depressive disorders in older adults to help unravel their etiology. This thesis first investigates the descriptive and physiological epidemiology of anxiety and depression (**Chapter 2 to 5**). In the second part, genetic epidemiological studies of anxiety and depression are reported (**Chapter 6 to 9**).

In **Chapter 2** the relation of current and past depression with anxiety disorders is investigated. **Chapter 3** examines mental health service use amongst people with

anxiety disorders and depression. In **Chapter 4** the cross-sectional association between anxiety disorders and cortisol is studied. **Chapter 5** describes the relation between atherosclerosis and incident depression. The study in **Chapter 6** validates the association between depression and a genetic variant in the *PCLO* gene. **Chapter 7** describes the results of a genome-wide association study of depressive symptoms. This study was performed in collaboration with other studies united in the CHARGE consortium. **Chapter 8** examines whether a large number of genetic variants with small effects predict depression and anxiety. This study was performed in collaboration with the Erasmus Rucphen Family study (ERF) and the Genetic Association Information Network Major Depressive Disorder study (GAIN-MDD). In **Chapter 9** the relation between obesity and depression is studied using a Mendelian randomization approach. In **Chapter 10** the results of Chapters 2 to 9 are summarized and discussed.

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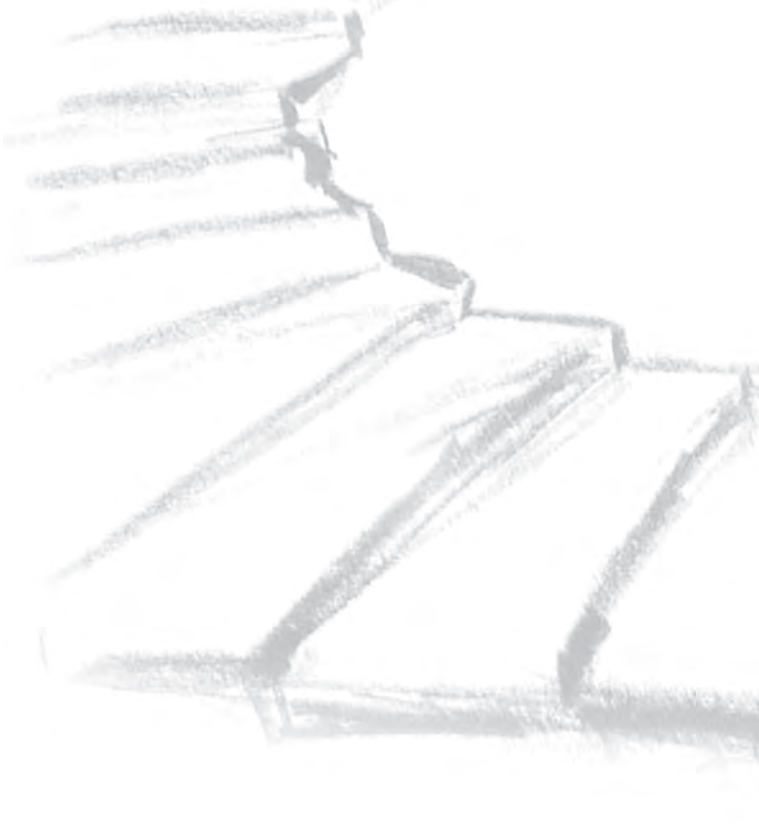

Chapter 2

Anxiety disorders and comorbid depression in community-dwelling older adults

Karin Hek, Henning Tiemeier, Rachel S. Newson,
Hendrika J. Luijendijk, Albert Hofman,
Cornelis L. Mulder

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Abstract

Anxiety disorder is a common psychiatric problem during late life, and frequently co-occurs with depression. High comorbidity between anxiety and depression may partly be explained by the definition of the disorders and the assessment of both disorders with one instrument at the same time. The current study investigates the relation of current and past depression with anxiety disorders in the Rotterdam Study, a large population-based cohort study of older adults in the Netherlands (n study population = 5565). DSM-IV anxiety disorder was ascertained with the Munich version of the Composite International Diagnostic Interview. DSM-IV depression was diagnosed with the Schedules for Clinical Assessment of Neuropsychiatry (SCAN) on a different day. Past depression was assessed from general practitioners' records, self-report, and a prior SCAN interview. Of the 457 persons with an anxiety disorder, 11.6% had a comorbid major depression, and another 6.3% had other depressive syndromes. However, 49.3% of persons with an anxiety disorder experienced or had in the past experienced a depressive episode. Our study suggests that comorbid depression in older adults with anxiety disorders may be less prevalent than previously suggested. However, the relation of current anxiety disorders with past depression is substantial.

Introduction

Anxiety disorder is a common psychiatric problem in late life, affecting 3 to 15% of older adults (1). It is associated with considerable distress, impairment in quality of life, disability, and increased mortality and frequently co-occurs with depression (2-6). Persons with both anxiety and depression experience more disability and distress than persons with a singular disorder (7).

Comorbidity of depression with anxiety was recently reported to be as high in older adults as in younger persons (8). However, the reported frequency of comorbid anxiety and depression in persons aged over 55 varies. In the French ESPRIT study, only 12% of persons with an anxiety disorder also reported major depression (9). In a large Canadian study 23% of older adults with an anxiety disorder had a comorbid major depression (7). However, generalized anxiety disorder (GAD), a common anxiety disorder in older adults (1), was not assessed. Beekman *et al.* (10), who included GAD in their study, found a comorbidity of 26% between anxiety and major depression. Kvaal *et al*. (11) reported an even higher comorbidity; 65% of older adults with anxiety disorders had a depressive disorder, an additional 25% of persons with anxiety disorder had a subthreshold depressive syndrome. However, in this study the Geriatric Mental State-Automated Geriatric Examination for Computer Assisted Taxonomy (GMS-AGECAT), and not the Diagnostic and Statistical manual of Mental Disorders (DSM) was used to define depression and anxiety. In a retrospective study on the overlap between lifetime diagnoses of anxiety and depression, 7 of the 19 persons (37%) with a lifetime diagnosis of anxiety disorder also had a lifetime diagnosis of major depression or dysthymia (12). Another 8 persons had a subthreshold depressive disorder (42%). Despite the great variability, all studies conclude that there is substantial comorbidity between anxiety and depression in the older adult population.

Inconsistencies in the extent of comorbidity may partly be determined by methodological differences between studies. Commonly used instruments to diagnose anxiety and depression, such as the Composite International Diagnostic Interview (CIDI), the Mini-International Neuropsychiatric Interview (MINI), GMS-AGECAT or Diagnostic Interview Schedule (DIS), assess anxiety in parallel to depression. Similar wording of the interview items is used to ascertain the shared symptoms of anxiety and depression. When asked about both disorders during one interview session, respondents may be tempted to give consistent answers (13). Consequently, comorbidity may be diagnosed more frequently if one assessment tool is used to diagnose anxiety and depression during one session, than if assessed by different instruments or in separate sessions. This "common method variance" could inflate the observed comorbidity between anxiety and depression (for a review see (14)).

In the present study, we examined the comorbidity between anxiety and depression in a large population-based cohort of older adults. We addressed the limitations of prior research by measuring current anxiety disorders and depression with different instruments on different days within a short time period to avoid common method variance. In addition, we used previously collected information on prior episodes of depression to estimate the comorbidity of current anxiety with current and past depression. This information was obtained by a clinical interview, self-report and continuous monitoring of general practitioners' records. We hypothesized that, on the one hand, comorbidity between anxiety and depression would be relatively low compared to previous studies if not assessed with the same instrument. On the other hand, we expected the overlap between current anxiety and depression to increase substantially, when past depressions were accounted for.

Method

Study population

This study was embedded in the Rotterdam Study; a prospective population-based cohort study of older adults to assess risk factors for chronic diseases (15-16). In 1990, all inhabitants of a district in Rotterdam aged 55 years and over were invited to participate, of which 7983 persons participated in the first round (baseline). This population was extended in 1999, with an additional cohort of 3011 persons aged 55 years and over. Every four years participants undergo an extensive home interview and physical examination at a research centre. The Medical Ethics Committee of the Erasmus MC approved the Rotterdam Study.

In the last examination round (2002-2005) anxiety disorders and depression were assessed. Of the 7609 eligible persons, 6007 participated in this round (Figure 1). We excluded 434 persons without valid anxiety or depression assessment and eight persons with bipolar disorder. The excluded persons (n = 442) were significantly older (mean age 75.5 versus 72.6) and more often female (68.8% versus 58.1%) than the included persons (n = 5565). For a selected sampe of 3374 persons, data were available not only on current anxiety and depression, but also on past depression. The persons without data on past depression were more often male and younger (45.1% males versus 39.9% males, mean age of 68.5 years versus 75.2 years).

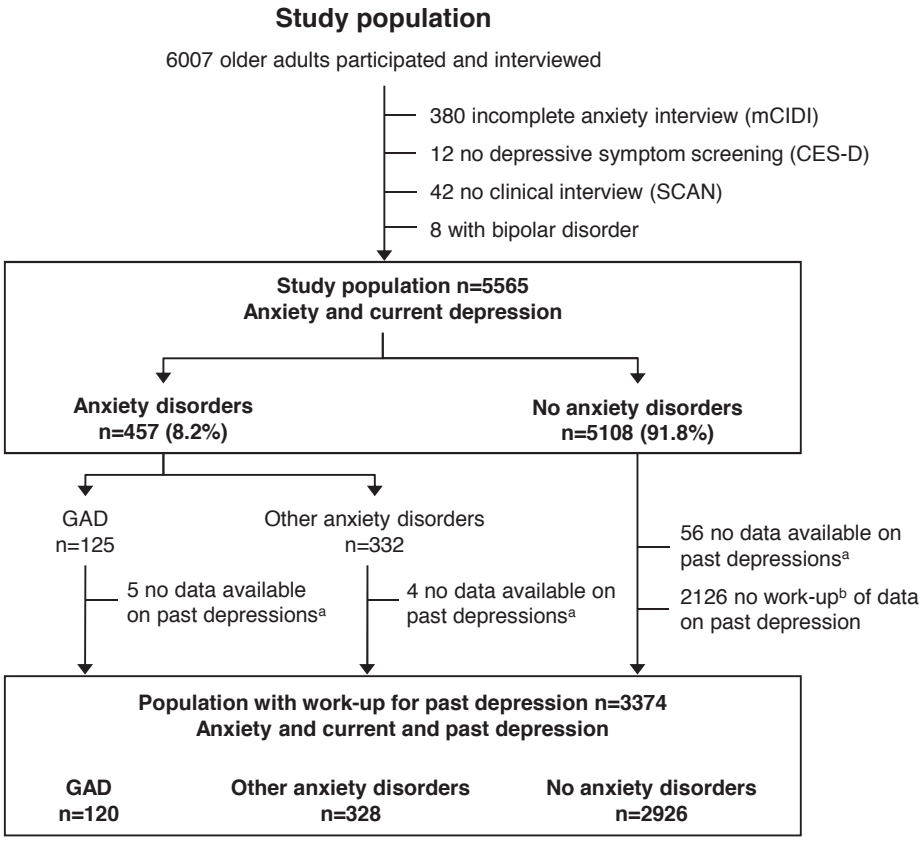

Figure 1 [a]Past depression as assessed by continuous monitoring of general practitioner (GP) records. [b]Work-up of GP records for the complete cohort was not feasible and unnecessary and was therefore performed for part of the total study population. This selected sample was used only for calculation of current anxiety comorbidity with current and past depression combined. All other calculations were based on the total, unselected, study population (n = 5565). Abbreviations: M-CIDI, Munich Composite International Diagnostic Interview; CES-D, Center for Epidemiologic Studies Depression scale; SCAN, Schedules for Clinical Assessment of Neuropsychiatry; GAD, Generalized Anxiety Disorder.

Measures

Anxiety disorders

Anxiety disorders were diagnosed as part of the home interview. Trained lay interviewers conducted a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI) to assess the following anxiety disorders with a computerized diagnostic algorithm according to DSM-IV criteria: GAD, panic disorder with or without history of agoraphobia, agoraphobia, social phobia and specific phobia. Obsessive compulsive disorder and post-traumatic stress disorder were not assessed. The M-CIDI was specifically designed to obtain DSM-IV diagnoses of mental disorders and test-retest reliability for anxiety disorders is good (kappa for

any anxiety disorder: 0.81) (17). Whereas the CIDI was not specifically designed for diagnosis in older adults, it is one of the most widely used instruments to diagnose anxiety disorders in older adults. In addition, the M-CIDI has an important adjustment to simplify questions. Visual response cards were used for the most complicated questions. The assessments of anxiety disorders were implemented to measure point prevalence. For GAD, problems to recall symptoms ending up to twelve months ago were expected. Therefore, we assessed one-month prevalence of GAD according to DSM-IV criteria. GAD is a chronic disease with low recovery rates. Average duration of more than ten years has been reported for GAD in older adults (18). One-month prevalence will presumably approximate one-year prevalence (18-19). For agoraphobia, social phobia, specific phobia and panic disorder "current" symptoms, such as avoidance of plane flights, were established more easily if ascertained over a longer period (the last year). Again, these disorders are generally chronic (20) and our one-year prevalence estimates are most likely good proxies of point prevalence.

Current depression

Assessment of depression has been described previously (21). In short, during the home interview, participants were screened for symptoms of depression with the Center for Epidemiologic Studies Depression scale (CES-D) (22). Screen-positive persons (CES-D-score ≥ 16) were invited for a semi-structured clinical interview with the Schedules for Clinical Assessment of Neuropsychiatry (SCAN) (23). This interview was conducted by a trained clinician at the participant's home one week to two months (median time interval: two weeks) after the screening procedure and the anxiety interview. We were able to use the SCAN in this population-based setting, because depression can be screened for with high sensitivity (24). With a computerized DSM-IV based diagnostic algorithm, major depression, minor depression and dysthymia during the past month were diagnosed. Although psychometric properties of the SCAN in older adults are unknown, it has been used repeatedly to diagnose depression in older adults in the Dutch population (25). The depression section of the CIDI was not applied to this population.

Past depression

In the years between baseline and the anxiety assessment, information on past depressions was ascertained prospectively in three ways. First, during the examination of 1997-2001 all participants were screened for depression by CES-D and screen-positive persons were assessed with the SCAN interview as described above. Second, physicians conducted repeated interviews to assess self-reported depression between subsequent assessment rounds (1997-2001 and 2002-2005). Third, GP-records and specialist letters were monitored actively for the occurrence of episodes of depressive

syndromes during a mean period of nine years prior to anxiety assessment. Monitoring of GP-records for the original cohort was almost complete. In the extended cohort, only depressive episodes of persons with an anxiety disorder were monitored. Monitoring is a time-consuming procedure as it requires screening and coding of (mostly non-digital) GP-records. Monitoring of persons without anxiety disorders in the extended cohort was considered unnecessary as the number of controls exceeded the number of cases by 5:1. Retrospective information on life-time episodes was collected at baseline. Depression comprised major depression, minor depression and dysthymia diagnosed with SCAN, self-reported depression if a health professional was consulted, and depression recorded by a GP or physician.

Assessment of other variables

Age, sex and marital status were recorded. Living status was coded as living alone compared to living with one or more people. Education was grouped into low level (primary education), intermediate (secondary education, vocational education) and high level education (higher education). Disability, or Activities of Daily Living, was assessed with the Stanford Health Assessment Questionnaire (26). This measures disability in eight fields (e.g., hygiene, eating, walking) with responses ranging from: 0 - perform without difficulty, to 3 - unable to do independently. The mean score of all fields constitutes the Disability Index and the standard cut-off of 0.5 indicated no disability versus mild to severe disability. We assessed functioning in basic daily activities with an adaptation of the Instrumental Activities of Daily Living (27). Six areas were assessed (e.g., maintaining finances, meal preparation) with responses ranging from: 0 - perform without difficulty, to 3 - unable to do independently. The mean score of the six items was calculated and a cut-off of 0.5 (upper quartile) was selected to distinguish between no instrumental disability versus mild to severe instrumental disability. Cognitive capacity was assessed with the Mini Mental State Examination (MMSE), which assesses six broad areas of daily cognitive functions (28). A cut-off of 26 indicated adequate cognitive capacity versus impaired cognitive capacity. Participants were continuously monitored for occurrence of coronary heart disease as was previously described (29). Diagnoses were assigned according to the International Classficiation of Diseases, 10th Revision (ICD-10). Coronary heart disease was defined as myocardial infarction (ICD-10 I21), a percutaneous transluminal coronary angioplasty, a coronary artery bypass graft and other forms of acute (I24) or chronic ischemic heart disease (I25). Diabetes mellitus cases were defined as having a fasting plasma glucose level of at least 7.0 mmol per liter, a nonfasting plasma glucose level of at least 11.1 mmol per liter, or the use of oral antidiabetes medication, use of insulin or treatment by diet and registered by the general practitioner as having diabetes (30).

Statistical analysis

Percentages and means were computed to describe the baseline characteristics of the study population. We performed chi-square tests and t-tests to compare these characteristics between persons with and without anxiety disorder.

The prevalence of anxiety disorders was calculated from the percentage of anxiety disorders in the total study population (n = 5565, Figure 1). We also studied the comorbidity of current anxiety with current (major) depression in this total study population. Only the comorbidity between current anxiety and current and past depression was estimated using the selected sample of 3374 persons (Figure 1). To assess comorbidity we compared the depression rate of anxiety and non-anxiety cases.

Comorbidity estimates are presented for all assessed subtypes of anxiety disorders separately. However, adjusted odds ratios were only calculated for GAD and agoraphobia separately. Specific phobia, social phobia and panic disorder were combined because numbers were too small to calculate stable estimates. In addition, we present analyses stratified by age.

Primarily, the one-month prevalence of depression was used in the analyses. However, we reran analyses including depressions observed in one and two years before anxiety assessment to facilitate comparison with studies that provide a one-year prevalence of depression. However, these results should be interpreted carefully as for GAD only a one-month prevalence was assessed.

Adjusted odds ratios were computed to compare occurrence of depression in persons with and without anxiety disorder. We adjusted odds ratios for age and gender. Odds ratios were further adjusted for the following sociodemographic variables: living status (living alone, yes or no), education and cognitive status (impaired cognitive status, yes or no) (31). These variables have been associated to anxiety and depression and could potentially affect the comorbidity estimates. Because the age and gender adjusted analyses yielded similar results as the fully adjusted analyses we only present the fully adjusted results. All covariates had less than 2% missing values; therefore complete case analyses were performed. Statistical analyses were performed in SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

Results

The point prevalence of anxiety disorders in this older adult population was 8.2% (n = 457). The most prevalent anxiety disorders were agoraphobia (4.0%, n = 221) and GAD (2.2%, n = 125). Sixteen percent of the agoraphobia cases (n = 35), almost 25% of the GAD cases (n = 31) and more than 20% of the persons with other anxiety disorders (n = 37) had another comorbid anxiety disorder.

The one-month prevalence of depression was 4.1%; about half fulfilled the DSM-IV criteria

for major depression. Characteristics of the study population are summarized in Table 1. Persons with a comorbid depression were more often living alone, and experienced more disability than persons with only an anxiety disorder. In addition, comorbid cases were more often diagnosed with coronary heart disease than non-comorbid cases.

Table 2 shows the comorbidity of current anxiety with current depression. Of the persons with a current anxiety disorder, 17.9% had a comorbid depression. In contrast, 2.8% of the persons without anxiety disorder had a current depression (adjusted OR for depression in persons with anxiety: 7.0, 95% CI 5.2-9.5). Next, we stratified the group of anxiety disorders into the following anxiety subtypes: GAD, agoraphobia, social phobia, specific phobia and panic disorder.

Table 1 Characteristics of study population (n = 5565)[a]

	No anxiety disorder	Anxiety disorder			
		Agoraphobia[b]	GAD[b]	Other Anxiety disorder[b,c]	All Anxiety disorders
	n=5108	n=221	n=125	n=166	n=457
Female (%)	56.3	79.6***	77.8***	75.3***	78.0***
Mean age (SD)	72.6 (7.8)	73.0 (7.4)	72.0 (7.9)	70.8 (7.1)**	72.0 (7.4)
Age range (minimum age - maximum age)	58.0-100.1	59.5-90.0	59.5-91.9	59.4-92.4	59.4-92.4
Living alone (%)	33.9	36.2	37.6	40.4	37.0
Education[d]					
Low level (%)	25.3	38.9***	32.8	33.9*	35.5**
Intermediate level (%)	61.3	54.6	59.5	53.3	55.6
High level (%)	13.4	6.5	7.8	12.7	8.9
Marital Status[d]					
Married (%)	64.2	61.5	61.6	57.8	61.3
Single (%)	5.1	3.6	4.0	12.7	3.9
Widowed (%)	24.0	26.2	26.4	24.7	25.4
Divorced (%)	6.7	8.6	8.0	4.8	9.4
Disability					
Functional - mild to severe (%)	40.2	62.4***	62.3***	61.8***	61.5***
Instrumental - mild to severe (%)	24.6	27.4	34.4*	30.9	29.1*
Physical health					
Coronary Heart Disease (%)	6.2	4.5	5.6	6.0	5.0
Diabetes Mellitus (%)	17.1	14.5	13.7	17.5	14.7
MMSE					
Impaired cognitive capacity (%)	13.5	15.2	25.0**	18.7	18.4**

[a]The population described in this table comprises the total study population.

[b]Agoraphobia, generalized anxiety disorder (GAD) and other anxiety disorders represent non-exclusive categories.

[c]Other anxiety disorders comprise social phobia, specific phobia and panic disorder.

[d]Overall test of significance was performed.

Note: Anxiety disorder groups were compared to the group without anxiety disorders with *t*-tests and chi-square tests.

MMSE, Mini Mental State Examination. *p < 0.05; **p < 0.01; ***p < 0.001.

Persons with GAD most often had a comorbid depression (40.8%), followed by persons with social phobia (25.8%). Persons with specific phobia least often had a comorbid depression (9.5%). Comorbidity estimates are also presented per age group in Table 2. The oldest age group included more anxiety cases with comorbid depression than the youngest age group ($\chi^2 = 5.5$, df = 1, $P = 0.019$).

Table 2 Current anxiety disorders and depression comorbidity[a]

	No current depression	Current depression[b]
	n (%)	*n* (%)
No anxiety disorder (n=5108)	4966 (97.2)	142 (2.8)
All anxiety disorders (n=457)	375 (82.1)	82 (17.9)***
GAD (n=125)[c]	74 (59.2)	51 (40.8)***
Agoraphobia (n=221)[c]	194 (87.8)	27 (12.2)***
Other anxiety disorders (n=166)[c,d]	141 (84.9)	25 (15.1)***
Social phobia (n=62)[c]	46 (74.2)	16 (25.8)***
Specific phobia (n=84)[c]	76 (90.5)	8 (9.5)**
Panic disorder (n=30)[c]	27 (90.0)	3 (10.0)
Stratified by age		
Age 58-70, n=2343		
No anxiety disorder (n=2144)	2102 (98.0)	42 (2.0)
All anxiety disorders (n=199)	170 (85.4)	29 (14.6)***
GAD (n=56)[c]	35 (62.5)	21 (37.5)***
Agoraphobia (n=84)[c]	77 (91.7)	7 (8.3)**
Other anxiety disorders (n=82)[c,d]	70 (85.4)	12 (14.6)***
Age 70-80, n=2165		
No anxiety disorder (n=1986)	1931 (97.2)	55 (2.8)
All anxiety disorders (n=179)	147 (82.1)	32 (17.9)***
GAD (n=43)[c]	27 (62.8)	16 (37.2)***
Agoraphobia (n=95)[c]	84 (88.4)	11 (11.6)***
Other anxiety disorders (n=62)[c,d]	51 (82.3)	11 (17.7)***
Age 80-100, n=1057[e]		
No anxiety disorder (n=987)	933 (95.4)	45 (4.6)
All anxiety disorders (n=79)	58 (73.4)	21 (26.6)***
GAD (n=26)[c]	12 (46.2)	14 (53.8)***
Agoraphobia (n=42)[c]	33 (78.6)	9 (21.4)***
Other anxiety disorders (n=22)[c,d]	20 (90.0)	2 (9.1)

[a]These numbers were based on the total study population (n = 5565).

[b]Current depression as assessed with the Schedules for Clinical Assessment of Neuropsychiatry (SCAN) interview.

[c]Generalized anxiety disorder (GAD), agoraphobia and other anxiety disorders represent non-exclusive categories.

[d]Other anxiety disorders comprised social phobia, specific phobia and panic disorder.

[e]The age 80-100 group comprised significantly more comorbid depression cases than the youngest age group (p = 0.019 as tested with a chi-square test).

Note: Anxiety categories were compared to the category without anxiety disorders with chi-square tests or Fisher exact tests. *p < 0.05; **p < 0.01; ***p < 0.001.

Comorbidity of anxiety and depression increased to 20.8% if we included all depressions established in the year before anxiety assessment. Including all depressions detected in the two years before anxiety assessment further increased this estimate of comorbidity to 22.1%.

Table 3 Comorbidity between current anxiety and all depressions during follow-up[a]

	No depression	Current or past depression[b]
	n (%)	n (%)
No Anxiety disorder (n=5108)	2190 (74.8)	736 (25.2)
All Anxiety disorders (n=448)	227 (50.7)	221 (49.3)***
GAD (n=120)[c]	38 (31.7)	82 (68.3)***
Agoraphobia (n=218)[c]	122 (56.0)	96 (44.0)***
Other anxiety disorders (n=164)[c,d]	83 (50.6)	81 (49.4)***
Social phobia (n=60)[c]	20 (33.3)	40 (66.7)***
Specific phobia (n=84)[c]	49 (58.3)	35 (41.7)***
Panic disorder (n=30)[c]	15 (50.0)	15 (50.0)***
Stratified by age		
Age 58-70, n=815		
No anxiety disorder (n=620)	457 (73.7)	163 (26.3)
All anxiety disorders (n=195)	106 (54.4)	89 (45.6)***
GAD (n=54)[c]	24 (44.4)	30 (55.6)***
Agoraphobia (n=83)[c]	51 (61.4)	32 (38.6)*
Other anxiety disorders (n=81)[c]	40 (49.4)	41 (50.6)***
Age 70-80, n=1740		
No anxiety disorder (n=1563)	1157 (74.0)	406 (26.0)
All anxiety disorders (n=177)	85 (48.0)	92 (52.0)***
GAD (n=42)[c]	6 (14.3)	36 (85.7)***
Agoraphobia (n=94)[c]	50 (53.2)	44 (46.8)***
Other anxiety disorders (n=61)[c]	31 (50.8)	30 (49.2)***
Age 80-98, n=819		
No anxiety disorder (n=743)	576 (77.5)	167 (22.5)
All anxiety disorders (n=76)	36 (47.4)	40 (52.6)***
GAD (n=24)[c]	8 (33.3)	16 (66.7)***
Agoraphobia (n=41)[c]	21 (51.2)	20 (48.8)***
Other anxiety disorders (n=22)[c]	12 (54.2)	10 (45.5)*

[a]These numbers were based on the selective sample of n = 3374.

[b]Current depression as assessed with the Schedules for Clinical Assessment and Neuropsychiatry (SCAN) interview and past depression as assessed by a clinical interview (SCAN), self-report and continuous general practitioner (GP) record monitoring.

[c]Generalized anxiety disorder (GAD), agoraphobia and other anxiety disorders represent non-exclusive categories.

[d]Other anxiety disorders comprised social phobia, specific phobia and panic disorder.

Note: Anxiety categories were compared to the category without anxiety disorders with chi-square tests.

*p < 0.05; **p < 0.01; ***p < 0.001.

For comparison with other studies, we also estimated comorbidity for major depression defined by DSM-IV criteria. Of the persons with anxiety disorder 11.6% had a comorbid major depression, but only 1.3% of persons without anxiety had a major depression (adjusted OR 8.9, 95% CI 6.0-13.1). Persons with GAD most often had a comorbid major depression (27.2%), followed by persons with social phobia (17.7%).

Table 3 shows the relation of current anxiety disorders and depression including past episodes of depression. 49.3% of the persons with an anxiety disorder had a past depression, compared to 25.2% of persons without anxiety disorder (adjusted OR 2.5, 95% CI 2.0-3.1, Table 4). Persons with GAD and social phobia most often had a current or past depression (68.3% and 66.7% respectively).

Table 4 Association of anxiety with current and past depression[a]

	Current depression[b]		Current or past depression[c]	
	n	OR (95% CI)	n	OR (95% CI)
No anxiety	5015	Ref	2890	Ref
All anxiety	448	7.0 (5.2-9.5)	439	2.5 (2.0-3.1)
GAD	121	22.1 (14.6-33.5)	116	5.5 (3.7-8.3)
Agoraphobia	216	4.4 (2.8-6.8)	213	2.0 (1.5-2.8)
Other anxiety disorders[d]	165	5.7 (3.6-9.2)	163	2.3 (1.7-3.2)

[a]Complete case logistic regressions were performed. Analyses were adjusted for age, gender, education, living alone, and cognitive status.
[b]Analyses were performed on the total study population of n = 5565.
[c]Analyses were performed on the selected sample of n = 3374.
[d]The category "Other anxiety disorders" comprised social phobia, specific phobia and panic disorder. Separate ORs were not calculated because of low numbers.
OR, odds ratio; CI, confidence interval; GAD, generalized anxiety disorder.

Discussion

In this population-based study of older adults, 18% of persons with a current anxiety disorder had a comorbid depression. Another one-third had experienced depression in the past. Only about half of the persons with an anxiety disorder had never experienced depression. Some studies observed similar comorbidity estimates, but many reported higher rates of comorbidity (7,10-11). This is despite the fact that previous studies generally applied a more stringent categorization of depression. In the current study, we estimated comorbidity of anxiety disorders with depressive syndromes (major depression, dysthymia and minor depression), whereas most previous research focussed on comorbid major depression only. Comorbidity of major depression with anxiety disorder in the current study was only 11.6%, which is lower than observed in other studies.

Several explanations for this discrepancy are conceivable. First, some studies in older

adults did not apply DSM-criteria to diagnose depressive disorders, but used GMS-AGECAT, a case definition specifically designed for older adults. GMS-AGECAT has been shown to identify more depressive cases than instruments applying DSM-IV criteria (32). Indeed, the prevalence of depression in the study of Kvaal *et al.* (9.7%) (11), who applied the GMS-AGECAT system, was much higher than the depressive syndrome prevalence in our study (4.1%). At the same time, the anxiety prevalence was much lower than in our study (3.1% versus 8.2%). Low anxiety prevalence in combination with high depression prevalence may account for the high observed comorbidity of 65% anxiety with depressive syndromes found in the study of Kvaal *et al.* Similarly, in the AMSTEL study, Schoevers *et al.* (33) also applied the GMS-AGECAT system and observed a depression prevalence of 12.9%. Not surprisingly, they found that as much as 60% of GAD cases had a comorbid depression. Most likely, the high comorbidity in these studies can be partly attributed to the diagnostic system used.

Second, differences in comorbidity estimates between studies may be explained by variation in the time frame of ascertainment. Most studies reported a one-year prevalence of anxiety and depression (7-8), and in some studies point prevalence was measured (9,11). An example of the latter is the ESPRIT study, Ritchie *et al.* (9) reported a one-month prevalence of anxiety and a fifteen-day prevalence of depression. This may explain the low comorbidity between anxiety and depression observed in this cohort (12%). In the current study, to best assess point prevalence, we primarily reported the one-year prevalence for anxiety disorders and the one-month prevalence for depression and for GAD. Although these disorders are generally chronic (20,34), this may have contributed to the relatively low comorbidity estimate we observed. Comorbidity increased only slightly if we accounted for depressions detected in the one or two years before anxiety assessment. To estimate one-year prevalence of depression we used multiple additional sources other than the SCAN interview. Also, for some extreme cases, depression assessment was two months after anxiety assessment. However, median time between depression and anxiety assessment was two weeks. Furthermore, both anxiety and depression in older persons are, as mentioned above, relatively chronic disorders. We therefore expect this time lag to hardly affect our comorbidity estimates.

Third, the studies described previously were, like the current study, population-based studies of older adults. However, sample size varied. The study by Heun *et al.* (12) for example, was a particularly small study, in which only 19 cases with anxiety disorder were identified. The study by Kvaal *et al.* (11) included less than 100 persons with anxiety disorder. The estimates of comorbidity from these studies may not be very precise. A small sample size may further increase the between-study-variability in observed comorbidity. Cairney *et al.* (7), Schoevers *et al.* (33) and the current study report more precise estimates based on large studies with many cases of anxiety disorder.

Fourth, unlike the commonly used WHO-CIDI, a structured interview performed by lay interviewers (35), we used the semi-structured SCAN interview conducted by clinicians to diagnose depression. Scoring of the SCAN interview does not rely on the participant's answers only, but also on the clinician's judgement. Structured lay interviews like the CIDI generally identify more cases than clinical interviews like the SCAN (35-36). This most likely reduced comorbidity of depression with anxiety in our study if compared to studies using the CIDI. In addition, the SCAN and the CIDI were designed for use in adults, not specifically for older adults. This might limit reliability and validity of the diagnosis in this older age group. However, both the CIDI and the SCAN have been used previously to diagnose anxiety and depression in older adults (7,25).

Finally, most studies used the same instrument to diagnose anxiety and depression. If disorders are assessed with one measurement instrument and during one session, comorbidity may be diagnosed more frequently than if assessed by different instruments or in different situations. This "common method variance" can artificially increase the observed comorbidity between anxiety and depression (14). To control the effect of common method variance, we diagnosed anxiety with the CIDI and depression with the SCAN and we conducted the interviews on different days. Merely using two different instruments on the same day, or the same instrument on different days to diagnose anxiety and depression might not be sufficient to abolish one of the causes of common method variance; the consistency effect (14). The ESPRIT study (9) used the same instrument to assess both anxiety and depression, but in addition, a team of psychiatrists and psychologists also considered medical history, medication use and neurological assessment. This design also reduced common method variance. Notably, the ESPRIT study and the present study reported a relatively low comorbidity between anxiety and depression compared to studies using the same instrument to diagnose anxiety and depression.

To assess anxiety and depression with the same and with different instruments during one assessment round would have enabled us to estimate the extent of this common method variance. In the current study we did not assess anxiety with SCAN or depression with CIDI. Our primary aim was to estimate the comorbidity without inflation by this potential problem to give an indication of its possible extent.

An additional observation in the current study was the relatively high prevalence of low MMSE levels in participants with GAD. This may reflect an underlying etiological process. Cognitive impairment can increase the likelihood that stressful circumstances or events result in continued worries or anxiousness. Response bias due to cognitive impairment cannot be ruled out either (37).

In the present study we examined prior depressions in persons with current anxiety disorders. Unlike most other studies assessing depression over longer periods of

time, we did not depend on retrospective depression self-report only. Prior episodes of depression were detected by continuous monitoring of GP-records over a mean period of nine years, a previous interview round and previous depression self-report. Combining multiple sources of retrospective and prospective ascertainment of past depression may reduce ascertainment bias often seen in other studies (38).

Continuous monitoring was, however, performed for most but not for the entire cohort. The original cohort was continuously monitored, whereas in the additional cohort persons without anxiety were not monitored. This selective sampling had some consequences for analyses on past depression. To overcome potential bias, we adjusted analyses for age and gender. In addition, we presented analyses stratified by age. These stratified analyses suggest that selection on age does not have a large effect on our results.

Half of all persons with an anxiety disorder had never experienced any depressive syndromes. Hence, in a substantial number of cases, pure anxiety disorders occurred. We observed few persons with pure GAD. In other studies GAD was also found to be highly comorbid with depression, whereas for example specific phobia and agoraphobia are known to be less comorbid with depression (7-8).

Although comorbidity between current anxiety and depression was lower than observed in other studies of older adults, half of all persons with an anxiety disorder had had depression in their history. This suggests a common vulnerability for the two disorders. Kendler *et al.* (39) demonstrated in a twin study that this vulnerability may comprise common genetic factors for anxiety and depression. A shared vulnerability might not necessarily lead to high comorbidity at one moment, but rather to an increased risk of any of the two disorders during life as observed in the current study. Environmental risk factors in susceptible persons may then determine which disorder will occur at a specific point in time (39).

An alternative explanation for the high comorbidity between the two disorders is that anxiety disorders increase the risk for developing depression. Anxiety disorders other than GAD are generally thought to have an onset early in life during childhood and adolescence, whereas the onset of depression is mostly during adulthood (40-41). Indeed, several longitudinal studies reported that anxiety precedes depression (42-43). However, the reverse was also observed (44-45). Whether anxiety and depression arise from the same diathesis or are risk factors for each other might differ per subtype of anxiety disorder.

In conclusion, our study implies that current comorbid depression in older adults with anxiety disorders may often be overestimated and less common than previously suggested. However, if past depression is taken into account, most persons with anxiety will have experienced depression.

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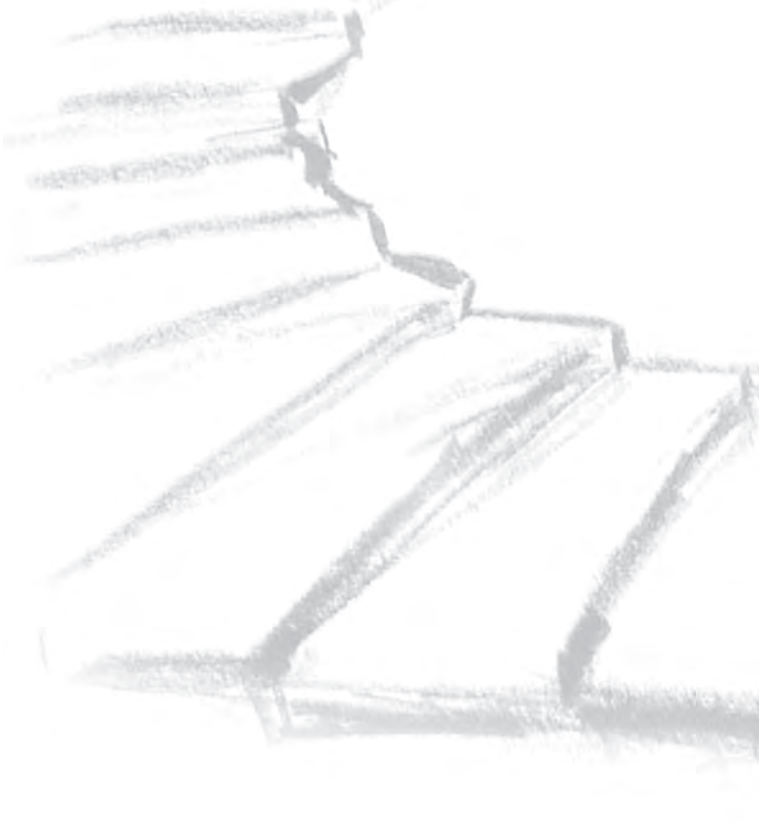

Chapter 3

Mental health service use of community-dwelling older adults with anxiety disorders

Karin Hek, Henning Tiemeier, Nikkie Aarts,
Bruno H. Ch. Stricker, Albert Hofman,
André I. Wierdsma, Cornelis L. Mulder

Abstract

Objective: To examine the frequency of mental health service use among older adults with prevalent anxiety disorders and compare this with mental health service use in older adults with depressive syndromes or comorbid anxiety and depression. **Methods:** The study population comprised 600 older adults with DSM-IV diagnoses of anxiety, depression or comorbid anxiety and depression from a population-based cohort. We screened general practitioner's (GP) records for anxiety and depression-related visits, used pharmacy records to assess medication use, and used a psychiatric case register to assess specialized mental health care utilization in the year before diagnosis. **Results:** Forty percent of the older adults with an anxiety disorder visited the GP with anxiety or depression-related symptoms, used specialized mental health services or used anxiolytic, anti-depressant or hypnotic and sedative medication. Sixty percent of those with a depressive syndrome or comorbid anxiety and depression used the studied services. The majority used medication only. **Conclusions:** This study confirms that mental health service use among older adults with anxiety disorders is low. Improved recognition in primary care but also improved awareness in the general population could contribute to mental health service use for these common and treatable disorders in the older adult population.

Introduction

Anxiety disorder is a common psychiatric problem in late life, affecting 3 to 15% of older adults (1). Although associated with considerable distress, impairment in quality of life, disability, and increased mortality (2-6), mental health service use amongst people with an anxiety disorder is low. A recent study amongst Canadian older adults reported that approximately one fifth of people with an anxiety disorder used mental health services (7), whereas of those with a mood disorder more than two fifth used mental health services and of the people with comorbid anxiety and mood disorder more than three quarters used such services.

Whereas anxiety at later age is common, studies focusing on mental health service use in older adults diagnosed with anxiety disorders are scarce. In the current study we compared mental health service use (in primary and specialized care) of older people with a prevalent anxiety disorder to such service use by older people with a prevalent depressive syndrome or comorbid anxiety and depression. Contrary to other studies, we did not depend on self-reported mental health service use, but we used multiple registries to assess mental health care use. We screened general practitioner's (GP's) records for anxiety and depression-related visits, used pharmacy records to assess anxiety and depression-related medication use, and used a psychiatric case register to assess specialized mental health care utilization. In addition, we described mental health service use by subtypes of anxiety and depressive syndromes.

Method

Study population

This study was embedded in the Rotterdam Study; a prospective population-based cohort study of older adults to assess risk factors for chronic diseases (8). All inhabitants of a district in Rotterdam aged 55 years and over were invited to participate. Every four years participants undergo an extensive home interview and physical examination at a research center. The medical ethics committee of the Erasmus MC approved the Rotterdam Study.

In the 2002-2005 examination round anxiety disorders and depressive syndromes were assessed. Of the 7609 eligible people, 6007 people participated (78.9%). We excluded 427 people without valid assessment of anxiety or depression, 10 people with bipolar disorder, 28 people without access to the GP record and 39 people with incomplete GP records. Of the 5503 remaining people, 600 had an anxiety disorder or a depressive syndrome.

Anxiety and Depression assessment

Anxiety disorders were diagnosed as part of the home interview. Trained lay interviewers conducted a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI) to assess the following anxiety disorders with a computerized diagnostic algorithm according to DSM-IV criteria: generalized anxiety disorder (GAD), panic disorder, agoraphobia, social phobia and specific phobia. The M-CIDI was specifically designed to obtain DSM-IV diagnoses and test-retest reliability for anxiety disorders is good (kappa for any anxiety disorder: 0.81) (9). For all anxiety disorders, except GAD, one-year prevalence was assessed. For GAD we assessed one-month prevalence, as problems to recall symptoms ending up to 12 months ago were expected. However, GAD is a chronic disease, with low recovery rates, and one-month prevalence presumably approximates one-year prevalence (10).

Depression was assessed in a two-step procedure. First, during the home-interview participants were screened for symptoms of depression with the Center of Epidemiologic Studies Depression scale (CES-D) (11). The CES-D detects depression in older adults with a high sensitivity (12). Screen-positive people (CES-D score ≥ 16) were invited for a semi-structured clinical interview with the Schedules for Clinical Assessment of Neuropsychiatry (SCAN). This interview was conducted by a trained clinician at the participant's home. With a computerized DSM-IV-based diagnostic algorithm, major depression, minor depression and dysthymia during the past month were diagnosed.

Mental health care use assessment

Mental health care use was assessed for the year before anxiety and depression diagnosis. We did not assess mental health care use after the interview date, because a diagnosis of anxiety or depression at the interview led to an increase of mental health care consumption. Records of GPs were monitored for symptoms of anxiety and depression. Coding of the records was performed blind from interview diagnosis. In addition, 80% of the patients had a GP that registered episodes of disease using the International Classification of Primary Care (ICPC) system. ICPC codes for anxiety and depressive syndromes were retrieved (p74, p76).

GP records and the Psychiatric Case Register (PCR) (13) were used to obtain information on outpatient and inpatient specialized mental health care. The PCR comprises data on outpatient and inpatient mental health service use in the Rotterdam area. Records from the PCR were matched to the study population using a probability link based on the first two letters of the family name, date of birth, gender, and the numeric part of the postal code.

Medication prescriptions were retrieved from pharmacies linked to the Rotterdam Study (coverage > 95%). The prescription of anti-depressants (N06A), anxiolytics (N05B) and

hypnotics and sedatives (N05C) were classified according to the Anatomical Therapeutic Chemical classification system (14).

Results

The study sample comprised 600 people, of whom 379 had an anxiety disorder, 139 had a depressive syndrome and 82 had comorbid anxiety and depression. The mean age was 72.8 years (range 59.4 to 98.7 years). More than three-quarters of the people were female (76.8%).

Table 1 shows mental health service use in primary care and specialized care over the past year in the three disorder groups. GPs documented anxiety or depressive complaints in only 9.5% of the people who fulfilled criteria of an anxiety disorder. Such complaints were recorded in 17.3% of those with a depressive syndrome, and 19.5% of those with comorbid anxiety and depression. The documentation of anxiety or depressive complaints was most frequent in people with a panic disorder (25.9%). A formal diagnosis of anxiety or depression was made by the GP in less than 2% of prevalent cases within the previous year.

Inpatient or outpatient mental health service use was infrequent: 2.9% of the people with an anxiety disorder, 6.5% of those with a depressive syndrome and 11.0% of those with comorbid anxiety and depression used specialized mental health services.

Anxiolytic or hypnotic and sedative medication was prescribed to 37.7% of people with anxiety, 56.8% of people with depressive syndrome and 57.3% of people with comorbid anxiety and depression. Selective serotonin reuptake inhibitor (SSRI) use was high in people with GAD (14.9%), social phobia (22.9%), panic disorder (25.9%), a depressive syndrome (18.7%) or comorbid anxiety and depression (23.2%), for which SSRIs were indicated.

When combining GP complaint documentation and diagnosis, inpatient and outpatient mental health service use, and medication prescription 40.6% of the older adults with an anxiety disorder, 59.0% of the people with a depressive syndrome and 62.2% of the people with comorbid anxiety and depression used the studied services.

Table 1 One-year mental health service use in older people with anxiety disorders and depressive syndromes

	Anxiety disorders[a]						Depressive syndromes[a]				Comorbid anxiety and depression[a]
	All anxiety disorders	Agora-phobia	GAD	Social phobia	Specific phobia	Panic disorder	All depressive syndromes	Major depression	Dysthymia	Minor depression	
	n=379	n=196	n=74	n=48	n=76	n=27	n=139	n=65	n=13	n=61	n=82
Mental health service use	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
No mental health service use	59.4 (225)	63.3 (124)	45.9 (34)	50.0 (24)	72.4 (55)	40.7 (11)	41.0 (57)	41.5 (27)	38.5 (5)	41.0 (25)	37.8 (31)
Use of mental health care services in primary care											
Symptoms documented by GP											
Anxiety or depressive symptoms	9.5 (36)	6.6 (13)	14.9 (11)	14.6 (7)	5.3 (4)	25.9 (7)	17.3 (24)	20.0 (13)	7.7 (1)	16.4 (10)	19.5 (16)
Anxiety symptoms	6.6 (25)	4.6 (9)	6.8 (5)	10.4 (5)	5.3 (4)	18.5 (5)	6.5 (9)	7.7 (5)	0 (0)	6.6 (4)	14.6 (12)
Depressive symptoms	3.7 (14)	3.1 (6)	8.1 (6)	6.3 (3)	0 (0)	7.4 (2)	14.4 (20)	15.4 (10)	7.7 (1)	14.8 (9)	9.8 (8)
Diagnosis by GP											
Anxiety disorder or depressive syndrome	2.4 (9)	1.5 (3)	2.7 (2)	4.2 (2)	0 (0)	11.1 (3)	2.9 (4)	4.6 (3)	0 (0)	1.6 (1)	7.3 (6)
Anxiety disorder	1.1 (4)	1.0 (2)	0 (0)	2.1 (1)	0 (0)	3.7 (1)	0 (0)	0 (0)	0 (0)	0 (0)	2.4 (2)
Depressive syndrome	1.3 (5)	0.5 (1)	2.7 (2)	2.1 (1)	0 (0)	7.4 (2)	2.9 (4)	4.6 (3)	0 (0)	1.6 (1)	4.9 (4)
Use of outpatient mental health services	2.9 (11)	1.0 (2)	8.1 (6)	4.2 (2)	0 (0)	11.1 (3)	6.5 (9)	6.2 (4)	0 (0)	8.2 (5)	11.0 (9)
Use of inpatient mental health services	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1.2 (1)
Medication prescription[b]											
Medication (N06A or N05B)[c]	32.5 (123)	30.1 (59)	41.9 (31)	41.7 (20)	21.1 (16)	51.9 (14)	47.5 (66)	46.2 (30)	53.8 (7)	47.5 (29)	50.0 (41)
Medication (N06A, N05B or N05C)	37.7 (143)	35.2 (69)	45.9 (34)	47.9 (23)	26.3 (20)	55.6 (15)	56.8 (79)	55.4 (36)	61.5 (8)	57.4 (35)	57.3 (47)
Benzodiazepines (N05B)	21.9 (83)	20.4 (40)	27.0 (20)	22.9 (11)	11.8 (9)	40.7 (11)	33.1 (46)	30.8 (20)	38.5 (5)	34.4 (21)	39.0 (32)
Anti-depressants (N06A)											
SSRIs (N06AB)	10.3 (39)	6.6 (13)	14.9 (11)	22.9 (11)	6.6 (5)	25.9 (7)	18.7 (26)	21.5 (14)	23.1 (3)	14.8 (9)	23.2 (19)
Other anti-depressants	7.4 (28)	9.2 (18)	4.1 (3)	12.5 (6)	5.3 (4)	3.7 (1)	12.2 (17)	13.8 (9)	7.7 (1)	11.5 (7)	6.1 (5)
Hypnotics and sedatives (N05C)	13.2 (50)	12.2 (24)	17.6 (13)	12.5 (6)	9.2 (7)	18.5 (5)	26.6 (37)	27.7 (18)	15.4 (2)	27.9 (17)	25.6 (21)

GAD, generalized anxiety disorder; GP, general practitioner; SSRI, selective serotonin re-uptake inhibitor.
[a]These categories are exclusive. The category of anxiety disorders and the category of depressive syndromes do not include people with comorbid disorders.
[b]Medication includes anti-depressant medication, anxiolytic medication and hypnotics and sedatives (categories N06A, N05B and N05C of the Anatomical Therapeutic Chemical classification system).
[c]This includes anti-depressants (N06A) and anxiolytics (N05B), but not hypnotics and sedatives (N05C).

Discussion

In the current study of older adults with a prevalent anxiety disorder, mental health service use in primary care and specialized care was low. Forty percent of those with an anxiety disorder visited the GP with anxiety or depression related symptoms, used inpatient or outpatient mental health services or used anxiolytic, anti-depressant or hypnotic and sedative medication. In comparison, sixty percent of those with a depressive syndrome and of those with comorbid anxiety and depression used medication or any of the studied mental health services. The majority used medication only. The findings of this study may suggest a recognition, treatment and referral gap in general practice. However, several other explanations need to be discussed.

First, although we assessed mental health care by multiple registers to ensure completeness, we may have underestimated mental health care use. Mental health care services by primary care psychologists (PCP) do not require referral from the GP and these were not included in the PCR. However, use of PCPs by older adults in the Netherlands is low (0.5% of the general population visits a PCP per year, of which about 7% is 60 years and older) and almost two thirds of the people visiting a PCP are referred by their GP (www.lve.nl/docs/jb2011.pdf).

Second, indication of medication use was unknown. In older adults without anxiety or depressive syndromes benzodiazepine use and sedatives use was also high (10.5% each in the Rotterdam Study). These medications are presumably often prescribed for e.g., sleeping or pain problems, which are common amongst older adults.

Third, this was a study of prevalent disorders in older adults. Anxiety and depression in older adults are generally chronic. GPs may have been aware of the anxiety and, or depressive syndrome, and they may have documented a diagnosis before the year that was studied here. This would explain the high medication use, but not the low mental health care use.

Fourth, although these people were diagnosed with an anxiety or depressive syndrome they may not themselves have felt the need to seek help (7). Nevertheless, one of the criteria for a DSM-IV anxiety or depression diagnosis is impairment.

In line with other studies, more people with a depressive syndrome than an anxiety disorder used mental health services; the group with comorbid anxiety and depression included most mental health service users (7,15-16). Of the people with an anxiety

disorder, the group with a panic disorder included the highest percentage of mental health service users. Given the high burden of the disorder, this was an expected finding. Inpatient and outpatient mental health service use was low across al subtypes of anxiety and depressive syndromes. This was also consistent with other studies (6,17).
In the current study medication use was high. In particular benzodiazepines and sedatives were frequently used by all groups. As noted above, these medications were presumably not prescribed for anxiety disorders and depressive syndromes only. Notably, SSRI use was much lower and more closely matched the numbers of people who reported anxiety or depressive problems.
In general, older people are less likely to use mental health services than younger people (16,18). A plausible explanation is that older adults tend to present their problems as somatic complaints, rather than psychiatric problems (19). In addition, they have more difficulties than younger people to identify anxiety and depressive symptoms as mental health problems (20).
In conclusion, this study confirms that mental health service use among older adults with anxiety disorders is low. Improved recognition in primary care but also improved awareness of these disorders in the general population could contribute to the use of services for these common and treatable disorders in the older adult population.

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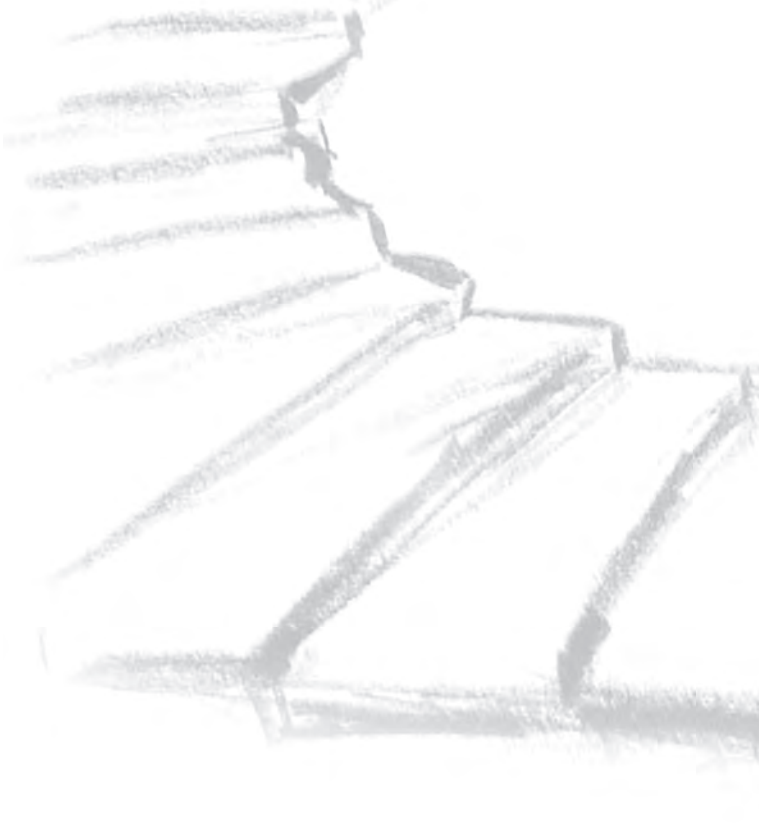

Chapter 4

Anxiety disorders and salivary cortisol levels in older adults: a population-based study

Karin Hek, Nese Direk, Rachel S. Newson, Albert Hofman,
Witte J.G. Hoogendijk, Cornelis L. Mulder,
Henning Tiemeier

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Abstract

Context: The hypothalamic-pituitary-adrenal (HPA) axis is one of the body's main systems that controls response to stress. It acts through the hormone cortisol. While the dysregulation of cortisol has been associated with anxiety disorders, the evidence is inconsistent. Moreover, only a few small studies have assessed this relationship in older adults. **Objective**: To determine whether in adults aged 65 years and over there is a difference in daily cortisol pattern between those with and without an anxiety disorder. **Methods:** The study population comprised 1788 older adults from a population-based cohort. The Munich version of the Composite International Diagnostic Interview was used to diagnose anxiety disorders (generalized anxiety disorder, social phobia, specific phobia, agoraphobia and panic disorder). The cortisol awakening response and total cortisol secretion over the day were calculated from cortisol levels in four saliva samples taken over the course of one day (at awakening, 30 minutes after awakening, at 1700h, at bedtime). **Results:** Older adults with an anxiety disorder (n = 145, median duration since first symptoms 41 years) had a lower cortisol awakening response ($P = 0.02$) than those without such a disorder (n = 1643). This association was most prominent in those with generalized anxiety disorder ($P = 0.008$), but was not associated with the extent of chronicity of anxiety disorders. **Conclusion:** Older adults from the general population with long-lasting anxiety disorders had a lower cortisol awakening response than those without. This is consistent with the notion that chronic anxiety may result in downregulation of HPA-axis activity. Longitudinal studies are needed to confirm this mechanism.

Introduction

The hypothalamic-pituitary-adrenal axis (HPA axis) is one of the body's main systems that controls response to stress. It acts through the hormone cortisol, which is produced in the adrenal cortex and affects many tissues including the brain. Cortisol is secreted in a distinct daily pattern whereby cortisol levels rise rapidly after awakening (the cortisol awakening response or CAR) and decrease slowly thereafter. Deregulation of the CAR, and total cortisol secretion over the day have been associated with various disorders, including anxiety disorders (1). In addition, age-dependent HPA-axis dysregulation may increase the vulnerability of older adults for psychiatric disorders (2).

Although numerous studies have described the relationship between the HPA axis and anxiety disorders, the evidence is inconsistent and only a few studies assessed this relationship in older adults. While one study reported that older adults (mean age 74, n = 111) with generalized anxiety disorder (GAD) had higher cortisol levels than those without GAD (3), another study (mean age 76, n = 48) reported no association of cortisol levels with an anxiety symptom score (4). A further study of older people (mean age 73, n = 201) also reported no difference in total cortisol levels during the day between people with and without lifetime GAD in a non-stressful condition, but the CAR was not assessed (5).

In the current study we assessed not only total cortisol secretion, but also the CAR. We jointly analysed anxiety disorders, but also present data on GAD, social phobia, specific phobia and agoraphobia separately. Furthermore, this study was no convenience sample, but comprised older adults (aged 65 and over) from the general population to minimize selection effects.

Method

Study setting

This study was set in the Rotterdam Study, a prospective population-based cohort study of older adults designed to assess risk factors for chronic diseases (6). In 1990, all residents in a district in Rotterdam who were aged 55 years and over were invited to participate. Every four years, participants undergo an extensive home interview and physical examination at a research centre. The Medical Ethics Committee of the Erasmus MC approved the Rotterdam Study.

The fourth examination round (2002-2004, n = 3550) assessed anxiety disorders and salivary cortisol levels. The study population comprised 1788 people after exclusion of people without a valid anxiety assessment (n = 287), people without the first two cortisol measurements (n = 1152), people using corticosteroids (n = 287), and people with dementia (n = 36). Almost two thirds of those who were excluded (n = 1762) were

female against 56.9% of the study participants. The excluded group was significantly older than the study population (mean age 77.3 versus 74.7) and had a lower education.

Assessment of anxiety disorders

Prevalent anxiety disorders were diagnosed during the home interview. Trained lay interviewers conducted a slightly adapted version of the Munich version of the Composite International Diagnostic Interview (M-CIDI) (7). The following anxiety disorders were assessed with a computerized diagnostic algorithm according to DSM-IV criteria: GAD, panic disorder, agoraphobia, social phobia and specific phobia. Age of symptom onset was recorded. Obsessive compulsive disorder and post-traumatic stress disorder (PTSD) were not assessed, because these disorders are relatively rare in the general population.

Salivary cortisol protocol

Saliva samples were collected on awakening (T1), 30 minutes after awakening (T2), at 1700h (T3), and at bedtime (T4). Cortisol levels in these samples were determined as previously described (8). The multiple measures of cortisol were combined in summary measures to provide valid information about the diurnal pattern of cortisol. We calculated the area under the curve with respect to the ground (AUCg) and the cortisol awakening response (CAR). The AUCg summarizes overall diurnal cortisol exposure. The CAR is a measure of the dynamics of the HPA-axis response upon awakening. The CAR and the AUCg are thought to be regulated differently (9). The AUCg was calculated as the total area under the curve from the individual cortisol measures on the y-axis and the time between cortisol measures on the x-axis. To not measure the effect of the CAR, we did not include T2 in the calculation. We corrected for total time awake and only calculated the AUCg for those with data on all three time points (n = 1664). The CAR was calculated as the difference between cortisol levels at T2 and T1 over two (n = 1788) (10). Analyses on the CAR were adjusted for the time between measurements.

Assessment of other variables

Age, sex, marital status, psycholeptics use, psychoanaleptics use, hormonal drug use, usual sleep duration, and alcohol, coffee and tea consumption were recorded. Education was grouped according to the Standard Classification of Education and rated on a scale from primary education (1) to university level (7). Height and weight were measured at the research centre to calculate body mass index (BMI). Smoking was coded according to current smoking status. Cognitive capacity was assessed using the Mini Mental State Examination. A cut-off of 26 indicated adequate cognitive capacity versus impaired cognitive capacity. Disability (Activities of Daily Living) was

assessed with the Stanford Health Assessment Questionnaire. The standard cut-off of a mean score of 0.5 indicated no disability versus mild to severe disability. Participants were continuously monitored for occurrence of coronary heart disease. International Classification of Diseases, 10th Revision was used to assign diagnoses of myocardial infarction (I21), percutaneous transluminal coronary angioplasty, coronary artery bypass graft and other forms of acute (I24) or chronic ischemic heart disease (I25). Diabetes Mellitus was diagnosed when a fasting plasma glucose level was at least 7.0 mmol/liter, a non-fasting plasma glucose level was at least 11.1 mmol/liter, or oral antidiabetes medication or insulin were used, or treated by diet and registered by the general practitioner (GP) as having diabetes. The number of sites with atherosclerotic plaques was identified by ultrasonography of both carotid arteries. Blood pressure was measured at the right brachial artery using a random-zero sphygmomanometer with the participant in sitting position.

In those who screened positive for depressive symptoms with the Centre for Epidemiologic Studies Depression scale (CES-D, cut-off score of 16), DSM-IV-defined major depression was assessed with a semi-structured clinical interview (Schedules for Clinical Assessment of Neuropsychiatry). The CES-D has a high sensitivity for major depression in older adults (11). Depressive syndromes during follow-up were assessed based on two clinical interviews, self-report depression and GP records. Month of cortisol sampling was categorized in months with less daylight (October-February) and months with more daylight (March-September). All variables, except education, were assessed during the same examination round as anxiety disorders and salivary cortisol.

Statistical analysis

Baseline characteristics for persons with and without anxiety disorder were compared using chi-square statistics, Student's t-tests and the Mann-Whitney U test. AUCg and CAR were normally distributed after excluding values above the 99th percentile. Covariates were imputed using the Expectation-Maximization algorithm. All covariates had less than 2% missing values. Differences in AUCg and CAR in persons with and without anxiety disorders were tested using linear regression analyses. Covariates were selected if the effect estimates of the association between cortisol levels and anxiety disorders changed more than 5% in the models. Subtypes of anxiety disorders were analysed jointly and separately with the exception of panic disorder which was too infrequent. We repeated all analyses excluding people with major depression. Data were analyzed using SPSS PASW version 17.

Results

The study population comprised 145 people with an anxiety disorder and 1643 people without an anxiety disorder. Agoraphobia (n = 71, 3.9%) and GAD (n = 39, 2.1%) were the most prevalent anxiety disorders. Thirty-five people were diagnosed with specific phobia (1.9%), sixteen had social phobia (0.9%) and four had a panic disorder (0.3%). Sixteen people (0.9%) had more than one anxiety disorder. The characteristics of the study population are shown in Table 1. The group with anxiety disorders comprised more females than the group without such a disorder (81% vs. 55%, $P < 0.001$). In addition, those with an anxiety disorder had a lower education ($P = 0.003$) and an eight-fold higher prevalence of depression than those without an anxiety disorder (8% vs. 1%, $P < 0.001$). Unadjusted cortisol levels at 30 minutes after awakening and at 1700h were lower in people with anxiety disorders. Supplementary Figure 1 shows the cortisol pattern over the day of older adults with and without anxiety disorders. After adjustment for covariates, cortisol level at 30 min after awakening was lower in those with an anxiety disorder (beta = -2.67; 95% CI = -4.44, -0.90; $P = 0.003$), but not cortisol level at awakening (beta = -0.28; 95% CI = -1.79, 1.23; $P = 0.72$), at 1700h (beta = -0.47; 95% CI = -1.06, 0.12; $P = 0.13$) or at bed time (beta = -0.18; 95% CI = -0.63, 0.28; $P = 0.44$). In addition, those with an anxiety disorder had significantly lower CAR than those without an anxiety disorder (beta = -1.19; $P = 0.02$, Table 2). Total cortisol secretion did not differ. When anxiety disorders were stratified by subtype, the association between GAD and the CAR was significant (beta = -2.50; $P = 0.008$, Table 2). Exclusion of people with major depression did not change these results (all anxiety disorders: beta = -1.18; 95% CI = -2.17; -0.18, $P = 0.021$, GAD: beta = -2.58; 95% CI = -4.66; -0.50; $P = 0.015$).

In a post-hoc analysis we tested the influence of chronicity of the anxiety disorders on the CAR by comparing the CAR of people with an anxiety disorder with duration shorter than 5.44 years (lowest quartile) to those with an anxiety disorder that lasted longer than 59.69 years (highest quartile). The groups did not differ significantly, but, if anything, the people with a more chronic anxiety disorder had a higher CAR (beta = 2.52; 95% CI = -0.86, 5.90; $P = 0.14$).

Discussion

In this population-based study of older adults, people with an anxiety disorder had a significantly lower CAR than those without anxiety disorder.

Three previous studies assessed the association between anxiety and cortisol levels in older adults. First, Mantella *et al.* (3) found higher cortisol levels (CAR as well as total cortisol secretion) in people with GAD than in healthy controls. This is opposite to our

Table 1 Characteristics of the study group

	Anxiety disorder* (n=145)	No anxiety disorder (n=1643)	*P*-value**
% Women	80.7	54.8	<0.001
Age, years, mean (SD; range)	74.7 (5.3; 66.1-90.0)	74.7 (5.6; 65.1-92.7)	0.93
BMI, kg/m^2, mean (SD; range)	27.7 (4.2; 18.6-41.3)	27.5 (4.0; 14.2-45.7)	0.44
% Current smokers	53.8	58.5	0.29
Alcohol drinking, units/day, median (IQR; range)	0.1 (1.0; 0-4.7)	0.5 (1.2; 0-5.0)	<0.001
Coffee drinking, cups/day, median (IQR; range)	3.0 (2.0; 0-23)	3.0 (2.0; 0-24)	0.14
Tea drinking, cups/day, median (IQR; range)	3.0 (2.1; 0-12)	3.0 (3.0; 0-16)	0.64
Mean sleep duration, hours, mean (SD; range)	6.5 (1.5; 2.5-10)	6.9 (1.2; 2-11)	0.013
Education, mean (SD; range)	2.6 (1.5; 1-6)	3.1 (1.7; 1-7)	0.003
Marital status			0.68
% single	4.1	5.2	
% married	60.7	64.5	
% widowed	29.0	25.3	
% divorced	6.2	5.2	
Cognitive status (MMSE), mean (SD; range)	27.4 (1.8; 19.0-30.0)	27.7 (1.8; 17.0-30.0)	0.09
% Impaired cognitive capacity	14.5	9.0	0.04
Functional disability, median (IQR; range)	0.6 (0.7; 0-2.3)	0.3 (0.5; 0-2.8)	<0.001
% Mild to severe functional disability	64.8	39.0	<0.001
% Major depression	8.3	1.1	<0.001
% Depression during follow-up	46.2	25.6	<0.001
Depressive symptoms (CES-D), median (IQR; range)	9.0 (16.1; 0.0-48.0)	3.0 (6.0; 0.0-44.0)	<0.001
Negative affect (CES-D), median (IQR; range)	2.0 (7.0; 0.0-20.0)	0.0 (1.0; 0.0-16.0)	<0.001
Anxiety duration, years, median (IQR; range)	40.6 (54.4; 0.3-73.2)	-	-
% Psycholeptics users	25.5	12.4	<0.001
% Psychoanaleptics users	9.7	3.6	<0.001
% Hormonal medication users	2.1	1.2	0.34
% Diabetes Mellitus	13.1	17.7	0.16
% Coronary Heart Disease	4.8	8.5	0.12
Systolic blood pressure, mean (SD; range)	156.6 (22.6; 106.0-217.0)	152.4 (21.3; 99.5-240.0)	0.02
No. of atherosclerotic plaques, mean (SD; range)	2.7 (1.8; 0-6)	2.9 (1.9; 0-6)	0.13
Cortisol levels, nmol/L, mean (SD; range)			
T1, at awakening	14.1 (7.7; 0.3-35.1)	14.7 (8.6; 0.0-60.6)	0.40
T2, 30 min after awakening	15.9 (8.3; 0.2-41.9)	18.4 (10.2; 0.1-64.2)	0.001
T3, 1700h	3.7 (2.4; 0.4-13.7)	4.2 (3.4; 0.0-29.3)	0.012
T4, at bedtime	2.2 (3.1; 0.1-26.7)	2.3 (2.4; 0.0-24.3)	0.71
AUCg	6.5 (3.0; 0.3-17.2)	7.0 (3.4; 0.4-26.3)	0.12
CAR	0.9 (5.1; -15.6-14.3)	1.8 (5.5; -27.0-28.0)	0.05
Time of the first sample, mean (SD; range)	0731h (48min; 0425-0941h)	0734h (55min; 0305-1245h)	0.60
% Sampling in months with more daylight	58.6	52.5	0.16

AUCg, area under the curve with respect to the ground; BMI, body mass index; CAR, cortisol awakening response; CES-D, Center for Epidemiologic Studies Depression scale; IQR, inter quartile range; MMSE, mini mental state examination; SD, standard deviation.

*The group of anxiety disorders comprised people with agoraphobia, social phobia, specific phobia, panic disorder and generalized anxiety disorder.

**t-tests, chi square tests or Mann-Whitney U tests were applied to test for difference.

Table 2 Associations between anxiety disorders and cortisol summary measures

	Cortisol summary measures					
	Area under the curve (AUCg)			Cortisol awakening response (CAR)		
	n	Beta (95% CI)	*P*-value	n	Beta (95% CI)	*P*-value
No anxiety disorder	1533	Reference		1643	Reference	
All anxiety disorders*	131	-0.39 (-1.02, 0.24)	0.23	145	-1.19 (-2.15, -0.23)	**0.02**
Subtypes of anxiety disorders**						
GAD	36	-0.02 (-1.22, 1.18)	0.97	39	-2.50 (-4.34, -0.65)	**0.008**
Social phobia	15	0.68 (-1.10, 2.44)	0.46	16	-1.48 (-4.19, 1.24)	0.29
Specific phobia	31	-1.26 (-2.48, -0.03)	**0.04**	35	-1.14 (-2.98, 0.70)	0.22
Agoraphobia	63	-0.62 (-1.48, 0.25)	0.16	71	-0.30 (-1.60, 1.01)	0.66

CI, confidence interval; GAD, generalized anxiety disorder.
All analyses were adjusted for age, gender, education, time of the first sample, months with more daylight, psychoanaleptic drug use, total sleep duration, disability (yes/no), major depression, logarithm of negative affect sum score. Analyses of the CAR were additionally adjusted for the time between the first and the second sample.
*All anxiety disorders comprised GAD, social phobia, specific phobia, agoraphobia and panic disorder.
**As some people have comorbid anxiety disorders, the numbers for GAD, social phobia, specific phobia and agoraphobia do not add up to the number for all anxiety disorders. The number of panic disorders was too low to assess separately. In bold: p-values that are significant at the 0.05 level.

finding and may result from recruitment differences. Mantella *et al.* used a convenience sample recruited via e.g., advertisements and mental health clinician referral that likely included more acute and help-seeking cases while we used a population-based sample with prevalent and chronic cases. Second, Heaney *et al.* (4) did not find a difference in cortisol levels between older adults with and without anxiety. While our study included 145 people with anxiety disorders, the study of Heaney *et al.* comprised 25 older people with anxiety symptoms. This study may not have had enough power to detect small differences in cortisol levels. Third, Chaudieu *et al.* (5) assessed total cortisol secretion and not the CAR in a population-based setting. They observed no difference in cortisol secretion between people with and without lifetime GAD in non-stressful conditions, which is compatible with our finding.

In the largest study of anxiety and cortisol in adults (age range 18-65), Vreeburg *et al.* (1) observed a higher CAR in people with anxiety disorders. This population was largely recruited from GPs and specialized mental health care institutions. Consequently, the study comprised many acute, help-seeking cases, whereas we included only prevalent, chronic anxiety cases from the general population. This difference is also reflected in the higher comorbidity of major depression (77.8%) and a higher prevalence of panic disorder cases (59.4%) in the sample of Vreeburg *et al.* Furthermore, the high CAR was driven by those with a panic disorder with agoraphobia and by those with a comorbid depression.

Numerous studies have indicated that stress activates the HPA axis, raising total cortisol levels and leading to a higher CAR. However, it has been hypothesized that the HPA

axis reacts to stress with temporal hyperactivity, but when stress persists the HPA axis becomes hypoactive (12). This mechanism has also been observed for depression. Oldehinkel *et al.* (13) observed lower urinary cortisol levels in chronically depressed older adults, not in more acutely depressed people. Thus while cortisol may be raised during acute anxiety, the HPA axis reacts to a chronic disorder with reduced cortisol levels. This change from hyperactivity of the HPA axis to hypoactivity is likely mediated through increased sensitivity to negative feedback from circulating cortisol (14). In our study, we observed no association between extent of chronicity of the anxiety disorder and cortisol levels. However, acute and recent onset cases are rare in community-dwelling older adults, and even the group with the shortest duration of symptoms (lowest quartile) had an average duration of more than 3 years.

In addition, we analysed the association between cortisol and subtypes of anxiety disorders. A lower CAR was observed for all subtypes, but only GAD was significantly associated with a lower CAR. This could not be attributed to impaired cognitive capacity or depression comorbidity. GAD is generally characterized by a chronic course and is closely related to depression for which similar findings were reported (13).

Older people are known to have a lower CAR (4,15). The exact function of the CAR is unknown, but it has been suggested to play a role in e.g., memory function, and the immune system. A low CAR has been associated with cardiovascular and auto-immune disorders (15), which in turn have been linked to anxiety disorders (16). Older adults with (chronic) anxiety disorders may thus have an increased vulnerability to a wide range of disorders.

Our findings need to be discussed in light of some limitations. First, non-compliance to the cortisol sampling protocol could explain the observed lower CAR, if more common in those with anxiety disorders (15,17). Second, cortisol samples were collected on only one day. This may have resulted in less precise estimates and reduced study power. Third, because this study was performed in a population-based setting, our results can not easily be generalized to a clinical patient group. Fourth, we did not assess PTSD or traumatic experiences, which are associated with blunted cortisol levels. However, PTSD has a relatively low prevalence and this effect is therefore not likely to explain our findings. Fifth, information on any remitted anxiety disorders or parental history was not available. Last, because this study was cross-sectional, we could not infer the causality of the associations we observed.

In conclusion, older adults with an anxiety disorder in this population-based study had a lower CAR than those without such a disorder. This is compatible with the notion that chronic anxiety may result in down regulation of HPA-axis activity. Longitudinal studies are needed to confirm this mechanism.

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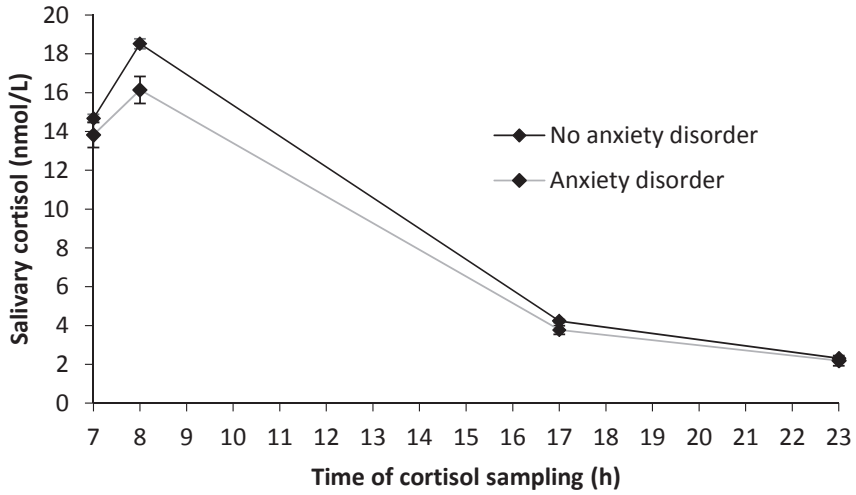
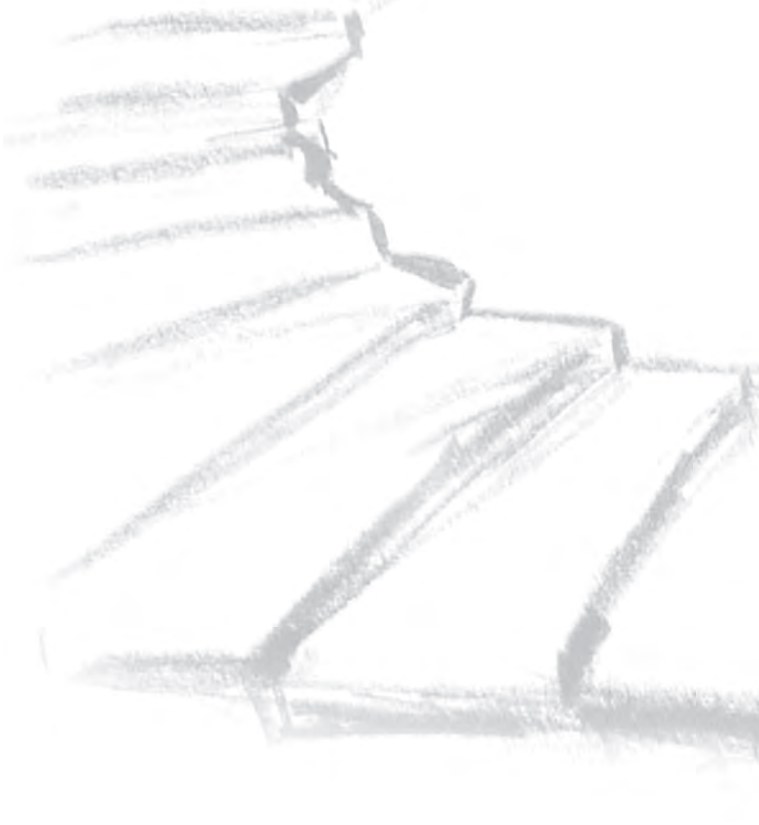
Supplementary Material



Supplementary Figure 1 Mean (standard error) diurnal cortisol pattern in older adults with and without anxiety disorders



Chapter 5

Atherosclerosis and incident depression in late life

Rachel S. Newson, Karin Hek, Hendrika J. Luijendijk,
Albert Hofman, Jacqueline C.M. Witteman,
Henning Tiemeier

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Abstract

Context: Depression is a prominent concern for older adults; therefore, it is important to identify causal mechanisms so that prevention and treatment strategies can be developed. The vascular depression hypothesis proposes that vascular factors precede the onset of depression in older adults. However, although cross-sectional associations have been established, owing to a lack of objective assessments and longitudinal data, the validity and temporal nature of this relationship is unclear. **Objective:** To examine whether atherosclerosis, an asymptomatic subclinical indicator of vascular burden, increases the risk of developing depression in older adults. **Design:** Prospective, population-based study. **Setting**: Set within the Rotterdam Study, participants were assessed on objective measures of generalized atherosclerosis at baseline (1997-1999) and followed up for an average of 6 years for incident depression. **Participants:** The baseline sample consisted of 3564 participants (56% female) with a mean age of 72 years who initially did not have depression or dementia. **Main Outcome Measures:** Depression was categorized into symptoms or syndromes and assessed in a multidimensional manner from physician and mental health specialist reports, pharmacy records (antidepressant usage), a clinical interview, and self-report. **Results:** During 21,083 person-years, 429 incidents of depressive symptoms and 197 incidents of depressive syndromes occurred. Individual atherosclerotic measures and a composite measure were not predictive of incident depressive symptoms (composite measure hazard ratio, 0.93; 95% confidence interval, 0.83-1.05), or incident depressive syndromes (composite measure hazard ratio, 0.97, 95% confidence interval, 0.81-1.16). An a priori power analysis indicated a sufficient sample size (α=.05; 0.95 power). **Conclusions:** Atherosclerosis does not appear to increase the risk of incident depression in older adults. These findings do not support the vascular depression hypothesis and, alternatively, taking findings from prior studies into account, suggest either that depression contributes to vascular burden or that both result from an underlying biological substrate.

Introduction

The vascular depression hypothesis postulates that depression in older adults manifests as a result of vascular disease (1-2). Vascular depression is proposed as an etiologically distinct subtype of depression that occurs with atypical clinical presentation (e.g., greater cognitive impairment, psychomotor retardation) and in the absence of psychobiological vulnerability or family history (1,3). The vascular depression hypothesis is clinically important, as identifying a mechanism of depression in older age would assist in developing clinical risk profiles and designing and implementing effective treatment and prevention strategies.

This hypothesis largely originates from brain imaging studies, which show increased cerebral white matter hyperintensities, gray matter lesions and brain atrophy, indicators of cerebral vascular burden, in people with depression (4-6). However, these studies have been largely cross-sectional (4-6), and the pathological basis of the abnormalities shown in imaging has not been reliably determined (7). Further, many older adults in the general population exhibit these imaging abnormalities, yet do not exhibit depressive symptoms or syndromes (8). A distinct clinical profile for vascular depression is proposed from hospital-based studies (1); however, studies in large nonclinical settings have failed to identify this distinct clinical profile (9-10). Further, vascular risk factors are purportedly associated with depression; however, this association has not been reliably found in longitudinal studies, with some studies showing no link (9,11-12), and other studies showing that only a few vascular risk factors such as smoking and antihypertensive medication were predictive of depression (13-14). If vascular disease caused incident depression, a more consistent pattern in risk factors would be expected. Another line of evidence often cited in support of the vascular depression hypothesis is depression following overt vascular events such as stroke (poststroke depression) (15). However, a recent prospective study examining this reported that vascular risk factors could not account for the relation between stroke and depression (16). This indicates that depression seen after stroke may not be vascular in origin and may alternatively result from the psychological impact of the brain damage induced by a stroke. The psychological and/or biological cause of poststroke depression is widely debated and still largely unclear (17).

Examining atherosclerosis in relation to depression provides an important validation of the vascular depression hypothesis. Atherosclerosis is the main cause of vascular disease and, as such, provides a unique marker of vascular burden. First, this is useful because measuring atherosclerosis provides a more sensitive preclinical indicator of vascular burden that is more accurate for detecting the presence of vascular disease than examining vascular risk factors or overt vascular events. Second, generalized atherosclerosis may be the mechanism through which vascular disease triggers

depression (1). Third, atherosclerosis is largely asymptomatic, which increases the potential that incident depression results from biological mechanisms rather than the psychological consequence of experiencing an overt disorder.

The first study to examine atherosclerosis within the context of the vascular depression hypothesis was a cross-sectional article showing that elderly people with severe levels of extracoronary atherosclerosis, aortic plaques, and coronary calcification were more likely to have depressive disorders (18). While these results support the vascular depression hypothesis, the temporal nature of this association is unclear. A longitudinal examination of atherosclerosis and depression demonstrated that atherosclerosis did not increase the risk of incident depression over a five-year period (19). However, atherosclerosis in this study was derived from a subjective estimate score based on cardiovascular outcomes, which may not provide a sensitive approximation of atherosclerosis. Further, only depressive symptoms were evaluated; intermittent depressive episodes were not examined; and the sample was restricted to the extreme end of the lifespan (85 and older).

Therefore, the current study aimed to clarify the validity and causal nature of the vascular depression hypothesis by prospectively examining the association between atherosclerosis and incident depressive symptoms and syndromes in a broad age range of older adults. Atherosclerosis was objectively examined with criterion standard measures at four sites and with electron-beam computed tomographic scans. Depression was continuously assessed using multiple methods.

Methods

Study Setting and Design

This study was based in the Rotterdam Study, a prospective, population-based cohort designed to examine the occurrence and risk factors of chronic diseases (20). The current study used the third examination round (1997-1999) as baseline, as thorough depression screening was introduced at this time. At this baseline examination, a home interview and research center visit were conducted. Additionally, during the follow-up period, an interim examination occurred (2002-2004). Follow-up data on incident depression was collected continuously from baseline until October 1, 2005. A flow diagram of the design and data collection is presented in Figure 1.

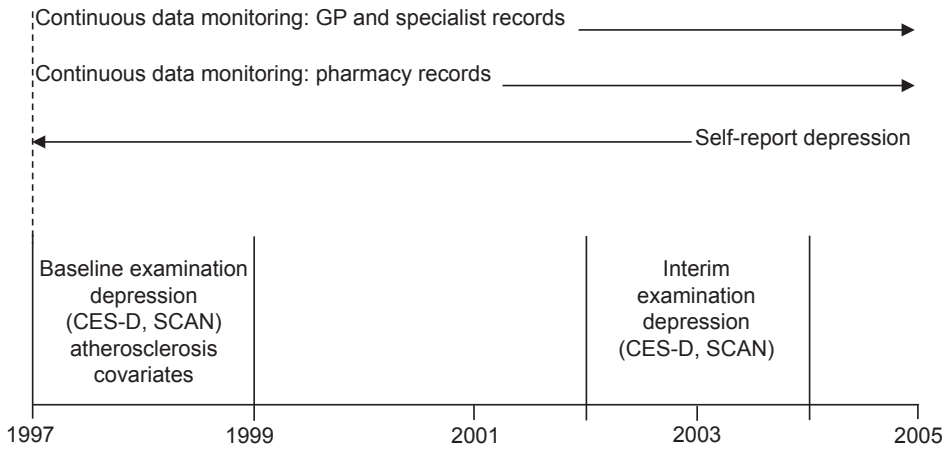

Figure 1 Assessment of depression (continuous monitoring, baseline, and interim examination). CES-D indicates Center for Epidemiologic Studies Depression scale; SCAN, Schedules for Clinical Assessment in Neuropsychiatry Interview.

Study Population

At the study baseline, 5990 residents were invited to attend and 4797 (80%) participated. Of these participants, 4597 (96%) had a valid screening for depression. Cases with depressive symptoms at baseline, as defined by a Center for Epidemiologic Studies Depression scale (CES-D) (21) score of 16 or more ($n = 362$), and those scoring below the cut off but with a positive indication for antidepressant usage ($n = 141$) were excluded from analyses. Further, cases with a diagnosis of bipolar disorder ($n = 5$) or dementia ($n = 122$) at baseline were excluded.

Participants were included in analyses if they had at least one measurement of atherosclerosis. Participants with no atherosclerosis assessment were (n = 403). Atherosclerosis measures were therefore available for the following participants: carotid plaques, $n = 3014$; aortic calcification, $n = 3026$; peripheral arterial disease, $n = 3408$; intima-media thickness, $n = 3256$. This provided an overall sample of 3564 participants (2005 females) for the current study. Participants who were excluded from the study were more likely to be female (71% vs 56%) and older (77 vs 72 years). A subset of community-dwelling participants younger than 85 years also participated in the Rotterdam Coronary Calcification Study, where they underwent a coronary atherosclerosis scan. Of the current study population, 1792 (50%) had available data on coronary calcification.

Incident Depression Assessment

Incident depression was assessed with multiple sources to increase the validity of diagnosis and to capture more events. This is a form of best estimate diagnosis, which

has been shown to be useful for psychiatric disorders (22-23). Data on depression was collected continuously from baseline and during the baseline and interval examination rounds. This method has been described previously (24).

Continuous Data

General practitioner and mental health specialist medical records were assessed continuously by trained research assistants who relayed the information about a potential depression. Two research physicians independently assessed this information in accordance with a predefined protocol and discussed discordant assessments. Antidepressant usage was determined from a cabinet check during the follow-up examination and continuously via digital pharmacy records from all pharmacies that serve the Ommoord area.

Examination Rounds – baseline and interval

The CES-D was completed during the home interview (21) of the third and fourth follow-up examinations. Participants with a positive depression score (≥16) were then invited for a subsequent assessment in which they completed the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) with clinicians (25). Self-reported history of depression was also recorded at each examination round. Standardized questions were used to determine if and when participants had experienced a depressive episode and if they sought treatment.

Incident depressive symptoms were recorded when clinically relevant depressive symptoms were reported that did not meet Diagnostic and Statistical Manual of Mental Disorders IV (DSM-IV) criteria for depression. This included participants who scored positive on the CES-D, were diagnosed with at least one core symptom of major depression in a medical record, self-reported depression where a physician or mental health professional was not consulted, or use of antidepressant drug treatments in the absence of medical records for depression.

Incident depressive syndromes included depressive syndromes recorded by a physician or self-reported depression for which a mental health specialist was consulted and DSM-defined minor and major depressive disorder and dysthymia reported by a mental health specialist or detected during the psychiatric SCAN interview.

The date of onset of depressive symptoms or syndromes was set at the day of the first report of symptoms or the first prescription date of an antidepressant drug, whichever came first.

Atherosclerosis Measurement

Extracoronary Atherosclerosis

Four measures were selected to assess extracoronary atherosclerosis at five sites in the body; they are strong predictors of vascular disease (26-29). Carotid plaques were assessed by B-mode ultrasound at the common, internal, and bifurcation sites of the carotid artery for the presence of atherosclerotic lesions. A point was given for each positive location of atherosclerotic plaque (irrespective of plaque size) (30). This was categorized into four groups: 0 plaques, 1 plaque, 2 to 3 plaques and 4 or more plaques. Aortic calcification of the posterior abdominal aortic wall was determined with a lateral x-ray of the lumbar spine (L1-L4). Calcification was considered present when linear densities were seen in an area parallel and anterior to the lumbar spine (29). Aortic calcified plaques were scored according to the length of the involved area along the lumbar spine (L1-L4) and categorized into four groups: less than 1 cm, 1 to 2.5 cm, 2.5 to 5 cm, and more than 5 cm. Peripheral arterial disease was assessed with ankle-brachial blood pressure. Systolic blood pressure of the right brachial artery was measured twice by a trained research assistant with a random-0 sphygmomanometer after the subjects had rested for 5 minutes. For analyses, the mean of the two blood pressure measurements was calculated. Systolic blood pressure was also measured while in supine position in the left and right posterior tibial artery; the lowest reading was used for analyses (8-MHz continuous-wave Doppler probe; Huntleigh 500D; Huntleigh Technology, Bedfordshire, England). In agreement with the recommendation of the Ankle Brachial Index Collaboration (28), peripheral arterial disease was considered present when the ankle-brachial blood pressure index was lower than 0.90. Intima-media thickness was measured as the mean of the far wall of both the right and left common carotid artery (millimetres). B-mode ultrasonography was conducted using a 7.5-MHz linear-array transducer (ATL Ultra-Mark IV; Advanced Technology Laboratories, Bethel, Washington) (31).

Coronary Calcification

Within a subset of the population, coronary calcification was measured as an indication of coronary atherosclerosis. This has been shown to be a highly sensitive predictor of coronary artery disease (32). A more detailed description of the Rotterdam Coronary Calcification Study is provided elsewhere (33). An electron-beam computed tomographic scan (C-150; Imatron, San Francisco, California) was used and measured from the level of the root of the aorta. Thirty-eight images were obtained with 100-millisecond scan time and 3-mm slice thickness. A calcium score was then obtained using the Agatston *et al.* method (34) in which the area (mm^2) of individual calcified lesions was multiplied by a factor based on the maximum density of the lesion. Calcium scores were divided into four categories: 0, more than 0 to 100, more than 100 to 400, and more than 400 (35).

Covariate Assessment

Age, sex, education, cognitive status, traditional cardiovascular risk factors, and history of overt vascular events were measured. Education was grouped according to the Standard Classification of Education and was rated from primary education (1) to university level (7). Cognitive status was measured with the Mini-Mental State Examination, which assesses six broad areas of daily cognitive functions (36). Several cardiovascular risk factors were considered as covariates: body mass index, smoking status, systolic and diastolic blood pressure, antihypertensive medication use, total and high-density lipoprotein cholesterol, diabetes mellitus, and overt vascular events. Height (meters) and weight (kilograms) were measured and body mass index was calculated as weight in kilograms divided by height in meters squared. Smoking status was coded in categories as never, former and current smoker. Antihypertensive use was verified during follow-up examination. A venipuncture was performed, and fasting blood samples were drawn and immediately frozen (-20ºC). Cholesterol values were determined with an automated enzymatic procedure (Boehringer Mannheim System; Mannheim, Germany). Diabetes Mellitus was determined by whether participants or pharmacy records reported using antidiabetic medication or if their fasting blood glucose concentrations were 200 mg/dL (to convert to millimoles per liter, multiply by 0.0555) or higher. History of overt vascular events (cerebrovascular accident, myocardial infarction) was obtained continuously through automated linkage with general practitioners files and medical specialist discharge reports. For each reported event, additional information was collected (e.g., hospital records, nursing home records). Two research physicians coded these events, and in the case of disagreement, a medical specialist was consulted. These covariates were selected, as they have been reported to be linked to both atherosclerosis and depression.

Statistical Analyses

Participants contributed to the current study from baseline until their first incident depression, death, or the end of follow-up (October 1, 2005). Intima-media thickness was used as a continuous variable and calculated so that an increase in hazard ratios reflected a 1-SD increase in the predictor. All other atherosclerotic measures were analyzed in their aforementioned categories to preserve their clinical meaning. However, they were also examined as continuous variables to assess for linear relations. The covariates of age, education, Mini-Mental State Examination, cholesterol, systolic and diastolic blood pressure, and cholesterol were examined as continuous variables. Sex, body mass index, smoking, and antihypertensive and antidepressant use were analyzed categorically. To increase the comparability of analyses and reduce bias, missing values on covariates used in analyses were imputed using single imputation

with expectation maximization algorithm (37). Variables were imputed using the entire baseline population (n = 4797) and an array of variables available from the Rotterdam Study. Missing values on covariates were minimal (maximum, 8%).

An a priori power analysis was performed to detect the minimum number of participants required to detect a significant difference. The minimum number of participants required to detect a hazard ratio of 1.2 ($P < .05$) at a power of 0.9 was 2000 participants. This indicates that the present study was sufficiently powered to detect a reasonable difference.

To examine the overall predictive capacity of atherosclerotic measures, a composite score was created from the four extracoronary measures. A factor analysis was conducted to combine the measures, which is a standard procedure for combining related variables and provides an increased power to detect relationships (38). The atherosclerosis measures were used in their continuous form, and a linear combination of these variables was used to construct a composite score. As participants with at least one measure of atherosclerosis were included in the study some participants had missing values for one or more atherosclerosis measures. To ensure the composite score was representative of the entire sample, the missing values were imputed using the aforementioned expectation maximization algorithm.

The association between atherosclerosis and risk of incident depressive symptoms and incident depressive syndromes was examined using Cox regression analysis. Two models were conducted for each measure of atherosclerosis (four extracoronary measures, composite score and coronary calcification assessment). The first model examined atherosclerotic predictors of incident depressive symptoms and then incident depressive syndromes, controlling for age and sex. The second model assessed the same relationship, but controlled for additional covariates and possible antecedents by using a multivariate model controlling for age, sex, education, Mini-Mental State Examination score, body mass index, smoking, total and high-density lipoprotein cholesterol, diabetes mellitus, systolic and diastolic blood pressure, and antihypertensive use. Proportional hazards assumptions were assessed with Schoenfeld residuals.

Secondary analyses were conducted to further examine the association. Analyses were first rerun in three subsets. The first was a subset of participants free from prevalent cardiovascular disease (n = 3079). These cases were originally included because this could be in the pathway between atherosclerosis and depression; however, it is important to analyze this subgroup to identify whether the depression is a psychological manifestation from an overt vascular event. Second, a subset of participants who were free from atherosclerosis five years prior to baseline, then developed atherosclerosis in the interim period, were evaluated. This enabled assessment of whether it is the development or the presence of atherosclerosis that is vital for vascular depression. Third,

the associations were evaluated in a subset free from history of depression. They were initially included to allow for atherosclerosis prior to baseline creating vulnerability for depression. Participants self-reported whether they had sought professional treatment for depression in the four years prior to baseline (n = 167). Additionally, analyses were run to evaluate sex by atherosclerosis interactions, as it is possible that a differential association between atherosclerosis and depression exists for men and women. Men have higher rates of atherosclerosis (39), and women are more likely to be depressed (40). Finally, it was evaluated whether atherosclerosis was a risk factor for incident DSM-IV-defined major depressive disorder (n = 56).

Results

Participants had a mean (SD) age of 71.9 (6.81) years at baseline and were followed for incident depressive events for a mean (SD) of 5.9 (2.1) years. Baseline characteristics of the sample are displayed in Table 1. During the study follow-up (21,083 person-years), 2938 participants (82.4%) experienced no incident depression, 429 (12.0%) experienced incident depressive symptoms, and 197 (5.5%) experienced incident depressive syndromes. Of the latter group 56 (1.6%) were diagnosed with a DSM-IV major depressive disorder.

Higher levels on individual measures of extracoronary atherosclerosis and their composite measure score did not increase the risk of incident depressive symptoms or syndromes after adjusting for age and sex (Table 2). This null relationship persisted after taking into account the effect of other potential depression and cardiovascular risk factors (results not shown, as they were highly comparable with unadjusted results). The only significant results from these models was for the association between moderate carotid plaques and incident depressive syndromes, which became significant (hazard ratio, 0.66; 95% confidence interval, 0.44-0.98; P = .04). However, it is notable that the predictive value of this hazard ratio is in an unexpected direction. Further, for this result to be meaningful within the vascular depression hypothesis, severe carotid plaques should match or increase this risk, while this was not the case in the current setting. Therefore, it is likely that this result is spurious. The proportional hazards assumption for a constant risk over time was met.

Within the subset of participants drawn from the Rotterdam Coronary Calcification Study, it was found that people with higher levels of coronary calcification did not have a greater risk of developing depressive symptoms or depressive syndromes. Age- and sex-adjusted models (Table 3a and 3b) did not differ greatly from multivariate models (results not shown, as they were highly comparable with unadjusted results).

Table 1 Baseline characteristics of study participants 1997-1999 (n = 3564)

Measures	
Demographics and background, mean (SD)	
Age, y	71.92 (6.81)
Sex female, No. (%)	2,005 (56.30)
Education, range 1-7	3.78 (1.87)
Mini-Mental State Examination score	27.76 (1.83)
Cardiovascular Risk Factors and History[a]	
Smoking, No. (%)	
Never	1,203 (33.80)
Former	1,796 (50.40)
Current	565 (15.90)
Body mass index, mean (SD)[b]	26.81 (4.07)
Systolic blood pressure, mm Hg, mean (SD)	143.97 (21.17)
Diastolic blood pressure, mm Hg, mean (SD)	75.46 (11.08)
Antihypertensive medication use, No. (%)	1,488 (41.80)
Total cholesterol, mg/dL, mean (SD)	5.82 (0.95)
HDL cholesterol, mg/dL, mean (SD)	1.39 (0.39)
Diabetes Mellitus, No. (%)	219 (6.10)
Prevalent MI, No. (%)	397 (11.10)
Prevalent CVA, No. (%)	116 (3.30)
Extracoronary Atherosclerosis, mean (SD)[c]	
Carotid plaques	1.25 (1.56)
Aortic calcification	2.13 (1.47)
Peripheral arterial disease, No. (%)[d]	598 (17.55)
Intima-media thickness, mm	0.87 (0.15)
Coronary Atherosclerosis[c]	
Coronary Calcification, mean (SD)	495.07 (950.76)

CVA, cerebrovascular accident; HDL, high-density lipoprotein; MI, myocardial infarction. SI conversion factors: to convert total and HDL cholesterol to millimoles per liter, multiply by 0.0259.
[a]Cardiovascular risk factors are based on imputed data (maximum, 8%).
[b]Calculated as weight in kilograms divided by height in meters squared.
[c]Atherosclerosis measures were available for the following participants: carotid plaques, n = 3014; aortic calcification, n = 3026; peripheral arterial disease, n = 3408; intima-media thickness, n = 3256.
[d]Peripheral arterial disease measured by ankle-brachial index (present, ≤ 0.900).

Table 2 Association between extracoronary measures of atherosclerosis and incident depression (age-and sex-adjusted model)[a]

	Incident depressive symptoms					Incident depressive syndromes				
	Total, No.	Cases, %	HR	(95% CI)	P-value	Total, No.	Cases, %	HR	(95% CI)	P-value
Carotid plaques										
Categorical										
None	865	14.1	1.00	[Reference]		809	8.2	1.00	[Reference]	
Mild	563	12.3	0.87	(0.65-1.17)	0.36	522	5.4	0.70	(0.45-1.09)	0.11
Moderate	938	11.8	0.86	(0.66-1.12)	0.27	872	5.2	0.72	(0.49-1.06)	0.10
Severe	484	13.2	1.02	(0.74-1.41)	0.89	445	5.6	0.89	(0.55-1.44)	0.63
P for trend					0.54					0.26
Continuous per 1 unit	2850	12.8	1.00	(0.93-1.07)	0.96	2648	6.2	0.96	(0.86-1.07)	0.46
Aortic calcification										
Categorical										
None	870	12.9	1.00	[Reference]		811	6.5	1.00	[Reference]	
Mild	785	10.8	0.84	(0.63-1.11)	0.22	742	5.7	0.94	(0.62-1.41)	0.75
Moderate	636	15.3	1.19	(0.90-1.58)	0.22	576	6.4	1.12	(0.73-1.73)	0.60
Severe	567	14.8	1.13	(0.84-1.52)	0.42	519	6.9	1.21	(0.77-1.88)	0.40
P for trend					0.09					0.69
Continuous per 1 unit	2858	13.2	1.07	(0.99-1.15)	0.08	2648	6.3	1.05	(0.94-1.17)	0.36
PAD										
None	2651	12.7	1.00	[Reference]		2473	6.4	1.00	[Reference]	
Present	566	13.8	1.05	(0.82-1.35)	0.70	520	6.2	1.00	(0.68-1.47)	0.99
IMT per 1-SD increase	3079	12.5	1.00	(0.89-1.12)	0.95	2871	6.2	1.05	(0.89-1.25)	0.56
Composite score per 1-SD[b]	3367	12.7	1.01	(0.91-1.12)	0.88	3135	6.3	1.04	(0.89-1.22)	0.63

CI, confidence interval; HR, hazard ratio; IMT, intime-media thickness; PAD, peripheral arterial disease measured by ankle-brachial index (present, ≤ 0.90); SD, standard deviation.

[a]Each measure was analyzed in a separate Cox regression multivariate model adjusted for age and sex.

[b]Composite score derived from the principal component analysis of the four extracoronary atherosclerotic measures (based on imputed data for missing values).

Secondary analyses conducted revealed comparable results (data not shown). The analyses outlined above were rerun in three subsets: (1) participants free from prevalent cardiovascular disease, (2) participants free from atherosclerosis five years prior to baseline but went on to develop atherosclerosis in the time leading up to baseline, and (3) participants free from history of depression. The results did not differ greatly in these population subsamples when compared with the original sample. Additionally, it was evaluated whether a differential relationship existed between atherosclerosis and depression in men and women. No differential association was found between men and women (all interaction terms $P > .05$). Finally, we sought to determine if atherosclerosis was a predictor of incident depression when depression was restricted to DSM-IV defined major depressive disorder. This analysis did not reveal the existence of a dose-response relationship.

Table 3a Association between coronary calcification and incident depressive symptoms (age- and sex-adjusted model)

	Incident depressive symptoms				
	Total, No.	Cases, %	HR	(95% CI)	P-value
Coronary Calcification					
Categorical					
None	157	12.1	1.00	[Reference]	
Mild	615	12.7	1.08	(0.65-1.79)	0.78
Moderate	381	11.8	1.06	(0.61-1.83)	0.84
Severe	537	11.7	1.12	(0.65-1.94)	0.68
P for trend					0.98
Continuous per 1 unit	1690	12.1	0.96	(0.81-1.14)	0.67

CI, confidence interval; HR, hazard ratio.

Table 3b Association between coronary calcification and incident depressive syndromes (age- and sex-adjusted model)

	Incident depressive syndromes				
	Total, No.	Cases, %	HR	(95% CI)	P-value
Coronary Calcification					
Categorical					
None	146	5.5	1.00	[Reference]	
Mild	581	7.6	1.54	(0.72-3.28)	0.27
Moderate	358	6.1	1.32	(0.58-3.00)	0.51
Severe	502	5.6	1.38	(0.60-3.15)	0.45
P for trend					0.71
Continuous per 1 unit	1587	6.4	1.09	(0.91-1.31)	0.34

CI, confidence interval; HR, hazard ratio.

Conclusions

In this population-based prospective cohort study, atherosclerosis was not predictive of incident depression. None of our measures of extracoronary atherosclerosis were linked to either incident depressive symptoms or depressive syndromes. Similarly, a composite score of these variables was not predictive of incident depression. Coronary calcification, measured in a subsample of participants, was also not associated with incident depression in older adults.

The vascular depression hypothesis proposed in the 1990s by Alexolopoulos and Krishnan (1-2) suggests that vascular disease precedes depression in older adults. To date, support for the vascular depression hypothesis has been inconsistent with several methodological limitations impairing interpretation of findings. Atherosclerosis is the main cause of vascular disease and a sensitive asymptomatic marker of vascular disease. Thus, examining atherosclerosis in relation to depression provides an important validation of the vascular depression hypothesis. However, most investigations into this hypothesis have been cross-sectional (18) or in small clinical samples (41-42). The only longitudinal study to examine this found that a subjective estimate of atherosclerosis was not predictive of incident depression (19). However, this study had several limitations. We sought to reexamine this hypothesis using standardized objective measures of atherosclerosis, an expanded age range and continuous assessment of incident depressive symptoms and syndromes, assessed from multiple sources. Interestingly, in support of the null association of the prior longitudinal study, yet in contrast to the prominent vascular depression hypothesis, we also found that atherosclerosis was not predictive of incident depression.

Reconciling these findings in this large body of literature does not provide strong support for the vascular depression hypothesis. However, the original cross-sectional investigation in the Rotterdam Study (18), and other cross-sectional research (41), did establish an association between atherosclerosis and depression. A plausible explanation for this is that a relation exists but such that depression contributes to the development of vascular disease. This proposition is supported by multiple population-based prospective studies showing that depression is prospectively linked to overt vascular events, such as myocardial infarction (43-45), stroke (46) and cardiovascular-related mortality (47). This has also been maintained by studies specifically on atherosclerois; for example, in the Cardiovascular Health Study, which showed that in 3781 participants aged 65 years and older, depressive symptoms were related to the development of atherosclerosis over a 3-year period, even after excluding for prevalent vascular disease (48). Depression has also been shown to predict the development of carotid plaques over a 10-year time period (49). An alternative plausible explanation is that both atherosclerosis and depression are clinical presentations of a shared biological substrate.

Most support for the vascular depression hypothesis has been derived from clinical

studies, while most community-based studies such as the current study fail to support the hypothesis. This could reflect that vascular factors are not specifically causative for depression but additionally for other symptoms or disorders. However, caution should also be taken when replicating clinical study findings within large cohort studies. Although this has many advantages, such as increased sample size to detect findings, standardized assessments and generalizability, it also can have drawbacks such as missing interim events and less specific symptom assessment.

The current study has several strengths of design which enhance the validity of the presented null results. The study had an adequate sample size with a large amount of cases, both for incident depressive symptoms (n = 429) and incident depressive syndromes (n = 197). This number of cases provides adequate power for the analyses conducted and allows for a large number of covariates (50). The study was also based within the general population, thus increasing the external validity and decreasing potential selection bias. Further, the range of atherosclerosis was heterogeneous, which may increase the chance of finding an association across all levels of atherosclerosis. The measurement of atherosclerosis was also objective, and well-validated assessment techniques were used (26-29,32). Additionally, we took into account cardiovascular risk factors and variables which are important in ageing and psychiatric research, thus allowing us to conduct multivariate models. Finally, a unique approach to measuring incident depression was employed which increases the chances of detecting events and increases the specificity (24). We collated multiple sources of information to detect cases of depression both continuously and within a clinical interview setting. The multiple sources of information are valid and monitored continuously, and the clinical interview is a criterion standard for assessing clinical events. Further, this method allows us to differentiate between depressive symptoms and syndromes. This is important for predictive studies as the degree of depression has been shown to present with a different clinical profile and potentially different underlying causal mechanisms.

Some limitations of the study should also be discussed. The current study included participants with at least one measure of atherosclerosis. Therefore, the main analyses were conducted on marginally different populations. However, the analysis of the composite score of these measures, which imputed missing values, revealed no difference in findings, suggesting this was not creating a large bias. This left only a small subsample of participants (n = 422) for whom we had no measures of atherosclerosis. It is possible that the reasons for non-assessment may create a bias. This subgroup may have a marginally different depression and/or cardiovascular risk profile, as they were more likely to be female and older. Similarly, the subsample analyzed from the Rotterdam Coronary Calcification Study was restricted to adults younger than 85 years. Nevertheless, the variation of atherosclerosis in this group was large, suggesting that if

a differential association was present, it would have been detected.

Points pertinent to the assessment of depression in the current study should also be considered. Between the baseline and intervening assessment, it is possible that a differential attrition occurred for those with a high vascular burden and a depression. However, given the use of continuous data from medical and pharmaceutical records in the current study, it is likely that serious cases were detected. The current study did not specifically assess the clinical profile ascribed to vascular depression (1); however, although this profile has not been consistently validated (10), it is likely that the broad definition of depression in the current study captured the proposed clinical syndrome. It is also possible that vascular depression is more chronic and refractory in nature, thus examining first incident event may not capture this. As examining repeated events would require knowledge of the end date of each depressive episode and a longer follow-up period, we are unable to examine this in the current data set, but the concept could be better examined in a closely observed clinical setting. Further, it is important to note that any cohort study of older adults includes many individuals with preexisting vascular disease and past depression. Although these disease entities can be controlled for, only incident and recurrent depression can be linked in their temporal sequence to vascular disease.

A final issue to be considered is that the present study focused on measures of extracerebral atherosclerosis. Given that a large body of support for the vascular depression hypothesis derives from brain imaging studies of cerebral atherosclerosis (4-6), it is consequently possible that only cerebral atherosclerosis increases the risk of incident depression. However, cerebral atherosclerosis, indirectly measured through the presence of white matter lesions and lacunar infarcts on brain magnetic resonance imaging, is highly associated with extracerebral atherosclerosis (51-52). Therefore, it is likely that the relationship between these two locations of atherosclerosis to depression is similar. A recent investigation from the Rotterdam Study supports this notion (53). In a subsample of 479 people, it was found that markers of vascular brain disease, detected from magnetic resonance imaging, were associated with depression cross-sectionally. However, in line with the findings from the current study, no relationship was detected between these markers and risk of depression longitudinally.

In summary, atherosclerosis did not increase the risk of depression in older adults. Given the strengths of the study, including a large sample, population-based setting, and criterion standard measurement, we are confident in the validity of these null findings. In light of this, the current study does not support the vascular depression hypothesis. Interpreting these results within the context of prior associational studies, which found a relationship between atherosclerosis and depression, and longitudinal studies showing depression as a predicator of vascular events, this may suggest that depression may contribute to, rather than result from, vascular burden in older adults.

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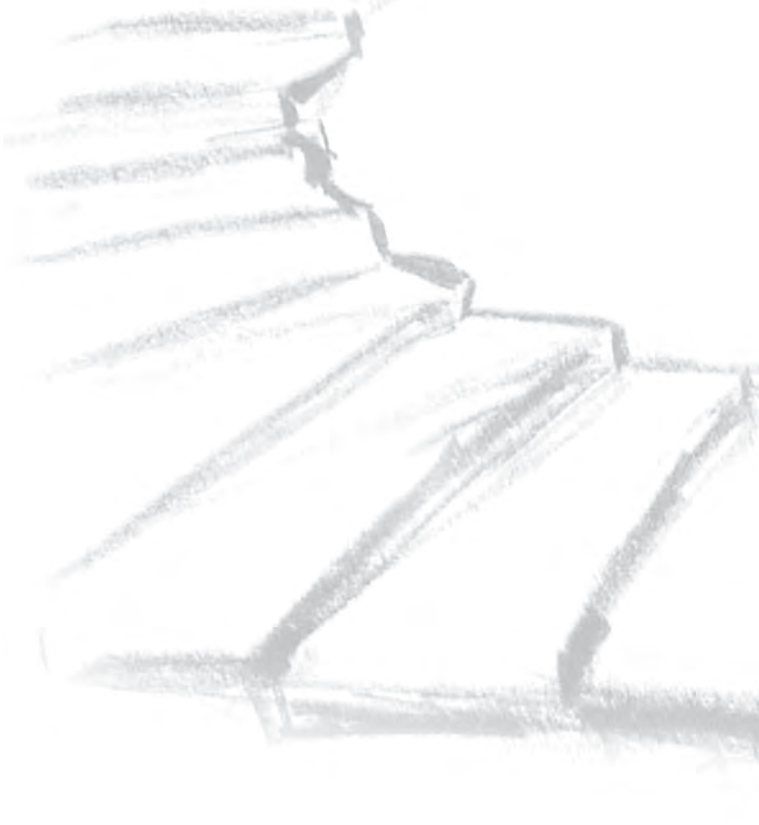

Chapter 6

The PCLO gene and depressive disorders: replication in a population-based study

Karin Hek, Cornelis L. Mulder, Hendrika J. Luijendijk,
Cornelia M. van Duijn, Albert Hofman,
André G. Uitterlinden, Henning Tiemeier

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Abstract

Previous genome-wide association analysis revealed a new putative candidate gene for major depression: the PCLO gene. Replication in one population-based cohort did not yield genome-wide significance and further replication efforts in clinical studies were unsuccessful. We aimed to validate the association of single-nucleotide polymorphism (SNP) rs2522833 in the PCLO gene with depression in the Rotterdam Study, a prospective population-based cohort of elderly persons. In the Rotterdam Study we identified 579 persons with a broad depression phenotype (depressive syndromes) of whom 178 cases with DSM-defined depressive disorder. The control group consisted of 912 persons free of depression during the follow-up period and in their histories. Logistic regression analysis showed an association between rs2522833 and depressive disorders ($P = 0.0025$). However, no association between the broader depressive syndrome group and this SNP was observed ($P = 0.20$). A meta-analysis combining all studies from the original publication and our study yielded a *P*-value of 2.16×10^{-3} for the association between SNP rs2522833 and depressive disorders. However, as in the previous publication, high heterogeneity between studies was observed. Thus, a meta-analysis with the findings from three population-based studies was performed. This demonstrated a genome-wide significant *P*-value ($P = 1.93 \times 10^{-9}$). In conclusion, this study provides additional evidence for an association between PCLO and depressive disorders in a population-based study; no association with a broader syndromal phenotype was observed.

Introduction

Depression is a common mental illness characterized by persistent loss of interest or depressed mood. Familial aggregation of depression and its heritability of 31-42% suggest a genetic role in the aetiology of depression (1). Although many candidate genes have been tested, only six have been replicated (2-3). Genome-wide association studies might help to identify new candidate genes for depression. So far, two genome-wide studies on major depressive disorder were performed (4-5). One putative candidate gene, PCLO, was identified (4).

This finding was replicated in one population-based study. However, in a joint meta-analysis genome-wide significance was not reached and the result could not be replicated in five clinical populations (4). A recent joint reanalysis of 29 correlated single-nucleotide polymorphisms (SNPs) in the PCLO region in the original and the replication study supported the role of PCLO as a causal risk factor for major depression (6).

The goal of the current study was to validate the association of depression with the SNP rs2522833 in the PCLO gene, in the population-based Rotterdam Study (7). In addition, we report the result of meta-analyses summarizing our result and the original findings.

Method

The study was embedded in the Rotterdam Study, a prospective population-based cohort of persons over 55 years. Ascertainment of depressive disorders and syndromes was described previously (8). Briefly, during follow-up visits, participants were screened for depression with the Center for Epidemiologic Studies Depression Scale (CES-D). Screen-positive persons (CES-D score ≥ 16) were invited for a semi-structured interview with the Present State Examination (PSE) by a clinician to diagnose current depression status. In addition, GP records and specialist letters were surveilled actively for the occurrence of depression. Furthermore, physicians conducted repeated interviews assessing self-reported history of depression and incidence of depression during the interval period between interviews.

Diagnostic and Statistical Manual of Mood Disorders (DSM)-defined depressive disorders were diagnosed only if assessed by a psychiatrist in routine care or with the PSE (major depression or dysthymia). Depressive syndromes comprised self-reported depression with consultation of a health professional, minor depression diagnosed with PSE, and depression recorded by a GP or physician.

Genotype data on SNP rs2522833 was available from the Infinium II HumanHap550K Genotyping BeadChip® version 3 (Illumina) in 5968 persons from a total of 5974 persons with genotype data (genotyping and quality control previously described) (9). Genotyping was performed blind to case-control status. The minor allele frequency

(MAF) of SNP rs2522833 in controls (0.43) matched HapMap CEU frequency (0.43) and the MAF in the controls of Sullivan *et al.* (0.43). We therefore did not further verify genotyping of SNP rs2522833 in an independent assay.

Of the 5968 successfully genotyped persons, 524 persons died before depression screening and 747 persons did not participate in depression screening. Five persons with bipolar disorder were also excluded from the analyses. Of the remainder, 178 persons were diagnosed with DSM-defined depression during follow-up (145 major depressions, 18 dysthymias and 15 depressions not otherwise specified), and 401 persons had a depressive syndrome. Past depression before baseline was not used to define a case, as this information is largely based on retrospective self-report and recall over a long period is not very reliable.

In the first analysis, DSM-defined depressive disorders and depressive syndromes were combined, in a second analysis only DSM-defined depressions were used as cases. In a third analysis, the case-group was further restricted to major depression only. The control group for both analyses consisted of 912 persons at low liability of depression. We excluded persons from the control group with a self-reported history of depression (symptoms and syndromes, $n = 695$) or clinically relevant depressive complaints during follow-up ($n = 714$), and those without information on history of depression ($n = 368$). To mirror the control group of Sullivan *et al.*, we also excluded persons based on CES-D scores. We included only persons scoring in the lowest 25% at depression screening. As 35% of the participants scored 0, we excluded the 1424 persons scoring above 0.

As mentioned above, we also excluded persons with bipolar disorder ($n = 5$) and neither cases nor controls had a diagnosis of schizophrenia. In contrast, a comorbid anxiety disorder which was assessed only during the last interview was no exclusion criterion. Again, this definition of the study population was in line with the report of Sullivan *et al.* Of the persons still alive and participating in the last interview 25 out of 701 controls (3.6%) and 38 out of 106 cases with depression (35.8%) had a prevalent anxiety disorder.

Genotype data on SNP rs2522833 was analyzed with logistic regression in SPSS. PLINK v.1.06 (10) was used to run a logistic regression analysis on all SNPs with a minor allele frequency greater than 5% in the PCLO region (base 82,225,378 to 82,630,133 on chromosome 7). The meta-analysis was based on Z-scores weighted by sample size. To adjust the sample size for asymmetric case/control ratio, we first estimated the power of the individual studies with the Genetic Power Calculator (11). Parameters were taken from the paper of Sullivan *et al.* (MAF = 0.45, genotypic relative risk = 1.14). Using the same parameters and power, we then determined the sample size for studies with equal numbers of cases and controls as was suggested by de Bakker *et al.* (12). In the meta-analysis, Z-scores were weighted by those effective sample sizes. We evaluated heterogeneity by using logistic regression parameters to determine Cochran's Q and I^2.

Results

Study population

The current study was set in the Rotterdam Study, a prospective population-based cohort of elderly persons. The study population consisted of all genotyped participants (n = 5974) with valid depression data. SNP rs2522833 was successfully genotyped in 5968 persons. The genotype frequency for this SNP was in Hardy-Weinberg equilibrium (pHWE = 0.57).

Depression cases were ascertained by continuous monitoring of GP records, depression self-report and depression screens followed by clinical interviews during follow-up visits to the research center. We identified 579 persons with a depressive syndrome (minor depression by clinical interview, depression self-report with consult of a GP, other health specialist or anti-depressant treatment, diagnosis by GP) of whom 178 persons had a depressive disorder (145 major depression, 15 dysthymia and 18 depression not otherwise specified diagnosed by clinical interview or diagnosis by a psychiatrist). The control group consisted of 912 persons at low liability for a depressive disorder: they had no history of depressive disorders, syndromes and complaints; neither did they experience depression or depressive complaints during follow-up, and they scored in the lowest quartile for depression screening.

PCLO SNP rs2522833 and depression

The association of polymorphism rs2522833 with depressive disorder and syndromes was tested with logistic regression assuming an additive effect. Like Sullivan *et al.*, we tested for the effect of the minor C allele. We first tested the association of SNP rs2522833 with the broad syndromal phenotype, also including self-reported depressions. No association of this syndromal phenotype with SNP rs2522833 was observed (P = 0.20). However, Sullivan *et al.* used a strict depression definition. Therefore, we restricted the case group to only those cases with Diagnostic and Statistical Manual of Mood Disorders (DSM)-defined depressive disorders. The association of depressive disorders with SNP rs2522833 was significant (P = 0.0025) (see Table 1). In addition, we further restricted the case group to those 145 cases with major depression, the case definition applied by Sullivan *et al*. This increased the strength of the association between depression and SNP rs2522833 [P = 0.0014, OR 1.503 (1.171-1.928)].

Like Sullivan *et al.*, we examined other SNPs in the gene region. The Illumina array holds 73 SNPs in the PCLO gene with minor allele frequency above 5% (base 82,225,378 to 82,630,133 on chromosome 7). Thirty-seven SNPs (51%) had a significant association (P < 0.05) with DSM-IV depressive disorders (the association of each SNP in the PCLO region with depressive disorders is listed in Supplementary Material, Table S1). After adjusting for SNP rs2522833, only two SNPs remained independently and significantly

associated with depression (results of this analysis for all SNPs in the PCLO region can be found in Supplementary Material, Table S1).

Table 1 Association of SNP rs2522833 with depression in the population-based Rotterdam Study

Phenotype	No of cases	No of controls	MAF[a] cases	MAF[a] controls	*P*-value	OR (95%CI)
DSM-IV depression or depressive syndrome[b]	579	912	0.45	0.43	0.20	1.10 (0.95-1.27)
DSM-IV depression only[b]	178	912	0.52	0.43	0.0025	1.42 (1.13-1.79)

DSM, Diagnostic and Statistical Manual of Mental Disorders; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]Minor allele: C, major allele: A.

[b]DSM-IV depression comprises persons diagnosed by a psychiatrist during routine care, and persons diagnosed with major depression or dysthymia in a clinical interview. Depressive syndromes include minor depression, self-reported depression and depression diagnosed by a general practitioner.

Meta-analysis

In addition, we performed a Z-score-based meta-analysis weighted by effective sample size. Combining results from all seven studies (NESDA-NTR, QIMR, Max-Planken Institute Psychiatry, West Germany, STAR-D, U Edinburgh and DeCC) from the publication of Sullivan *et al.*, with results of the present study, yielded a *P*-value of 2.16×10^{-3}. However, Sullivan *et al.* indicated high inter-study heterogeneity of the case groups. They showed in a principal component analysis that the Australian QIMR study was most similar to the original sample. The QIMR was the only study that, like the original sample and our study, ascertained their cases from the population. Extending the *post hoc* analyses from Sullivan *et al.*, we thus performed a second meta-analysis. For this analysis, we included only the results of studies with population-based ascertainment of cases. The result of the original cohort (NESDA-NTR, Z = 5.01), a population-based replication cohort (QIMR, Z = 2.20), and the Z-score for the association with depressive disorders observed in our study (Z = 3.01) were included. This resulted in a genome-wide significant *P*-value of 1.93×10^{-9}. Meta-analyzing the five clinical studies only, yielded a *P*-value of 0.39 for the association between SNP rs2522833 and major depressive disorders. The effect was in the opposite direction of the effect in the population-based studies.

We formally evaluated heterogeneity between studies by calculating Cochran's *Q* and I^2. Combining all eight studies, 79% of all variation could be explained by heterogeneity ($Q = 32.90$, df = 7, $P < 0.0001$, $I^2 = 0.79$), compared with only 31% when including the three population-based studies ($Q = 2.90$, df = 2, $P = 0.23$, $I^2 = 0.31$). The five clinical studies were most alike ($Q = 2.92$, df = 4, $P = 0.57$, $I^2 = 0$).

Discussion

Candidate gene studies on major depression so far, have not been very successful (3,13). However, our results suggest that the PCLO gene may qualify as a new and replicated candidate gene for major depression. The PCLO protein (piccolo) is part of a pathway that was not investigated in any of the more than 100 candidate gene studies reviewed by Lopez-Leon *et al.* (3). This underlines the importance of further and larger genome-wide association studies to identify more putative genes and disease mechanisms (5).

Our results also highlight the importance of two features of successful replication that are particularly challenging for large-scale psychiatric genetic research, namely standardized clinical phenotype assessment and similar setting. Both have been pointed out previously as important criteria for positive replication (14), besides the existence of a real effect.

The semi-structured interview used in the Rotterdam Study, combining clinical expertise with standardized assessment, reduced misclassification of depressive disorders. With this approach, we successfully replicated the finding of Sullivan *et al.* Including syndromal depression more than doubled the case group, but syndromal depression was ascertained less rigorously and replication was unsuccessful.

Furthermore, it is important to note that Sullivan *et al.* confirmed their finding only in the one population-based sample (the Australian QIMR) and not in any of five clinical samples. Like Sullivan *et al.*, we observed high heterogeneity between studies, which was substantially reduced when analyzing only the clinical or population-based samples. This suggests that it is more appropriate and informative to meta-analyze the three population-based studies and the five clinically based studies separately than to combine the results of the eight studies. In the population-based Rotterdam Study, we replicated the result of Sullivan *et al.* Combined with the original results from population-based studies, a genome-wide significant association between depressive disorders and SNP rs2522833 was observed. The meta-analysis of clinical studies did not show an association between major depressive disorders and this polymorphism. Sullivan *et al.* argued that cases, but not controls, were different between clinical and population-based samples. Case ascertainment from clinical settings may introduce confounding, for example by co-morbidity, or by population stratification as was already discussed by Sullivan *et al.* In addition, selection bias, or referral filters may explain case differences, for example cases from clinical studies are more often recurrent and typically have an earlier onset than cases from the population-based studies (4). Hence, the characteristics of depression observed in clinical studies and population-based studies may not be very similar, and may, in part, have a different genetic susceptibility.

In conclusion, within a population-based study, we validated the association between depressive disorder and the PCLO gene. Further research is required to elucidate the mechanism of PCLO function in depression.

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Supplementary Material

Table S1 Associations of single-nucleotide polymorphisms (SNPs) in the PCLO region on chromosome 7 with depression, unadjusted and adjusted for SNP rs2522833

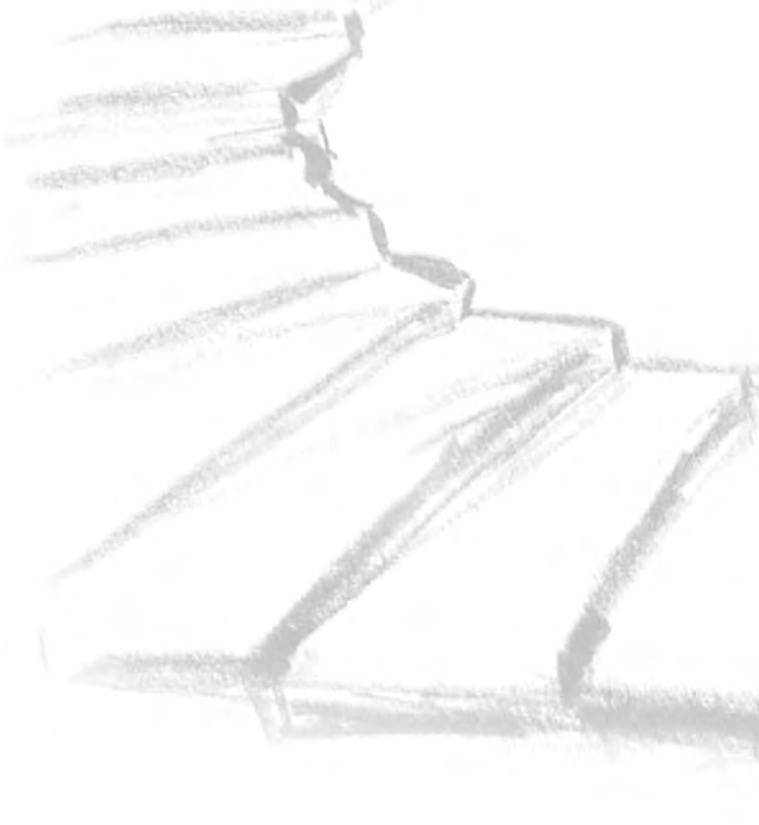
					Unadjusted for SNP rs2522833			Adjusted for SNP rs2522833		
SNP ID	Position	Minor allele	MAF cases	MAF controls	OR	95% CI	P-value	OR	95% CI	P-value
rs6959361	82227185	A	0,4916	0,4169	1,346	1,073-1,688	0,01015	1,109	0,8163-1,506	0,5084
rs10954689	82240309	A	0,5646	0,4694	1,469	1,166-1,849	0,00108	1,424	0,8338-2,431	0,1957
rs10808300	82246080	A	0,5646	0,4694	1,469	1,166-1,849	0,00108	1,424	0,8338-2,431	0,1957
rs7792186	82257077	A	0,2921	0,3661	0,7183	0,5615-0,9189	0,008467	0,8617	0,6215-1,195	0,3721
rs6979066	82260873	A	0,4157	0,341	1,379	1,092-1,742	0,007039	1,063	0,7152-1,58	0,7626
rs7782629	82270834	A	0,1264	0,1415	0,8784	0,6263-1,232	0,4523	1,076	0,7454-1,554	0,6954
rs6955106	82272200	A	0,4185	0,506	0,7043	0,5597-0,8863	0,002797	0,8408	0,5231-1,351	0,4738
rs12668093	82272969	A	0,1638	0,153	1,084	0,7968-1,475	0,607	1,023	0,7493-1,397	0,8863
rs2715147	82286341	G	0,4339	0,5138	0,7325	0,5829-0,9203	0,007529	1,015	0,5927-1,739	0,9563
rs2522833	82291644	C	0,5169	0,4293	1,422	1,131-1,786	0,002544	NaN	NaN-NaN	NaN
rs2023847	82299229	C	0,2825	0,3464	0,7479	0,5834-0,9587	0,02186	0,9371	0,6752-1,301	0,6979
rs12707524	82324551	G	0,2725	0,2112	1,44	1,098-1,89	0,008476	1,176	0,8372-1,651	0,3503
rs13229774	82333348	C	0,3792	0,2985	1,458	1,144-1,858	0,002339	1,228	0,8452-1,784	0,2814
rs13233504	82335767	A	0,2753	0,2126	1,445	1,104-1,893	0,007453	1,187	0,8474-1,661	0,3193
rs12707530	82335908	A	0,4114	0,3348	1,399	1,103-1,774	0,005657	1,212	0,8789-1,672	0,2407
rs2888018	82339226	C	0,4153	0,3372	1,409	1,112-1,786	0,004529	1,192	0,8672-1,639	0,279
rs2888019	82341345	A	0,4298	0,5158	0,7141	0,5692-0,8958	0,003601	0,8777	0,5464-1,41	0,5897
rs2371366	82346874	A	0,1236	0,1186	1,051	0,7368-1,499	0,7843	0,8237	0,5615-1,208	0,3213
rs13237603	82352074	G	0,339	0,2758	1,354	1,059-1,731	0,01569	1,159	0,8743-1,536	0,3052
rs17282616	82354868	G	0,2949	0,2234	1,476	1,138-1,913	0,003288	1,245	0,8993-1,725	0,1864
rs17156818	82354930	G	0,1264	0,1208	1,057	0,7427-1,503	0,7596	0,8422	0,5771-1,229	0,3733
rs17282763	82358102	G	0,273	0,3391	0,7403	0,571-0,9599	0,02328	0,912	0,66-1,26	0,5768
rs2189972	82362599	G	0,1949	0,1926	1,015	0,7583-1,359	0,9201	0,9353	0,6944-1,26	0,6598

(Table S1 continued)

					Unadjusted for SNP rs2522833			Adjusted for SNP rs2522833		
SNP ID	Position	Minor allele	MAF cases	MAF controls	OR	95% CI	P-value	OR	95% CI	P-value
rs2371368	82370307	A	0,1938	0,1922	1,011	0,7559-1,351	0,9437	0,9327	0,693-1,255	0,6457
rs10808302	82372651	C	0,3989	0,4879	0,7022	0,5582-0,8834	0,002537	0,8219	0,5686-1,188	0,2968
rs17156844	82382923	G	0,191	0,1929	0,9877	0,7378-1,322	0,9335	0,9107	0,6757-1,227	0,5392
rs12539066	82391885	A	0,3118	0,2916	1,103	0,8603-1,413	0,4401	0,9525	0,7286-1,245	0,7219
rs10954695	82395164	A	0,4326	0,359	1,365	1,082-1,722	0,008648	1,147	0,8534-1,543	0,3625
rs9690648	82399260	G	0,05337	0,06011	0,8836	0,5379-1,452	0,6252	0,9771	0,5923-1,612	0,9278
rs17235831	82399897	A	0,08708	0,1005	0,8511	0,5695-1,272	0,4317	1,001	0,6586-1,522	0,995
rs6467917	82407593	G	0,4011	0,4923	0,6944	0,5513-0,8748	0,001964	0,7922	0,5691-1,103	0,1676
rs6467919	82407643	G	0,3989	0,4928	0,6883	0,547-0,8662	0,001447	0,7825	0,5624-1,089	0,1456
rs12669254	82411034	G	0,118	0,0929	1,3	0,9109-1,855	0,1483	1,099	0,7539-1,602	0,6237
rs976714	82419795	A	0,4242	0,3478	1,374	1,092-1,73	0,006797	1,176	0,8836-1,565	0,2665
rs10954696	82420782	A	0,4242	0,3475	1,375	1,093-1,73	0,006636	1,175	0,8845-1,562	0,2655
rs2107829	82452185	A	0,1573	0,1822	0,84	0,6178-1,142	0,2661	0,976	0,7029-1,355	0,8847
rs41567	82457564	A	0,1742	0,2082	0,8085	0,6042-1,082	0,1526	0,9444	0,6888-1,295	0,7222
rs1001594	82468642	A	0,4045	0,482	0,7312	0,5805-0,921	0,007853	0,8367	0,6371-1,099	0,1998
rs41590	82478173	A	0,2949	0,3645	0,7372	0,5779-0,9404	0,0141	0,8249	0,6331-1,075	0,1538
rs41593	82480045	A	0,2966	0,3628	0,7487	0,5869-0,9551	0,01981	0,8361	0,642-1,089	0,1845
rs41601	82484733	G	0,4944	0,4929	1,006	0,8015-1,263	0,9586	1,021	0,8119-1,283	0,8604
rs41610	82490956	G	0,3202	0,3399	0,921	0,7292-1,163	0,49	0,9525	0,7516-1,207	0,6873
rs6977045	82491185	A	0,1573	0,09737	1,763	1,264-2,459	8,36E-04	1,511	1,05-2,174	0,02623
rs12707538	82497792	A	0,2219	0,1508	1,639	1,227-2,189	8,13E-04	1,436	1,041-1,982	0,02756
rs7810390	82500033	G	0,3933	0,377	1,075	0,8464-1,364	0,5545	0,9897	0,7744-1,265	0,9338
rs10274504	82503766	G	0,1124	0,1273	0,8665	0,6057-1,24	0,4329	0,9986	0,6871-1,451	0,9942
rs10257151	82504643	A	0,1124	0,129	0,8545	0,598-1,221	0,3878	0,9854	0,6785-1,431	0,9386
rs6979844	82507280	A	0,05085	0,05982	0,8426	0,5053-1,405	0,5113	0,9447	0,5632-1,584	0,8292

(Table S1 continued)

					Unadjusted for SNP rs2522833			Adjusted for SNP rs2522833		
SNP ID	Position	Minor allele	MAF cases	MAF controls	OR	95% CI	P-value	OR	95% CI	P-value
rs6948626	82511108	G	0,1124	0,1243	0,8906	0,6219-1,275	0,5271	1,026	0,7051-1,492	0,8941
rs1978177	82526516	G	0,2697	0,3383	0,7255	0,5637-0,9337	0,01269	0,808	0,6165-1,059	0,1225
rs4732493	82527837	A	0,1601	0,1508	1,073	0,7869-1,463	0,6561	0,974	0,7088-1,339	0,8712
rs6950504	82542501	G	0,5562	0,4885	1,315	1,045-1,655	0,01948	1,238	0,9782-1,567	0,07564
rs10954701	82548284	C	0,1788	0,1663	1,088	0,8033-1,474	0,5853	1,012	0,7405-1,383	0,9409
rs10487657	82551084	A	0,5562	0,4874	1,32	1,049-1,66	0,01782	1,243	0,9831-1,573	0,06916
rs11562069	82559678	G	0,4382	0,3749	1,284	1,026-1,607	0,02904	1,181	0,935-1,491	0,1629
rs2023986	82565386	A	0,5337	0,4749	1,256	1,004-1,572	0,04618	1,167	0,9256-1,471	0,1917
rs17819684	82570519	A	0,4298	0,3583	1,337	1,065-1,679	0,01229	1,229	0,9701-1,558	0,0875
rs10264030	82573687	A	0,5337	0,4753	1,254	1,002-1,569	0,04812	1,165	0,9244-1,468	0,1958
rs929356	82576472	C	0,4831	0,4223	1,264	1,012-1,578	0,03928	1,186	0,9442-1,49	0,1424
rs10954710	82584373	G	0,368	0,3235	1,209	0,9581-1,525	0,1099	1,142	0,9018-1,446	0,2703
rs2371541	82593482	A	0,4052	0,3756	1,126	0,8954-1,417	0,3092	1,088	0,863-1,371	0,4767
rs6946469	82598161	A	0,514	0,4661	1,202	0,9619-1,501	0,1057	1,135	0,9053-1,424	0,2716
rs10954712	82599053	A	0,4326	0,4383	0,9766	0,7743-1,232	0,8416	0,9465	0,7493-1,196	0,6443
rs6950738	82599186	G	0,427	0,3982	1,119	0,8944-1,401	0,3247	1,113	0,8887-1,395	0,3505
rs2158654	82604537	G	0,3427	0,365	0,9091	0,7178-1,151	0,4288	0,9046	0,7137-1,147	0,4073
rs11978731	82605635	A	0,2086	0,2119	0,9818	0,7492-1,287	0,8941	0,9803	0,7471-1,286	0,8856
rs7783211	82609331	A	0,4607	0,4355	1,105	0,8815-1,386	0,386	1,105	0,8804-1,386	0,3895
rs7783351	82609420	A	0,07303	0,07377	0,9891	0,6378-1,534	0,9609	1,032	0,6649-1,602	0,888
rs10954714	82619032	A	0,3785	0,3463	1,141	0,9076-1,435	0,2583	1,132	0,8991-1,425	0,2918
rs7807790	82621782	G	0,2373	0,2711	0,8398	0,6456-1,093	0,1934	0,8417	0,6469-1,095	0,1995
rs12668225	82627841	C	0,07102	0,06861	1,038	0,6645-1,621	0,8697	1,087	0,6953-1,7	0,7138
rs12532785	82627858	G	0,3034	0,2618	1,218	0,9542-1,554	0,1134	1,238	0,9686-1,583	0,08813
rs6467937	82628178	G	0,1156	0,1013	1,147	0,8084-1,627	0,4423	1,06	0,7409-1,516	0,7506



Chapter 7

A genome-wide association study of depressive symptoms

Karin Hek, Ayse Demirkan, Jari Lahti, Antonio Terracciano, Alexander Teumer, Marilyn C. Cornelis, Najaf Amin, Erin Bakshis, Jens Baumert, Jingzhong Ding, Yongmei Liu, Kristin Marciante, Osorio Meirelles, Michael A. Nalls, Yan V. Sun, Nicole Vogelzangs, Lei Yu, Stefania Bandinelli, Emelia J. Benjamin, David A. Bennett, Dorret Boomsma, Alessandra Cannas, Laura H. Coker, Eco de Geus, Philip L. De Jager, Ana V. Diez-Roux, Shaun Purcell, Frank B. Hu, Eric B. Rimm, David J. Hunter, Majken K. Jensen, Gary Curhan, Kenneth Rice, Alan D. Penman, Jerome I. Rotter, Nona Sotoodehnia, Rebecca Emeny, Johan G. Eriksson, Denis A. Evans, Luigi Ferrucci, Myriam Fornage, Vilmundur Gudnason, Albert Hofman, Thomas Illig, Sharon Kardia, Margaret Kelly-Hayes, Karestan Koenen, Peter Kraft, Maris Kuningas, Joseph M. Massaro, David Melzer, Antonella Mulas, Cornelis Mulder, Anna Murray, Ben A. Oostra, Aarno Palotie, Brenda Penninx, Astrid Petersmann, Luke C. Pilling, Bruce Psaty, Rajesh Rawal, Eric M. Reiman, Andrea Schulz, Joshua M. Shulman, Andrew B. Singleton, Albert V. Smith, Angelina R. Sutin, André G. Uitterlinden, Henry Völzke, Elisabeth Widen, Kristine Yaffe, Alan B. Zonderman, Francesco Cucca, Tamara Harris, Karl-Heinz Ladwig, David J. Llewellyn, Katri Räikkönen, Toshiko Tanaka, Cornelia M. van Duijn, Hans J. Grabe, Lenore J. Launer, Kathryn L. Lunetta, Thomas H. Mosley Jr., Anne B. Newman, Henning Tiemeier, Joanne Murabito

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Abstract

Background: Depression is a heritable trait that exists on a continuum of varying severity and duration. Yet, the search for genetic variants associated with depression has had few successes. We exploit the entire continuum of depression to find common variants for depressive symptoms. **Methods:** In this genome-wide association study, we combined the results of 17 population-based studies assessing depressive symptoms with the Center for Epidemiologic Studies Depression Scale (CES-D). Replication of the independent top hits ($P < 1 \times 10^{-5}$) was performed in five studies assessing depressive symptoms with other instruments. In addition, we performed a combined meta-analysis of all 22 discovery and replication studies. **Results:** The discovery sample comprised 34,549 individuals (mean age of 66.5) and no loci reached genome-wide significance (lowest $P = 1.05 \times 10^{-7}$). Seven independent single-nucleotide polymorphisms were considered for replication. In the replication set (n = 16,709), we found suggestive association of one single-nucleotide polymorphism with depressive symptoms (rs161645, 5q21, $P = 9.19 \times 10^{-3}$). This 5q21 region reached genome-wide significance ($P = 4.78 \times 10^{-8}$) in the overall meta-analysis combining discovery and replication studies (n = 51,258). **Conclusions:** The results suggest that only a large sample comprising more than 50,000 subjects may be sufficiently powered to detect genes for depressive symptoms.

Introduction

Major depressive disorder (MDD) is a complex disease with an underlying heritable component. Family and twin studies report a high familial tendency of the disorder and heritability estimates of 31% to 42% (1-2). However, the long search for genetic variants associated with depression has had few successes. Several linkage studies for major depressive disorder have been performed and these identified only one relevant locus (3-4). In addition, hundreds of candidate genes have been investigated in association studies, but only six variants have been confirmed in meta-analyses (5-6). Recent efforts to find new candidate genes via genome-wide association studies (GWAS) have also been largely unsuccessful (7-15). Genome-wide association studies identified interesting regions, but associations with MDD reached standard levels of genome-wide significance at only one locus (15). Furthermore, only few previously reported candidate genes were replicated in genome-wide association studies (7,13,16).

Depression exists on a continuum of varying severity and duration. Depressive symptoms (measured on a continuous scale) and MDD (measured on a dichotomous scale) are associated with similar patterns of risk factors suggesting shared etiology with varying severity (17). The ability to detect genetic predictors might, therefore, be improved by analyzing depression quantitatively (18), defining MDD as a diagnostic entity applied to the extreme of the depression continuum (19). Using the phenotypic variation within cases and control subjects by analyzing depression quantitatively has been shown to greatly increase the power to detect genetic variants (20). In fact, a GWAS of the depression facet of personality (a continuous trait), identified several candidate genes. However, the sample size was small and findings remain to be confirmed (21).

In the current study, we exploit the entire continuum of depression, defined as the number and severity of depressive symptoms a person experiences. We assessed depressive symptoms with one of the most widely used instruments in the general population, namely the Center for Epidemiologic Studies Depression (CES-D) scale. This scale assesses the following major dimensions of depression: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite and sleep disturbance. The CES-D detects cases of MDD with high sensitivity and specificity (22) and has proven to be relatively stable over time (82% of older adults had stable CES-D scores over four measurement rounds in ten years) (23-24). In addition, a high CES-D score, like a diagnosis of MDD, is associated with cardiovascular disease and mortality (25-26). Moreover, heritability estimates of depressive symptoms, as measured with the CES-D, range from 15% to 34% (27-29).

We present the results of a meta-analysis combining genome-wide association results of depressive symptoms from 17 population-based studies of European ancestry (n = 34,549). In addition, we sought to replicate our findings in five samples that used

instruments other than the CES-D to quantify depressive symptoms (n = 16,709). Finally, we performed a combined meta-analysis of all discovery and replication studies that included 51,258 individuals.

Methods

Discovery samples

This discovery set included results from 17 population-based studies comprising a total of 34,549 persons of European descent. The following studies collaborating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (30) in the United States and Europe were included: the Atherosclerosis Risk In Communities study (ARIC1 and ARIC2) (31), the Cardiovascular Health Study (CHS) (32), the Framingham Heart Study (FHS) (33-34), and the Rotterdam Study I, II and III (RS-I, RS-II and RS-III) (35). The following population-based studies joined the discovery analyses: the Baltimore Longitudinal Study of Aging (BLSA) (36), the Erasmus Rucphen Family (ERF) (37) study, the Health, Aging and Body Composition study (Health ABC), the Invecchiare in Chianti (Aging in the Chianti area, InCHIANTI) (38) study, Helsinki Birth Cohort Study (HBCS) (39), Multi-Ethnic Study of Atherosclerosis (MESA) (40), Nurses' Health Study (NHS) (41), Rush Memory and Aging Project (MAP) (42), Religious Orders Study (ROS) (43), and SardiNIA study (44). All studies were approved by their local institutional review boards and all participants provided written informed consent.

Phenotype definition

Depressive symptoms were measured with the CES-D scale (10-item version [CHS, NHS, Rush MAP, Rush ROS], 11-item version [ARIC1], or 20-item version [ARIC2, BLSA, ERF, FHS, HBCS, Health ABC, InCHIANTI, MESA, RS-I, RS-II, RS-III, SardiNIA]). The CES-D scale was designed for use in the general population. All three CES-D versions used here detect the same four latent factors (45): depressed affect, somatic symptoms, positive affect and interpersonal problems. Each item is scored from 0 to 3 depending on the frequency of the symptoms during the past week. A higher score corresponds to more depressive symptoms. Scores from one examination round per study were used, but CES-D scores have been shown to be relatively stable over time (23-24). In studies with multiple CES-D assessments, the round with the largest number of participants (generally the first examination round) was chosen. Persons with schizophrenia or bipolar disorder were excluded based on records, interviews, or medication use (these disorders probably have a distinct genetic component). In addition, persons with a Mini-Mental State Examination score < 22, indicative of dementia, were excluded. We included persons with genotype data and depressive symptom score who were aged 40 years and older.

Adjustment for use of anti-depressants

In the search for common variants for depressive symptoms in a population-based sample, persons using antidepressants, who most likely had depression or depressive symptoms, increase genetic information. We, thus, did not exclude these persons from the analysis, but we chose to adjust their total depressive symptoms score for medication use. However, response to antidepressants is highly variable. In addition, information on compliance is often not available in population-based studies. We therefore used a nonparametric imputation algorithm to adjust the CES-D score for treatment effect. We made two assumptions: the CES-D score of a person using antidepressants is a right-censored value, i.e., the score is lower than the untreated value would be; and persons with a high CES-D score, on average, responded less to their medication than persons with a lower CES-D score. We replaced the score of a person on antidepressants with the mean depressive symptom score of all persons using antidepressants that had the same or a higher depressive symptom score. This procedure was performed separately for men and women and was based on an algorithm used for adjustment of blood pressure for persons on antihypertensive drugs (46). Antidepressant medication was defined by each study separately to account for differences between countries.

Genotyping and imputation

Genome-wide genotyping was performed by the individual studies on Illumina (Illumina, Inc., San Diego, California) or Affymetrix (Affymetrix, Santa Clara, California) platforms. All studies imputed their genotype data to ~2.5 million single-nucleotide polymorphisms (SNPs) to account for the different genotyping platforms. HapMap release 22 CEU (HapMap sample comprised of Utah residents with Northern and Western European ancestry) build 36 was generally used as reference for imputation (two studies used build 35). Genotype and imputation quality control were performed in each study separately. Genotype and quality control procedures for each study can be found in Supplementary Table S1.

Data analysis

A linear regression was performed on total depressive symptom score, adjusted for age and gender. The distribution of CES-D scores is skewed, but linear regression is fairly robust to nonnormallity. CHS and ARIC additionally adjusted for field study site, NHS for disease status, SardiNIA for self-report versus tester-read and reported answers, FHS for cohort (offspring, generation 3). Furthermore, FHS used linear mixed effect models to account for familial correlations. In the ERF study, kinship matrix was used to correct for relatedness.

Meta-analysis

We performed a *P*-value based meta-analysis weighted by sample size. This is a valid approach to account for the different CES-D versions to measure depressive symptoms and for the different distributions of depressive symptoms. The meta-analysis test-statistic was computed as follows:

$$z_{meta} = \sum_i \frac{\beta_i}{SE_i} \times \sqrt{\frac{N_i}{N_{total}}}$$

The meta-analysis was performed with METAL (http://www.sph.umich.edu/csg/abecasis/metal/) (47). The beta (β) of each individual study *i* was matched to a common coded allele (the minor allele) for each SNP across all studies. Single-nucleotide polymorphisms with a minor allele frequency less than 2.5% or an observed to expected variance ratio (imputation quality) less than 0.30 were excluded on a per study basis. Single-nucleotide polymorphisms for which the total sample size was lower than 5000 were removed from the results. Genomic control correction was applied to each study's results.

Replication

Independent top SNPs with a *P*-value < 1 x 10^{-5} in the discovery meta-analysis were selected with the clumping function in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (48) (R^2 < 0.05, 500 kilobase [kb]) for replication in five studies that measured depressive symptoms with other instruments (total n = 16,709). Persons included in the replication studies were independent from those in the discovery studies. Although replication with other instruments than the CES-D might introduce some heterogeneity, all instruments measure depressive symptoms. Further, a positive replication would ensure that our top hits are not instrument-dependent.

Age, Gene, Environment Susceptibility–Reykjavik Study (AGES-RS) (49), the ARIC study (ARIC3) (31), Monitoring of Trends and Determinants of Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg F3 and F4 (MONICA/KORA F3 and F4) (50), and the Study of Health in Pomerania (SHIP) (51-52) measured depressive symptoms with the Geriatric Depression Scale (GDS), Maastricht Questionnaire (MQ), Patient Health Questionnaire (PHQ-9) and the Beck Depression Inventory-II (BDI-II), respectively. The BDI-II, GDS and PHQ-9 aim to screen for depression and are highly correlated (53-54). The BDI-II is based on the DSM-IV criteria for MDD and comprises 21 items on a scale of 0 to 3 with higher scores indicating more severe depressive symptoms over the past two weeks. The PHQ-9 is, like the BDI-II, based on the DSM-IV criteria for MDD, but it consists of nine items on a scale of 0 to 3 to assess depressive symptoms over the past two weeks. The GDS was specifically designed to screen for depression in older adults and comprised 15 items answered with 'yes' or 'no'. The Maastricht Questionnaire

(21 items), although designed to measure vital exhaustion, correlates with measures of depressive symptoms (55) and was previously used to assess depressive symptoms (56-57).
Replication was considered significant if the Bonferroni-corrected *P*-value for testing seven SNPs was ≤ 0.050 (uncorrected *P*-value ≤ 7.1×10^{-3}).

Pathway analysis

Protein ANalysis THrough Evolutionary Relationships (PANTHER) (58) was used to identify and classify biological processes among the SNPs associated with *P*-values < 10^{-4} from the overall meta-analysis (n = 51,258). After SNP selection, SNPs were annotated to genes and/or flanking genes with the SCAN SNP and CNV Annotation Database (http://www.scandb.org). PANTHER then compares this genelist to a reference list (Homo Sapiens gene list from the National Center for Biotechnology Information) using the binomial test. Results were Bonferroni-corrected to account for multiple testing.

Candidate gene search

Altogether, 17 SNPs previously reported to be associated to depression were selected: 1 SNP that has been found genome-wide significantly associated with depressive phenotypes after replication (7,59), 4 top SNPs from the largest MDD meta-analysis so far (13), and 12 top SNPs from the only published GWAS that studied a depressive trait continuously (21). Single-nucleotide polymorphisms were tested for association in the discovery meta-analysis (n = 34,549) and in the overall meta-analysis including all studies that measured depressive symptoms (n = 51,258).

Results

Meta-analysis of depressive symptoms

Table 1 shows the characteristics of the study populations. Mean age in the discovery studies ranged between 55.9 and 80.8 years. The percentage of women varied between 44.6% and 100%. In line with the population-based design of the studies, median depressive symptoms scores ranged between 2 and 10 for the CES-D 20-item version. This is well below the cut-off of 16 at which major depression cases in older adults can be identified with high specificity and sensitivity (22). The percentage of persons scoring above this cut-off varied between 4.7% and 27.1%. Distributions of CES-D scores differed between studies and therefore a Z-score based meta-analysis was used to combine the individual study results. Antidepressant use ranged from 3.0% to 14.0%. On average, CES-D scores for persons on antidepressants more than doubled after imputation.

Table 1 Study sample characteristics of discovery and replication samples

			Depressive symptom score										International Standard Classification of Education[b]				
Sample	Instrument	N	Mean	(SD)	Median	(Range)	≥ 16 %[a]	Anti-Depressant users %	Mean Age	(SD)	Female %	Current smokers %	Level 0/1 %	Level 2 %	Level 3 %	Level 4 %	Level 5/6 %
Discovery Studies (n = 34,549)																	
ARIC1	CES-D 11	393	3.80	(3.57)	3	(0-18)	9.92	14.0	72.7	(5.46)	59.5	19.6	2.0	8.1	35.4	7.9	46.6
ARIC2	CES-D 20	614	8.52	(7.41)	6	(0-34)	16.1	11.1	71.0	(5.60)	49.7	19.7	3.1	8.3	34.7	11.7	42.2
BLSA	CES-D 20	764	6.90	(6.5)	5	(0-55)	8.51	NA	71.6	(13.8)	44.6	3.0	0.4	1.5	11.0	12.4	74.8
CHS	CES-D 10	3155	4.27	(4.29)	3	(0-26)	11.3	3.11	72.2	(5.29)	61.2	11.0	2.5	12.3	38.6	9.3	37.2
ERF	CES-D 20	1297	12.7	(10.9)	10	(0-59)	27.1	8.20	55.9	(10.1)	56.7	43.2	40.4	42.5	13.6	NA	3.5
FHS	CES-D 20	4956	7.25	(8.21)	4	(0-53)	10.3	10.4	56.1	(10.5)	53.3	14.7	0.5	3.1	32.2	24.9	39.2
HABC	CES-D 20	1654	4.93	(5.78)	3	(0-43)	4.70	3.60	73.8	(2.80)	47.1	6.4	11.9	NA	34.4	53.6	NA
InCHIANTI	CES-D 20	942	11.8	(8.24)	10	(0-46)	24.6	3.40	70.4	(9.85)	52.8	18.5	73.5	11.2	7.3	4.6	3.4
RSI	CES-D 20	3791	4.86	(7.35)	2	(0-52)	7.30	3.80	72.7	(7.21)	58.5	16.4	31.4	29.0	29.8	NA	9.8
RSII	CES-D 20	2093	5.81	(7.90)	3	(0-48)	9.70	5.00	64.8	(8.03)	54.5	19.6	21.6	35.6	27.1	NA	15.7
HBCS	CES-D 20	1386	9.58	(8.68)	7	(0-53)	19.4	4.70	63.4	(2.86)	59.7	23.0	33.0	18.4	26.0	NA	22.5
MESA	CES-D 20	2423	6.93	(6.87)	5	(0-50)	10.0	12.2	62.7	(10.2)	52.2	11.4	1.6	3.4	16.5	28.4	50.1
NHS	CES-D 10	5891	6.36	(4.50)	6	(0-26)	15.9	13.3	71.7	(6.70)	100	5.5	0	0	0	72.6	27.4
RSIII	CES-D 20	2041	6.32	(8.22)	3	(0-53)	9.90	6.90	56.0	(5.67)	56.1	22.4	9.8	35.0	28.4	NA	26.8
RUSH MAP	CES-D 10	825	1.38	(1.75)	1	(0-8)	20.1	13.6	80.8	(6.53)	73.0	2.4	1.7	27.4	19.9	42.8	8.2
RUSH ROS	CES-D 10	778	1.10	(1.51)	1	(0-8)	13.9	9.00	75.5	(7.24)	66.5	2.1	1.3	5.4	3.1	46.0	44.2
SardiNIA	CES-D 20	1438	11.9	(8.20)	10	(0-53)	25.2	3.00	58.0	(11.4)	59.5	NA	28.9	50.3	16.1	NA	4.8
Replication studies (n = 16,709)																	
AGES-RS	GDS	2855	2.58	(2.26)	2	(0-15)	9.92	13.8	76.4	(5.46)	58.0	12.7	22.1	16.8	NA	33.3	27.8
ARIC3	MQ	8918	10.2	(8.79)	8	(0-42)	9.39	4.04	57.2	(5.67)	52.7	23.8	4.8	10.2	36.4	9.2	39.4
MK F3	PHQ-9	1433	3.52	(3.54)	3	(0-26)	6.80	NA	60.5	(9.13)	51.3	14.3	12.1	56.4	17.6	0.8	13.1
MK F4	PHQ-9	1807	3.36	(3.3)	3	(0-27)	5.50	NA	60.9	(8.85)	51.5	14.6	10.0	52.4	22.6	1.1	14.0
SHIP	BDI-II	1696	6.44	(7.11)	4	(0-58)	8.90	NA	59.4	(11.6)	51.4	25.5	5.1	0.3	60.4	15.9	18.4

ARIC1, ARIC2, ARIC3, RSI, RSII, RSIII and MKF3, MKF4 included unique individuals.
AGES-RS, Age, Gene, Environment Susceptibility–Reykjavik Study; ARIC, Atherosclerosis Risk in Communities study; BDI-II, Beck Depression Inventory–II; BLSA, Baltimore Longitudinal Study of Aging; CES-D, Center for Epidemiologic Studies Depression scale; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family study; FHS, Framingham Heart Study; GDS, Geriatric Depression Scale; HABC, Health, Aging and Body Composition study; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti; MESA, Multi-Ethnic Study of Atherosclerosis; MK, Monitoring of trends and determinants of cardiovascular disease/cooperative health research in the region of Augsburg (MONICA/KORA); MQ, Maastricht Questionnaire; NA, not applicable; NHS, Nurses' Health Study; PHQ-9, Patient Health Questionnaire-9 items; RS, Rotterdam Study; Rush MAP, Rush Memory and Aging Project; Rush ROS, Rush Religious Orders Study; SardiNIA, SardiNIA study; SHIP, Study of Health In Pomerania; BDI-II, SD, standard deviation.
[a]Cut-off for screen positives was 9 for ARIC1, 8 for CHS, 9 for NHS, 3 for Rush MAP and Rush ROS, 6 for AGES-RS, 24 for ARIC3, and 17 for SHIP.
[b]Level 0: pre-primary education, level 1: primary education or first stage of basic education, level 2: lower secondary education or second stage of basic education, level 3: (upper) secondary education, level 4: post-secondary non-tertiary education, level 5: first stage of tertiary education, level 6: second stage of tertiary education.

The genomic control inflation factor lambda (λ_{gc}) for each study ranged between 0.997 and 1.024. A meta-analysis of 17 studies (n = 34,549) with depressive symptoms measured by CES-D was performed (Q-Q and Manhattan plots in Supplementary Figure S1). The total number of SNPs analysed was 2,391,896. No association reached the pre-specified genome-wide significance level of 5×10^{-8} for the association with the depressive symptom score. However, we identified 117 SNPs with a *P*-value < 1×10^{-5}, which included seven independent top SNPs ($R^2 < 0.05$ in 500 kb, Table 2). The SNP with the lowest *P*-value was rs8020095 ($P = 1.05 \times 10^{-7}$) and maps to an intronic region of *GPHN* on chromosome 14. Of the seven top SNPs, none had a heterogeneity *P*-value (tested by Cochran's *Q*) below 0.05 in the discovery meta-analysis.
We reran the analysis for the independent top SNPs excluding people on antidepressants. *P*-values of the top SNPs shifted towards one (e.g., rs8020095 *P*-value 1.56×10^{-6}, rs161645 *P*-value 1.71×10^{-3}). Adding five points to the total score for people using antidepressants in a subsample (RS-I, RS-II, RS-III, n = 7925) resulted in the same top SNPs and similar *P*-values for the top SNPs tested here.

Replication

Table 2 presents the results of the replication analysis and the overall meta-analysis across discovery sample and replication sample. The mean observed to expected variance ratio for the seven top SNPs across all cohorts ranged between 0.91 and 0.98 (Supplementary Table S2). In the replication sample, a SNP on chromosome 5 showed an association with depressive symptoms (5q21, rs161645, $P = 9.19 \times 10^{-3}$, Table 2), but this association did not reach the predefined threshold for multiple testing (corrected for multiple testing *P* = 0.064). This SNP resides in a gene desert, with the closest gene *NUDT12* more than 1000 kb away.

Table 2 Meta-analysis results of CES-D depressive symptom score in discovery studies, replication of results in studies that measured depressive symptoms with other instruments, and overall meta-analysis of all studies

								Discovery meta-analysis CES-D		Replication other instruments		Overall meta-analysis	
								N = 34,549		N = 16,709		N = 51,258	
SNP[a]	Chr	Position	SNPs (n)[b]	Closest Gene	Distance (base pair)	Allele	MAF	Overall direction (per study)	*P*-value	Overall direction (per study)	*P*-value	Overall direction	*P*-value
rs8020095	14	66,523,611	2	GPHN	intron	A/G	0.17	+(+++-+++++++-+++-+?)	$1.05x10^{-7}$	-(-?--+)	0.79	+	$3.04x10^{-6}$
rs8038316	15	52,560,732	3	UNC13C	intron	A/G	0.05	-(-?----------+-----+-)	$1.24x10^{-6}$	-(-----+)	0.42	-	$9.64x10^{-6}$
rs161645	5	104,097,816	3	NUDT12	1,171,427	A/G	0.34	+(++++++++++++-+++-+?)	$2.32x10^{-6}$	+(+++-+)	**$9.19x10^{-3}$**	+	**$8.39x10^{-8}$**[c]
rs357282	5	38,904,792	0	OSMR	intron	T/G	0.13	+(++++++++++-++++-++)	$7.56x10^{-6}$	+(-+---+)	0.87	+	$1.60x10^{-4}$
rs4653635	1	223,662,313	3	LBR	intron	A/G	0.16	-(---+-----++--------)	$8.14x10^{-6}$	+(-++--)	0.55[d]	-	$8.89x10^{-4}$
rs4594522	20	30,718,645	5	COMMD7	35,508	C/T	0.36	-(----+---+------+--)	$9.29x10^{-6}$	-(-++--)	0.80	-	$1.56x10^{-4}$
rs13137117	4	94,673,387	9	GRID2	intron	T/A	0.25	+(+++-+++-+++++++--)	$9.77x10^{-6}$	+(-+-+-)	0.97	+	$2.63x10^{-4}$

In the overall meta-analysis including discovery and replication samples (n = 51,258), SNP rs40465 reached genome-wide significance ($P = 4.78 \times 10^{-8}$). This SNP is in high LD with SNP rs161645 ($r^2 = 0.80$). Rs40465 had a *P*-value of 2.58×10^{-6} in the discovery meta-analysis and a *P*-value of 5.00×10^{-3} in the meta-analysis of replication studies. An association plot of the 5q21 region is presented in Figure 1.

Direction of effect discovery: FHS, CHS, RS-I/RS-II/ RS-III, ARIC1, ARIC2, ERF, InCHIANTI, Health ABC, BLSA, HBCS, MESA, NHS-Brc, NHS-CHD, NHS-KID, NHS-T2D, RUSH-MAP, RUSH-ROS, SardiNIA. Direction of effect replication: AGES-RS, ARIC3, MK F3, MK F4, SHIP. Allele = minor/major on the + strand, the minor allele is the coded allele.

?, not tested; CES-D, Center for Epidemiologic Studies Depression scale; Chr, chromosome; MAF, minor allele frequency; SNP, single-nucleotide polymorphism.

[a]Independent SNPs with a *P*-value < 1×10^{-5} in the discovery meta-analysis. The total n in the overall meta-analysis for SNP rs8020095 was 40,902, for rs8038316 48,103, for rs161645 49,820 and for the other SNPs 51,258. The mean observed versus expected variance ratio (measure of imputation quality) for imputed SNPs ranged between 0.91 and 0.99. Supplementary Table S2 includes this information detailed per SNP.

[b]Supporting SNPs: Number of SNPs in linkage disequilibrium with the topSNP ($R^2 > 0.8$), with a *P*-value < 10^{-4}.

[c]Lowest *P*-value for the overall meta-analysis $P = 4.78 \times 10^{-8}$ for SNP rs40465 (G/T) that is in linkage disequilibrium ($R^2 = 0.80$) with rs161645, discovery $P = 2.58 \times 10^{-6}$ (+++++++++++++-+?), replication $P = 5.00 \times 10^{-3}$ (+++-+).

[d]Heterogeneity *P*-value < 0.05.

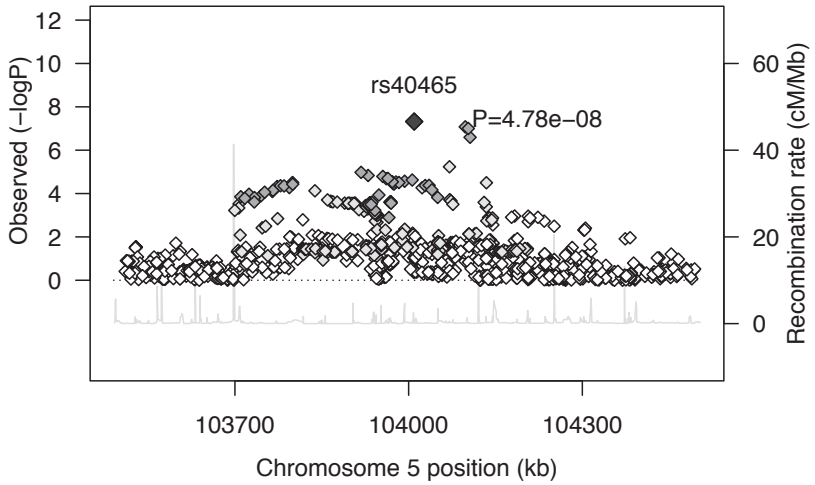

Figure 1 Association results in the 5q21 region. Summary of the association of single-nucleotide polymorphisms (SNPs) on chromosome 5 (base 103,500,000 to 104,500,000) with depressive symptoms from the overall meta-analysis (n = 51,258). The SNP with the strongest association (rs40465) is highlighted in blue and its corresponding P-value is given. Other SNPs are colored according to their degree of linkage disequilibrium (LD) with rs40465, ranging from high LD (orange, R2 0.5 to 1.0) to low LD (white, R2 < 0.2). cM, centimorgan; kb, kilobase; Mb, megabase.

In contrast, the strength of the associations of the other top SNPs with depressive symptoms was attenuated as judged by the *P*-value. All SNPs with a *P*-value < 1×10^{-4} from the overall meta-analysis (n = 51,258) are presented in Supplementary Table S3.

Pathway analysis

One hundred four functional genes of the 170 genes that were annotated were mapped to biological processes. Relevant processes that were overrepresented amongst top SNPs (*P*-value < 10^{-4}) of the overall meta-analysis were neurotransmitter secretion (Bonferroni-corrected *P*-value = 9.84×10^{-3}), vitamin transport (Bonferroni-corrected *P*-value = 0.014), and synaptic transmission (Bonferroni-corrected *P*-value = 0.037). A complete list of biological processes that were significantly overrepresented is presented in Table 3.

Table 3 Pathway analysis

Biological Process	NCBI	Observed	Expected	Over/under	Adjusted *P*-value[a]
Neurotransmitter secretion	346	6	1.81	+	$9.84x10^{-3}$
Vitamin transport	95	3	.50	+	0.014
Protein metabolic process	3240	26	16.92	+	0.015
Synaptic transmission	594	7	3.10	+	0.037
Transport	2857	22	14.92	+	0.038
Vesicle-mediated transport	1160	11	6.06	+	0.040
Cation transport	621	7	3.24	+	0.045
Cell-cell signaling	1331	12	6.95	+	0.045
Protein transport	1646	14	8.60	+	0.048
Intracellular protein transport	1646	14	8.60	+	0.048

Enrichment of biological processes among our top results (overall meta-analysis *P*-value < 10^{-4}) was statistically tested with a binomial test.
NCBI: number of genes in a biological process (reference). Observed: number of genes that belong to a biological process among the genome-wide association study (GWAS) results. Expected: expected number of genes that belong to a biological process in the GWAS results. Over/under: overrepresentation or underrepresentation of the genes in the results.
[a]A Bonferroni-correction was applied to correct for multiple testing.

Candidate gene search

None of the 17 tested candidate genes were replicated in the current study (Supplementary Table S4). Nine out of seventeen associations had the same direction in our overall meta-analysis as in the published study, and none of the nine was significant (uncorrected for multiple testing).

Discussion

In this GWAS of depressive symptoms, we combined the results of 17 population-based studies with 34,549 individuals to find common variants for depressive symptoms. Including the five replication studies, this effort comprised data from 51,258 independent individuals. Of the seven SNPs we attempted to replicate, we found suggestive evidence for the observed association of one SNP in the 5q21 region with depressive symptoms. This region reached genome-wide significance when tested over all studies (n = 51,258). Although evidence shows that depression can be well represented by a continuum of depressive symptoms we observed a genome-wide significant hit in this large GWAS only when pooling all studies with depressive symptoms. This difficulty of finding signals is in line with GWAS of major depression. Nine GWAS of depression, of which the largest comprised ~6000 MDD cases and ~7000 control subjects, yielded only one genome-wide significant finding (15).

The approach of studying depression on a continuum has the advantage that not only information on extremes is used but that all available information is exploited. Van

der Sluis *et al.* (20) showed that if the phenotypic variation among cases, as well as the variation among control subjects, is used, this greatly increases the power to detect genetic variants. However, studying depression along a continuum in population-based studies implies that many individuals have a low depressive symptoms score and that few persons score high. Therefore, it remains to be validated whether the results presented here are generalizable to clinical depression cases. In addition, the CES-D measures current depressive symptoms and not remitted depressive symptomatology. This introduced false-negatives, but in this population-based approach in which low depressive symptomatology is overrepresented, the resulting bias would be conservative. Furthermore, the distribution of depressive symptoms differed between cohorts. We therefore performed a *P*-value based meta-analysis, which is a valid approach, but has the consequence that we cannot draw conclusions on effect sizes.

Differences in depressive symptoms distribution do not impact on the validity of the findings. People with high depressive symptoms are more likely to carry risk variants, but this should not depend on the number of people with a high score. Furthermore, the distribution of I^2, a measure of heterogeneity (60), of the results combining all samples did not differ from the distribution of I^2 of the results when samples with high or low depression prevalence were meta-analyzed separately. No excess heterogeneity was observed, which suggests that depressive symptoms can be analyzed linearly. However, some genetic main effects may be more detectable in very homogeneous populations. Observed differences in distributions of depressive symptoms may have resulted from environmental factors, and if these, in turn, interact with specific genetic variants, only very homogeneous studies could also detect a genetic main effect.

Environmental factors, like education level, differed among cohorts. In observational research, one would have controlled for such possible confounders. In genetic studies, confounding by environmental factors is unlikely to occur (61), but controlling for environmental factors can also be done to increase precision, i.e., reduce the variance in depressive symptoms (62). However, environmental factors explain very little variance in depressive symptoms. Therefore, the benefit of performing additional controlled analyses will be negligible and offset by running several models with the risk of multiple testing.

In the current study, depressive symptom scores for people using antidepressants were imputed to take into account the high variability in response to antidepressants. In an analysis of depressive symptoms, people on antidepressants, who most likely had depression or depressive symptoms, are particularly informative. Therefore, excluding this group a priori may have changed the results. In a subsample, the imputation algorithm used in the current study yielded similar results as adding an arbitrary score of five points to the depressive symptom scores of people using antidepressants.

This study was performed in older adults. Cerebrovascular burden and cognitive impairment, which have a relatively high prevalence in old age, are known to be associated with depressive symptoms. In addition, while a high CES-D score indicates depressive symptoms it can also be suggestive of, for example, anxiety (63). In other words, the level of depressive symptoms is a clinically heterogeneous phenotype. However, the genetic background of clinically heterogeneous phenotypes like anxiety and depression may be more uniform than the clinical presentation suggests (64). In addition, while nongenetic determinants of depression may differ with age, genetic determinants were shown to be stable at different ages (65-66). Therefore, the results presented here are presumably generalizable to younger populations.

We combined results from studies that measured depressive symptoms with instruments other than the CES-D to replicate the association between depressive symptoms and seven independent top SNPs. In an overall meta-analysis, we tested whether any variation introduced by different instruments was offset by the increased power. In the replication effort, one SNP (5q21 region) reached a *P*-value below 0.05 but did not pass this threshold when controlling for multiple testing. Another SNP in the 5q21 region, however, reached genome-wide significance when the association across discovery and replication studies was tested (n = 51,258). The 5q21 region resides in a gene desert with the closest gene, *NUDT12*, lying more than 1000 kb away, and which has not been previously implicated in psychiatric disorders.

Although we observed suggestive association of the 5q21 region with depressive symptoms, genome-wide significance was observed only after pooling the results of the discovery and replication studies. Also, we could not replicate associations with candidate genes that previously have been reported to be associated with depression. Several explanations are plausible.

A first explanation for these observations is that the top SNPs identified in this study are false-positive findings. However, the discovery set was large and although we did not find any genome-wide significant hits, true hits are expected to be found among the top findings. A pathway analysis on the results of the overall meta-analysis showed that biological processes that play a role in depression were overrepresented among our top hits.

Second, the replication sample was smaller than the discovery sample and may be underpowered to detect true effects with moderate effect sizes, which might have been overestimated in the discovery analysis (winner's curse). Indeed, we found suggestive evidence of association for only one of seven SNPs, but the direction of association was compatible for five out of seven SNPs.

Third, lack of replication might be related to heterogeneity of the replication phenotype. In the replication approach, we combined the results of studies that measured

depressive symptoms with different instruments. Instruments were also administered at different time points across studies. However, the instruments have been reported to be highly correlated (correlations between 0.77 and 0.86) and relatively stable genetic determinants over the life span were observed in an Australian Twin study (53-54,65,67-68).

Several other factors can hinder the search for common variants associated with depressive symptoms. Population stratification, for example, can result in false-positive findings. To avoid population-stratification, only individuals from European descent were included. Including only individuals from European descent also minimized measurement error caused by cultural differences in responses to the CES-D (69). Other possible explanations are the presence of genetic heterogeneity (70), gene-gene interactions (71), and gene-environment interactions. The interaction between candidate genes and life events has been repeatedly studied for depression (72). However, to study this phenomenon in a genome-wide approach requires much larger data sets (13). In addition, it is suggested that the gain of gene-environment interaction studies over studies of main effects for complex diseases like depression is minimal (73). The study described here focused on common genetic variation, but rare variants or copy number variations not tagged by SNPs might play a role in depression (74-75). Using a larger reference panel, like the haplotypes generated by the 1000 Genomes Project, would have improved the yield of rare variants. Harmonizing imputation reference and imputation tools might have further increased the power of the study to detect associations. Also, not single SNPs, but many SNPs collectively, each with a very small effect, may affect the susceptibility for depressive symptoms (66).

In conclusion, the efforts of a large collaboration to identify common variants associated with depressive symptoms yielded no genome-wide significant hit in the discovery sample. In the replication approach, we found suggestive evidence for a SNP in the 5q21 region. When analyzing the discovery and replication samples, one genome-wide significant hit in this region was observed. Further investigation of the 5q21 region is necessary to verify the association with depressive symptoms and to pinpoint the possible functional variant. Such a future study of depressive symptoms could analyze this phenotype stratified by gender and incorporate longitudinal information with repeated measures of depressive symptoms to provide more power to our search for potential candidate genes.

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Supplementary Material

Table S1 Genotyping and quality control information

			Sample QC		Imputation QC				Imputation		Analysis	
Cohort	Platform	Calling algorithm	Call rate	Other exclusions	MAF	HWE P-value	Call rate	Other	Software	Build	Software	Adjustment*
AGES-RS	Illumina 370 K	BeadStudio	<95%	Sex mismatch, sample failure, genotype mismatch with reference panel	>1%	>10-6	>97%		MACH	HapMap release 22 CEU (build 36)	ProbABEL	
ARIC1	Affymetrix 6.0	Birdseed	<95%	sex mismatch, 1st degree relatives, cryptic relatedness, genotype discordance, outliers in PCA	>1%	>10-6	>95%	-	MACH	HapMap release 22 CEU (build 36)	ProbABEL	study site
ARIC2	Affymetrix 6.0	Birdseed	<95%	sex mismatch, 1st degree relatives, cryptic relatedness, genotype discordance, outliers in PCA	>1%	>10-6	>95%	-	MACH	HapMap release 22 CEU (build 36)	ProbABEL	study site
ARIC3	Affymetrix 6.0	Birdseed	<95%	sex mismatch, 1st degree relatives, cryptic relatedness, genotype discordance, outliers in PCA	>1%	>10-6	>95%	-	MACH	HapMap release 22 CEU (build 36)	ProbABEL	study site
BLSA	Illumina HumanHap 550K	BeadStudio	<98.5%	Non-European, sex mismatch	≥1%	≥10-4	>99%	-	MACH	HapMap release 22 CEU (build 36)	Merlin-offline, mach2dat	PCA

(Table S1 continued)

Cohort	Platform	Calling algorithm	Sample QC		Imputation QC				Imputation		Analysis	
			Call rate	Other exclusions	MAF	HWE P-value	Call rate	Other	Software	Build	Software	Adjustment*
CHS	Illumina 370 CNV	BeadStudio	<95%	sex mismatch, sample failure	≥1%	≥10-5	>97%	>2 replicate errors or Mendelian inconsistencies (for reference CEPH trios), heterozygote frequency=0, not in HapMap	BimBam10	HapMap release 21A CEU (build36)	R	site
ERF	Illumina 6k, Illumina 318K, Illumina 370K and Affymetrix 250K	BeadStudio, BRLMM	-	-	>1%	>10-6	>98%	-	MACH	HapMap release 22 CEU (build 36)	Probable, R	Relatedness
FHS	Affymetrix 500K and MIPS 50K combined	BRLMM	<97%	subject heterozygosity >5 SD away from the mean, large Mendelian error rate	>1%	>10-6	>97%	SNPs: mishap *p*>10^-9, <100 Mendelian errors, SD from the mean	MACH	HapMap release 22 CEU (build 36)	R packages kinship, GEE	Relatedness
HABC	Illumina Infinium Human1M-Duo BeadChip	BeadStudio	-	-	≥ 1%	≥10-6	≥97%	-	MACH	HapMap release 22 CEU (build 36)	R	none
HBCS	Illumina Infinium 610K Quad (Custom modified)	BeadStudio	-	HWE > 10-5	>1%	>10-6	>95%	-	MACH	HapMap release 22 CEU (build 36)	PLINK & ProbABEL	none
InCHIANTI	Illumina HumanHap 550K	BeadStudio	-	gender mismatch, IBD analysis to exclude related individuals	>1%	>10-4	>98%	-	MACH	HapMap release 22 CEU (build 36)	Mach-2qtl & Mach-2dat	none

(Table S1 continued)

			Sample QC		Imputation QC				Imputation		Analysis	
Cohort	Platform	Calling algorithm	Call rate	Other exclusions	MAF	HWE P-value	Call rate	Other	Software	Build	Soft-ware	Adjust-ment*
MESA	Affyme-trix Ge-nome-Wide Human SNP Array 6.0	Birdseed	<95%	Sample Level: gen-der mismatches, cryptic duplicates, and SNP level: monomorphic SNPs, SNPs with observed heterozygosity > 53%, and SNPs with missing rate > 5%	>1%	>10-4	>95%		IMPUTE	NCBI Build 36	SNPTest	first 10 PCs
MKF3	Affymetrix 500K	BRLMM	<93%	sex mismatch	-	-	-	-	MACH	HapMap release 21 CEU (build 35)	ProbA-BEL	none
MKF4	Affymetrix 6.0 (1000K)	Birdseed2	<93%	sex mismatch	-	-	>93%	-	MACH	HapMap release 22 CEU (build 36)	ProbA-BEL	none
NHSBrC	Illumina 550K	BeadStudio	<90%	SNP QC: MAF <0.01; Sample QC: call rate <90%, duplicates and first/second degree relatives, ancestry outliers	-	-	-	-	MACH	HapMap release 22 CEU (build 36)	ProbA-BEL	disease status, top 4 EVs
NHSchd	Affymetrix 6.0	Birdseed	<98%	SNP QC: pHWE<10E-4, MAF <0.02; Sample QC: call rate <98%, sex discrepancy with genetic data from X-linked markers, duplicates and first/second degree relatives, ancestry outliers, heterozygosity, missing phenotype information	-	-	-	-	MACH	HapMap release 22 CEU (build 36)	ProbA-BEL	disease status, top 3 EVs

(Table S1 continued)

Cohort	Platform	Calling algorithm	Sample QC		Imputation QC				Imputation		Analysis	
			Call rate	Other exclusions	MAF	HWE P-value	Call rate	Other	Software	Build	Software	Adjustment*
NHSkid	Illumina 610Q	BeadStudio	<95%	SNP QC: MAF <0.01, pHWE<10E-5; Sample QC: call rate <95%, duplicates and first/second degree relatives, ancestry outliers	-	-	-	-	MACH	HapMap release 22 CEU (build 36)	ProbABEL	disease status, top 4 EVs
NHSt2d	Affymetrix 6.0	Birdseed	<98%	SNP QC: pHWE<10E-4, MAF <0.02, >1 discordance/12 replicates, significant plate associations; Sample QC: call rate <98%, -sex discrepancy with genetic data from X-linked markers, duplicates and first/second degree relatives, ancestry outliers, heterozygosity, autosomal chromosome aberrations	-	-	-	-	MACH	HapMap release 22 CEU (build 36)	ProbABEL	disease status, top 3 EVs
RS1	Version 3 Illumina Infinium II HumanHap 550 SNP chip array	BeadStudio	<98%	sex mismatch, excess autosomal heterozygosity >0,336, outliers identified by IBS clustering analysis	≥1%	>10-6	≥98%	-	MACH	HapMap release 22 CEU (build 36)	SPSS, ProbABEL, GRIMP, R	none
RS2	Version 3 Illumina Infinium II HumanHap 550 SNP chip array	BeadStudio	<98%	sex mismatch, excess autosomal heterozygosity >0,336, outliers identified by IBS clustering analysis	≥1%	>10-6	≥98%	-	MACH	HapMap release 22 CEU (build 36)	SPSS, ProbABEL, GRIMP, R	none

(Table S1 continued)

			Sample QC		Imputation QC				Imputation		Analysis	
Cohort	Platform	Calling algorithm	Call rate	Other exclusions	MAF	HWE P-value	Call rate	Other	Software	Build	Software	Adjustment*
RS3	Version 3 Illumina Infinium II HumanHap 550 SNP chip array	BeadStudio	<98%	sex mismatch, excess autosomal heterozygosity >0,336, outliers identified by IBS clustering analysis	≥1%	>10-6	≥98%	-	MACH	HapMap release 22 CEU (build 36)	SPSS, ProbABEL, GRIMP, R	none
RUSH (MAP)	Affymetrix 6.0	Birdsuite	<95%	genotype-derived gender discordant with reported gender, inbreeding coefficient *F*>0.04	≥1%	>10-6	>95%	Exclusion: mishap test <10-9	MACH	HapMap release 22 CEU (build 36)	SAS, ProbABEL, R	First 3 principal components of eigenstrat
RUSH (ROS)	Affymetrix 6.0	Birdsuite	<95%	genotype-derived gender discordant with reported gender, inbreeding coefficient *F*>0.04	≥1%	>10-6	>95%	Exclusion: mishap test <10-9	MACH	HapMap release 22 CEU (build 36)	SAS, ProbABEL, R	First 3 principal components of eigenstrat
SardiNIA	Affymetrix 10K, 500K, 1000K	BRLMM	<95%	sex mismatch, Mendelian error	>1%	>10-6	>90%	-	MACH	HapMap CEU (build 36)	MERLIN	dummy variable: self-report vs. tester read and reported answers
SHIP	Affymetrix Human SNP Array 6.0	Birdseed V2	<92%	duplicate samples (by IBS), reported/genotyped gender mismatch	-	-	-	-	IMPUTE	HapMap release 22 CEU (Build 36)	SNPTEST, QUICKTEST, R, PLINK	none

EV, Eigenvector ; HWE, Hardy-Weinberg equilibrium; IBD, identity by descent; IBS, identity by state; MAF, minor allele frequency; PCA, principal component analysis; QC, quality control; SNP, single-nucleotide polymorphism.

*Adjustment additional to age and sex.

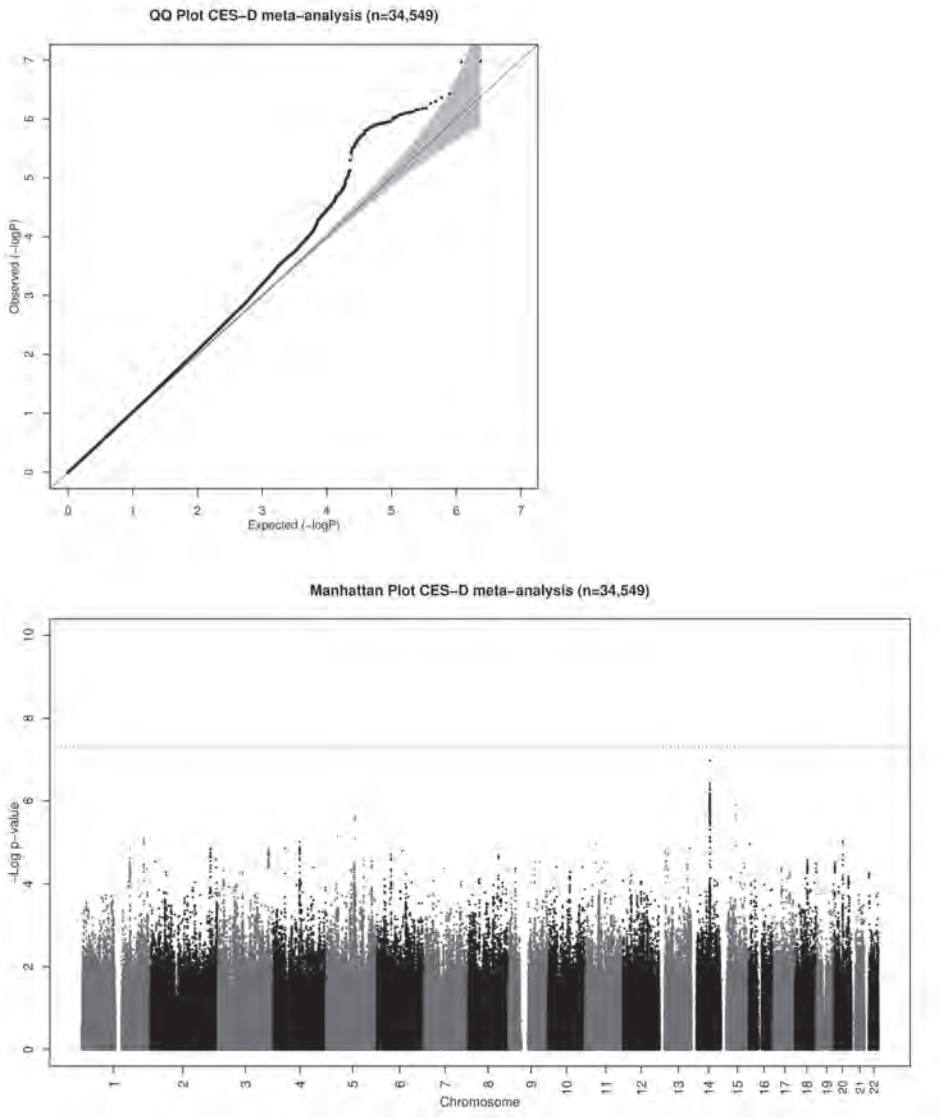

Figure S1 Genome-wide association study results for depressive symptoms in the discovery sample. Quantile-Quantile plot (**top**) and Manhattan plot (**bottom**) of total depressive symptoms score meta-analysis of discovery samples (*n* = 34,549). CES-D, Center for Epidemiologic Studies Depression scale.

Table S2 Additional top SNP information

SNP	# genotyped[a]	# imputed[b]	R^{2c}
rs13137117	1	24	0.91
rs161645	0	24	0.97
rs357282	12	13	0.93
rs40465	1	23	0.93
rs4594522	9	16	0.97
rs4653635	1	24	0.99
rs8020095	9	14	0.91
rs8038316	0	24	0.98

SNP, single-nucleotide polymorphism.
[a]Number of cohorts that genotyped this SNP
[b]Number of cohorts that did not genotype this SNP
[c]Observed versus expected variance ratio (measure of imputation quality) R^2 is based on SNPs that had not been genotyped.

Table S3 Single-nucleotide polymorphisms (SNPs) with a P-value < 10^{-4} from the overall meta-analysis (discovery + replication, n = 51,258).

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs40465	t	g	0,652	49820	-5,459	4,78E-08	----------+-?---+-	0	0,601
rs161645	a	g	0,337	49820	5,359	8,39E-08	++++++++++-++-+?+++-+	0	0,752
rs6421926	t	c	0,338	49820	5,327	9,96E-08	++++++++++-++-+?+++-+	0	0,739
rs60271	a	c	0,339	51258	5,155	2,54E-07	++++++++++-++-+-+++-+	0	0,652
rs1383605	a	t	0,206	51258	4,766	1,88E-06	+-+++++++-++++++--++++++	0	0,723
rs10279132	t	g	0,715	51256	4,735	2,19E-06	++++++++++-++++++++++	0	0,514
rs2242277	t	c	0,793	51258	-4,727	2,28E-06	-+-------+-----++------	0	0,732
rs12679544	t	c	0,793	51258	-4,685	2,80E-06	-+-------+-----++------	0	0,688
rs8020095	a	g	0,161	40902	4,668	3,04E-06	+++-++++++-++++-+?-?---+	33,5	0,069
rs7152001	c	g	0,839	40902	-4,665	3,08E-06	---+-------+---+-?+?++-	33,1	0,072
rs11914750	t	c	0,671	51258	-4,542	5,58E-06	---++-+----------+--	0	0,527
rs1008813	a	g	0,519	51255	4,535	5,77E-06	++++-+++-++++++-+++++++	15,1	0,256
rs1008812	a	g	0,482	51257	-4,534	5,80E-06	----+---+------+-------	15,5	0,250
rs1976423	a	c	0,502	47397	-4,533	5,82E-06	------+--+?-+--+-?---+-	10,6	0,321
rs17026230	a	g	0,330	51258	4,531	5,86E-06	+++--+-++++++++++++--++	0	0,495
rs1873213	t	g	0,969	45537	-4,525	6,05E-06	+--??---?--?+?-+-----+?	25,1	0,165
rs8072065	a	g	0,829	51257	-4,523	6,10E-06	--------+++----------	0	0,679
rs1008814	a	t	0,519	51257	4,521	6,16E-06	++++-+++-++++++-+++++++	15,3	0,253
rs8000066	t	c	0,519	51257	4,506	6,60E-06	++++-+++-++++++-+++++++	14,5	0,263
rs7587554	t	c	0,474	51258	4,498	6,85E-06	+++---++++-++-+++++++++	0,9	0,447
rs10958604	t	g	0,786	51258	-4,497	6,88E-06	-+-------+-----++------	0	0,736
rs7339176	a	g	0,518	51257	4,493	7,01E-06	++++-+++-++++++-+++++++	15	0,257
rs12452091	c	g	0,830	51257	-4,492	7,07E-06	--------+++----------	0	0,677
rs12451111	t	c	0,169	51257	4,473	7,72E-06	++++++++-+-++++++++-++	0	0,750
rs12793618	a	g	0,043	41709	-4,469	7,86E-06	??---+--+--------?--+-+	0	0,883
rs9900677	a	g	0,830	51257	-4,467	7,94E-06	--------+++----------	0	0,694
rs17488749	a	g	0,145	49818	-4,461	8,16E-06	------+-----++---?-----	39	0,033
rs17488784	a	t	0,145	49818	-4,458	8,28E-06	------+-----++---?-----	38,9	0,033

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs9468252	a	g	0,969	45537	-4,454	8,42E-06	+--??---?--?+?-+-----+?	18,7	0,235
rs11784532	t	c	0,785	51258	-4,45	8,58E-06	-+-------+-----++------	0	0,656
rs12451588	c	g	0,832	51257	-4,44	8,98E-06	---------+-+---------+--	0	0,753
rs8038316	a	g	0,050	48103	-4,425	9,64E-06	-?---------+----+-----+	19,4	0,204
rs1592757	c	g	0,364	51258	4,406	1,05E-05	++++++-++--+-++-+-+++--	28,7	0,099
rs1503389	t	c	0,931	48099	4,405	1,06E-05	+?+++-++-++-+++-+++++++	0	0,631
rs12452510	a	t	0,166	51257	4,402	1,07E-05	++++++++-+-++++++++++++	0	0,813
rs10091355	t	c	0,846	51258	-4,386	1,16E-05	-+-------+-----++------	0	0,679
rs6900413	a	g	0,031	47317	4,382	1,18E-05	-++??+++?+--?+-++++-?	23	0,182
rs12449501	a	g	0,167	51253	4,371	1,24E-05	++++++++-+-++++++++++	0	0,815
rs2409064	a	g	0,932	48103	4,36	1,30E-05	+?+++-++-++-+++-+++++++	0	0,640
rs16966168	a	g	0,150	50250	4,359	1,31E-05	+++??++-++++-+-+++++++	0	0,551
rs7485858	t	c	0,369	50251	-4,349	1,37E-05	+--??----------------+	0	0,728
rs1421669	c	g	0,628	51258	-4,334	1,47E-05	------+--++-+--+-+---+-	28,8	0,098
rs2168312	a	g	0,261	51258	4,333	1,47E-05	++++-++-++-++++--+++-+	11,2	0,309
rs7978337	a	t	0,369	50250	-4,329	1,50E-05	+--??----------------+	0	0,730
rs2139680	a	t	0,369	50251	-4,316	1,59E-05	+--??----------------+	0	0,722
rs2447838	t	c	0,436	51258	4,311	1,63E-05	+++++++-+++++++-+-++++--	29,3	0,094
rs9535050	a	g	0,478	51257	4,299	1,72E-05	+++--+-++++--+-+++++-	0	0,827
rs2312971	c	g	0,516	51257	-4,293	1,76E-05	---++-+-----++-+------+	0	0,683
rs9959343	t	c	0,261	51258	4,293	1,77E-05	+-++-++-++-++++--+++-+	13,4	0,278
rs10101533	a	g	0,151	51258	4,292	1,77E-05	+-+++++++-++++--++++++	0	0,691
rs4942783	c	g	0,522	51257	-4,282	1,85E-05	---++-+-----++-+------+	0	0,836
rs4754128	a	g	0,932	48102	4,282	1,86E-05	+?+++-++-++-++--+++++++	0	0,617
rs7004479	t	c	0,154	51258	4,28	1,87E-05	+-+++++++-++++--++++++	0	0,670
rs6493686	c	g	0,950	48103	4,278	1,89E-05	+?++++++++-++-+-++++-	22,6	0,167
rs8033074	a	c	0,950	48103	4,276	1,90E-05	+?++++++++-++-+-++++-	22,4	0,169
rs7107383	a	t	0,072	48103	-4,276	1,91E-05	-?---+--+--+---+-------	0	0,730
rs937055	t	c	0,919	51258	-4,267	1,99E-05	------+-++--+----+--+-+	11,9	0,298
rs254035	a	t	0,437	51258	4,264	2,01E-05	+++++++-+++++++-+-++++--	31,1	0,079
rs12453488	a	g	0,173	51254	4,262	2,02E-05	++++++++-+-++++++++-++	0	0,670
rs2447832	t	c	0,437	51258	4,259	2,05E-05	+++++++-+++++++-+-++++--	30,9	0,080
rs323105	c	g	0,965	43679	-4,247	2,17E-05	-?-------?-++--???+-++-	27,4	0,136
rs2312972	t	c	0,521	51257	-4,245	2,18E-05	---++-+-----++-+------+	0	0,845
rs1520550	a	g	0,631	51258	4,24	2,23E-05	-++--+++++++++++++++++-	0	0,762
rs7833452	a	g	0,847	51258	-4,236	2,27E-05	-+-------+-----++------	0	0,702
rs10785027	a	t	0,369	51258	-4,234	2,30E-05	+--++----------------+	0	0,757
rs2077781	t	g	0,261	51258	4,228	2,36E-05	+-++-++-++-++++--+++-+	11,9	0,298
rs33817	a	g	0,437	51258	4,226	2,38E-05	+++++++-+++++++-+-++++-	27,1	0,115
rs8030855	c	g	0,056	51258	4,221	2,43E-05	+++++-+++++++-+-+++++++	0	0,599
rs16870152	t	c	0,843	51256	4,219	2,46E-05	++++++-++++-+-+++++++++	31,7	0,074
rs2276203	a	g	0,261	51258	4,214	2,51E-05	+-++-++-++-++++--+++-+	12,7	0,288
rs7182991	t	g	0,055	51258	4,208	2,58E-05	+++++-+++++++-+-+++++++	0	0,684
rs4636213	a	g	0,200	51258	4,207	2,59E-05	++++-+--++++-+++++++++	0	0,966
rs7182611	c	g	0,055	51258	4,2	2,67E-05	+++++-+++++++-+-+++++++	0	0,679

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs185260	a	c	0,563	51258	-4,197	2,71E-05	-------+-------+-+---++	26,7	0,118
rs4489949	a	g	0,944	51258	-4,194	2,74E-05	-----+-------+-+----+--	0	0,605
rs2414196	c	g	0,738	51258	4,191	2,78E-05	+-++++++-+++++++-++++++--	35,7	0,047
rs1356893	t	c	0,605	51258	4,185	2,85E-05	-+++-++-+++++++++++++++-	0	0,603
rs254025	c	g	0,563	51258	-4,184	2,87E-05	-------+-------+-+---++	27,8	0,107
rs7177816	a	g	0,054	51258	4,183	2,88E-05	+++++-+++++++-+-++++-++	0	0,679
rs11664693	c	g	0,262	51258	4,183	2,88E-05	+-++-++-++-++++++-+++-+	6,7	0,369
rs12667152	t	g	0,156	49818	-4,182	2,88E-05	------+----+-+---?-----	37,3	0,041
rs4941210	t	c	0,262	51258	4,179	2,92E-05	+-++-++-++-++++++--+++-+	8,5	0,346
rs254020	t	c	0,438	51258	4,178	2,94E-05	+++++++-+++++++-+-+++--	27,6	0,110
rs1073839	t	g	0,850	51257	-4,176	2,96E-05	---+--+------+-+-------	0	0,613
rs1353416	a	g	0,355	51258	4,176	2,97E-05	+++--+-+++++++++++++-++	0	0,480
rs2414195	a	g	0,738	51258	4,175	2,98E-05	+-++++-+++++++-++++++--	36,9	0,040
rs11927424	a	g	0,644	51258	-4,172	3,02E-05	---++-+-------------+--	0	0,522
rs9596054	a	g	0,478	51257	4,17	3,04E-05	+++--+-++++++-+-++++++-	0	0,768
rs3111816	a	g	0,352	51239	-4,17	3,04E-05	----------+-----------+	0	0,964
rs4073665	a	g	0,873	51258	-4,169	3,05E-05	----------++------+-----	26,1	0,124
rs1397164	a	c	0,637	49820	4,168	3,08E-05	-++-++---++++++++?++-++	22,3	0,170
rs7170422	t	c	0,945	51258	-4,167	3,08E-05	-----+-------+-+----+--	0	0,664
rs10851526	t	c	0,261	51258	-4,167	3,09E-05	-+-----+-------+-----++	35,1	0,050
rs11977246	t	c	0,844	49818	4,166	3,10E-05	++++++-++++-+-+++?+++++	37,4	0,041
rs254023	t	c	0,563	51258	-4,166	3,11E-05	-------+-------+-+---++	27,6	0,109
rs2919955	a	g	0,352	51257	4,165	3,12E-05	++++++-++--++++++-+++-+	0	0,660
rs7641985	a	c	0,644	51258	-4,163	3,14E-05	---++-+-------------+--	0	0,476
rs13155692	t	c	0,748	49820	-4,161	3,18E-05	-------------++-+-?--+-++	17,1	0,233
rs9554349	t	c	0,033	38528	4,16	3,18E-05	+?+++--++++++++++??+??+	0	0,517
rs11147450	t	c	0,521	51257	-4,16	3,18E-05	---++-+-----++-+------+	0	0,795
rs2125659	t	c	0,946	51258	-4,158	3,21E-05	-----+-------+-+----+--	0	0,698
rs10505424	a	c	0,199	51258	4,156	3,24E-05	++++-+--+++++-+++++++++	0	0,977
rs17750582	a	c	0,055	51258	4,155	3,25E-05	+++++-+++++++-+-++++-++	0	0,659
rs17553281	a	t	0,822	51257	-4,155	3,25E-05	----++-+----+----+----+	23,5	0,152
rs1442111	t	g	0,352	51258	4,155	3,26E-05	++++++-++--++++++-+++-+	0	0,721
rs10879604	a	t	0,374	50251	-4,155	3,26E-05	+--??--+---------------+	0	0,670
rs1587150	a	t	0,355	51258	4,154	3,27E-05	+++--+-+++++++++++++-++	0	0,481
rs12955929	t	g	0,958	46834	-4,153	3,28E-05	---------?-++--???+-++-	16	0,258
rs8026763	t	c	0,262	51239	-4,152	3,30E-05	-+-----+-------+-----++	35,5	0,048
rs7308693	t	c	0,631	51258	4,149	3,33E-05	-++--++++++++++++-++++-	0	0,724
rs1011947	a	g	0,262	51258	4,148	3,36E-05	+-++-++-++-++++-++++-+	5	0,393
rs10785028	t	g	0,631	51258	4,147	3,38E-05	-++--++++++++++++-++++-	0	0,723
rs7137885	t	c	0,368	51258	-4,144	3,42E-05	+--++-------------+----+	0	0,735
rs2161097	t	c	0,438	51258	4,143	3,43E-05	+++++++-+++++++-+-+++--	29,4	0,093
rs7555997	a	c	0,101	50232	-4,141	3,46E-05	---??-+--+---+---------	0	0,808
rs2447828	t	c	0,438	51258	4,138	3,51E-05	+++++++-+++++++-+-+++--	29,9	0,088
rs11927001	a	g	0,869	51258	4,131	3,62E-05	-+++-++++-++-++-+++++++	27,2	0,113
rs2125657	t	c	0,054	51258	4,131	3,62E-05	+++++-+++++++-+-++++-++	0	0,695

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	Het-ISq	Het-PVal
rs2112163	t	c	0,438	51258	4,13	3,62E-05	+++++++-+++++++-+-++++--	30,2	0,086
rs6551366	c	g	0,355	51258	4,128	3,66E-05	+++--+-+++++++++++++++	0	0,487
rs1589595	t	c	0,738	51258	-4,128	3,66E-05	-+---+---+---+-----+----+-	5,8	0,382
rs1522116	t	c	0,441	51257	4,126	3,69E-05	++++++-++-++++--+++-++++-	0	0,791
rs920623	a	g	0,352	51258	4,125	3,71E-05	++++++-++--++++++-+++-+	0	0,649
rs7205464	t	g	0,097	51256	4,122	3,75E-05	++++++++-+++++++---++++	0	0,945
rs7295470	t	c	0,372	51258	-4,122	3,75E-05	+--++-----------------+	0	0,723
rs1426134	c	g	0,353	51258	-4,12	3,78E-05	----------+----------+	0	0,915
rs16893023	a	c	0,194	51258	4,119	3,80E-05	++++-+--+++++-+++++++++	0	0,952
rs12050204	t	g	0,080	51252	-4,119	3,81E-05	-----+---+-++--------+-	0	0,759
rs9903859	a	g	0,160	51257	4,118	3,82E-05	++++++++-+-+++++++++-++	0	0,495
rs1045301	t	g	0,082	51258	-4,111	3,94E-05	-----+---+-++--------+-	0	0,810
rs2337127	a	c	0,946	51258	-4,11	3,95E-05	-----+-------+-+----+--	0	0,698
rs1687128	t	g	0,081	51258	4,105	4,05E-05	++++++-+--++-++++-++-+-	9	0,339
rs9303295	a	g	0,850	51257	-4,103	4,09E-05	---++---+-----+-+-------	0	0,654
rs4776080	t	c	0,269	51258	-4,101	4,12E-05	-+----++--------------++	34,6	0,053
rs10879605	t	c	0,374	51258	-4,1	4,13E-05	+--++---+--------------+	0	0,744
rs2414218	t	c	0,276	51257	-4,1	4,13E-05	-+-----+--------------++	31,8	0,073
rs325501	c	g	0,587	51258	-4,097	4,19E-05	-------+---------+---++	17,3	0,227
rs2414217	t	c	0,276	51258	-4,093	4,26E-05	-+-----+--------------++	30,6	0,083
rs325481	a	g	0,586	51258	-4,092	4,27E-05	-------+---------+---++	19,4	0,200
rs1583953	t	c	0,353	51258	4,087	4,37E-05	++++++-++--++++++-+++-+	0	0,858
rs988542	a	g	0,628	51258	4,086	4,39E-05	-++--++-++++++++++++++-	0	0,759
rs1106420	a	t	0,318	51258	-4,086	4,39E-05	++---+-+-+-----+-+-----	1,4	0,442
rs8079016	t	c	0,161	51257	4,086	4,40E-05	++++++++-+-++++++++-++	0	0,494
rs7953276	a	c	0,377	51258	-4,084	4,43E-05	---++---+--------------+	0	0,868
rs12955292	a	g	0,036	46834	4,083	4,44E-05	+++++++++?+--++???-+--+	22,3	0,184
rs768792	a	g	0,647	51258	-4,083	4,45E-05	------+--++------+---+-	0	0,868
rs1687119	a	g	0,919	51258	-4,08	4,51E-05	------+-++--+----+--+-+	8,9	0,339
rs9535127	t	c	0,481	51257	4,076	4,59E-05	+++-++-++++--+-++++++-	0	0,864
rs10748226	t	g	0,628	51258	4,075	4,60E-05	-++--++++++++++++++++-	0	0,778
rs4760780	t	c	0,629	51258	4,075	4,60E-05	-++--++-++++++++++++++-	0	0,740
rs6445194	t	g	0,489	51258	-4,075	4,61E-05	-+----+------+--+-+----	5,9	0,381
rs12441046	t	g	0,724	51258	4,072	4,65E-05	+-++++-++++++++++++--	30,9	0,080
rs13177473	a	g	0,647	51258	-4,068	4,75E-05	------+--++------+---+-	0	0,852
rs1542727	a	g	0,174	51258	4,065	4,81E-05	+++++-+++-++-++++++++-+	0	0,536
rs2203976	t	c	0,628	51258	4,062	4,87E-05	-++--++-++++++++++++++-	0	0,738
rs6582151	t	c	0,371	51258	-4,06	4,91E-05	+--++--+---------+----+	0	0,728
rs2139675	t	g	0,628	51258	4,059	4,93E-05	-++--++-++++++++++++++-	0	0,737
rs13250310	a	t	0,732	48103	-4,056	4,99E-05	+?--++-+--------+------	8,7	0,344
rs1394309	a	g	0,932	48437	-4,053	5,05E-05	---+-----?+++--++?--++-	38,6	0,038
rs139265	a	g	0,833	51256	-4,049	5,15E-05	+--+---------+-+--+---+	0,4	0,454
rs16955611	a	g	0,949	51258	-4,044	5,25E-05	-----+-------+-+----+--	0	0,773
rs7899547	t	g	0,360	51258	4,043	5,27E-05	+-+++++++++-++-+++++++++	0	0,918
rs2028526	t	c	0,648	51251	-4,042	5,31E-05	------+--++------+---+-	0	0,748

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs9645898	a	c	0,706	50250	-4,041	5,33E-05	---??-----+-+----------	0	0,784
rs3922857	t	c	0,097	51256	4,039	5,36E-05	+++++++-+++++++---++++	0	0,921
rs2363065	t	c	0,628	51258	4,039	5,37E-05	-++--++-+++++++++++++-	0	0,744
rs7976937	t	c	0,361	51258	-4,039	5,38E-05	+---+--+--------------+	0	0,862
rs325485	a	g	0,388	51257	4,037	5,42E-05	+++++++-++-+-++++-++---	12,8	0,287
rs6964185	a	g	0,126	51256	-4,032	5,53E-05	-+----+-+--+--++++-----	0	0,675
rs2836021	t	c	0,836	51256	4,03	5,59E-05	+-+--++++++++-++-+++-++	0	0,788
rs11179680	a	g	0,362	51258	-4,027	5,64E-05	+---+--+--------------+	0	0,864
rs10984257	t	c	0,869	51258	-4,024	5,72E-05	----+---++----+--------	37,6	0,037
rs11683777	t	c	0,151	44606	4,022	5,77E-05	?++++++++-+--++++++++-?	0	0,823
rs12824659	a	g	0,333	51258	-4,021	5,79E-05	-------+--------------+	0	0,954
rs4889796	t	c	0,781	49819	4,02	5,83E-05	-+-++++++++++-++-?+++++	26,8	0,121
rs11611073	a	g	0,362	51258	-4,018	5,86E-05	+---+--+--------------+	0	0,865
rs508760	t	g	0,070	48103	-4,018	5,87E-05	-?---+--------+++-+-----	0	0,631
rs7975033	a	t	0,372	51258	-4,015	5,94E-05	+--++--+---------+-----+	0	0,704
rs6875442	a	c	0,625	51258	4,012	6,01E-05	+++++++-+++-+-+++-+++++	0	0,958
rs9365900	a	g	0,071	40276	4,011	6,05E-05	??+-+-++++++-+++?-+?++	23,5	0,171
rs2211846	a	g	0,165	50250	-4,01	6,08E-05	-+-??--------+------+--	0	0,769
rs10742719	a	c	0,525	50249	-4,008	6,13E-05	-+-??--+-------+-+---+-	0	0,590
rs2043475	t	c	0,168	51257	4,007	6,14E-05	++++++++++-++++++-++-++	0	0,604
rs2995807	t	c	0,140	51256	-4,007	6,15E-05	+---------------+--+-----	0	0,948
rs7964705	t	c	0,629	51258	4,006	6,17E-05	-++--++-+++++++++-++++-	0	0,697
rs7199995	t	c	0,140	51256	4,005	6,20E-05	++++++++--++++++-+-++++	0	0,964
rs2836012	a	t	0,165	51255	-4,005	6,21E-05	-+-++--------+------+--	0	0,833
rs2296561	a	t	0,879	49820	-4,005	6,22E-05	----------+----+-?++-+-	32,9	0,069
rs2084919	t	c	0,515	51258	4,004	6,23E-05	+-++++-+-+-+++-++++++--+	1,6	0,439
rs1915293	t	c	0,629	51258	4,004	6,24E-05	-++--++-+++++++++-++++-	0	0,699
rs12367407	a	t	0,637	51258	4,002	6,27E-05	-+++-++-++++++++++++-	0	0,858
rs10942087	t	c	0,637	51258	4	6,33E-05	+++++-+-+++++-+++-+++++	0	0,832
rs323097	a	c	0,959	46834	-4	6,33E-05	----------?-++--???+-++-	13,7	0,286
rs2047268	a	c	0,363	51258	-3,999	6,36E-05	-----+-+-----+---+-----	0	0,826
rs13157155	c	g	0,626	51258	3,998	6,38E-05	+++++++-+++-+-+++-+++++	0	0,958
rs2148473	a	g	0,869	51258	-3,998	6,39E-05	----+---++-+--+--------	39,2	0,029
rs11917572	t	c	0,959	47020	3,997	6,42E-05	+++++-+-+?+-+--+++?+-+-	0	0,605
rs4627955	a	g	0,625	51258	3,996	6,43E-05	+++++++-+++-+-+++-+++++	0	0,955
rs12654558	t	c	0,636	51258	3,996	6,45E-05	+++++-+-+++++-+++-+++++	0	0,830
rs2836014	a	c	0,165	51257	-3,995	6,48E-05	-+-++--------+--+---+--	0	0,806
rs1472763	c	g	0,318	50246	3,994	6,49E-05	+++??++++-++-++++--++++	0	0,684
rs12657561	t	c	0,364	51258	-3,992	6,55E-05	-----+-+-----+---+-----	0	0,831
rs10984272	a	t	0,870	51258	-3,991	6,57E-05	----+---+--+--+--------	40,5	0,024
rs10984285	t	c	0,124	51258	3,99	6,61E-05	++++-+++--+-++-++++-++	39,9	0,026
rs9516233	t	c	0,648	51257	3,987	6,69E-05	+++++-+++-+----++-+++++	31,1	0,078
rs1443737	a	t	0,363	51258	-3,987	6,70E-05	+---+--+---------------+	0	0,860
rs13013073	c	g	0,388	43950	-3,986	6,73E-05	-?---?--+-+--+----?--++	0	0,552
rs7825010	a	g	0,842	51258	-3,985	6,74E-05	-+------++-----++------	0	0,689

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs11179697	a	g	0,363	51258	-3,985	6,74E-05	+---+--+--------------+	0	0,851
rs13163964	a	g	0,625	51258	3,983	6,80E-05	+++++++-+++-+-+++-+++++	0	0,953
rs325506	c	g	0,433	49820	3,983	6,81E-05	+++++++-+-++++++-+?+++--	10,5	0,320
rs6551361	t	c	0,645	51258	-3,981	6,87E-05	---++-+-------+-----+--	0	0,541
rs11038193	a	t	0,529	50250	-3,979	6,91E-05	-+-??--+---+---+-+---+-	0,6	0,450
rs9524069	a	c	0,648	51257	3,978	6,94E-05	+++++-+++-+----++-++++	31,5	0,075
rs13339086	a	g	0,097	51256	3,977	6,99E-05	++++++++-+++++++---++++	0	0,930
rs995431	a	t	0,637	51258	3,976	7,00E-05	+++++-+-+++++-+++-++++	0	0,831
rs9949310	t	c	0,959	46834	-3,976	7,02E-05	---------?-++--???+-++-	13,4	0,291
rs4072224	a	g	0,903	51256	-3,975	7,05E-05	--------+-------+++----	0	0,860
rs139263	a	g	0,166	51256	3,972	7,13E-05	-++-+++++++-+-++-+++-	0	0,465
rs10413178	t	c	0,094	51258	-3,972	7,14E-05	---+--+---+----------	0	0,921
rs11179681	a	g	0,638	51258	3,972	7,14E-05	-+++-++-++++++++++++-	0	0,867
rs13181679	a	g	0,352	51258	3,971	7,15E-05	++++++-++--++++++-+++-+	0	0,896
rs1443738	t	g	0,363	51258	-3,97	7,18E-05	+---+--+--------------+	0	0,863
rs7467375	t	g	0,125	51258	3,97	7,20E-05	++++-+++--+-++-++++-++	39,7	0,027
rs7177989	t	c	0,726	51258	3,968	7,26E-05	+-++++++++++++++++++--	36,2	0,044
rs973303	t	g	0,353	51257	-3,966	7,31E-05	-----+---+-++++--+-----	29,5	0,092
rs6881764	a	g	0,482	51258	3,961	7,48E-05	+++++++-+--+-++-+-++---	22,7	0,161
rs8008773	a	t	0,873	51258	-3,96	7,48E-05	----------+---+-++++-	46,6	0,008
rs12541821	a	g	0,139	51258	3,96	7,51E-05	+-+++++++-++-++++-++++++	0	0,573
rs12134580	t	c	0,971	35673	-3,959	7,52E-05	----??+?-?????-???---+-	50,7	0,022
rs12368237	a	g	0,375	51258	-3,956	7,61E-05	+---+--+--------------+	0	0,856
rs6421241	t	c	0,609	51258	3,955	7,65E-05	-++--++-++++++++++++-	0	0,740
rs1443742	t	c	0,362	51258	-3,955	7,66E-05	+---+--+--------------+	0	0,863
rs4297682	a	g	0,904	51256	-3,955	7,67E-05	--------+-------+++----	0	0,867
rs2049103	a	g	0,958	47020	3,954	7,68E-05	+++++-+-+?+-+-++++?+-+-	0	0,687
rs11923274	a	g	0,958	47020	3,953	7,73E-05	+++++-+-+?+-+--+++?+-+-	0	0,656
rs10180695	t	c	0,424	51258	3,951	7,78E-05	++++++-+++++-+++-+++-++	0	0,940
rs4971723	t	c	0,435	51258	3,951	7,79E-05	++++++-+++++-+++-+++-++	0	0,904
rs11783005	t	c	0,284	48103	3,951	7,79E-05	+?++--+-++-++++++--++++	10,3	0,322
rs2414188	t	c	0,267	51258	-3,951	7,80E-05	-+----++--------------++	33,7	0,059
rs11618590	t	c	0,837	51257	-3,95	7,81E-05	-------+++-+--------+--	0	0,613
rs1545292	a	g	0,625	51258	3,95	7,83E-05	-+++-++-++++++++++++-	0	0,855
rs4412846	a	t	0,353	51257	-3,949	7,84E-05	-----+---+-++++--+-----	30	0,088
rs7974278	c	g	0,624	51258	3,948	7,87E-05	-+++-++-++++++++++++-	0	0,855
rs12521551	a	g	0,364	51258	-3,947	7,90E-05	-----+-+-----+---+-----	0	0,831
rs7651475	t	c	0,042	47020	-3,947	7,93E-05	-----+-+-?-+-++---?-+-+	0	0,664
rs2881577	a	g	0,958	47020	3,946	7,94E-05	+++++-+-+?+-+--+++?+-+-	0	0,664
rs4738700	t	c	0,662	51258	-3,945	7,98E-05	------+-+----------+--++	16,2	0,241
rs1373834	a	g	0,648	51257	3,944	8,01E-05	+++++-+++-+----++-+++++	33,1	0,064
rs13162928	t	c	0,648	51258	-3,943	8,04E-05	------+--++------+---+-	0	0,894
rs1363179	t	g	0,583	51258	-3,943	8,05E-05	----+-+---+--+-+----+-+	30,4	0,085
rs6582152	c	g	0,368	51258	-3,941	8,11E-05	+--++--+----------+----+	0	0,667
rs8091788	a	g	0,829	51258	-3,94	8,14E-05	-+--+--+----------+-----	0	0,585

(Table S3 continued)

SNP	Allele1	Allele2	Freq1	Weight	Zscore	*P*-value	Direction	HetISq	HetPVal
rs7628116	c	g	0,042	47020	-3,938	8,23E-05	-----+-+-?-+-+----?-+-+	0	0,699
rs1421908	t	c	0,417	51258	3,937	8,25E-05	++++-+-+++-++-+-++++-+-	30,8	0,081
rs7907283	a	g	0,087	51258	3,936	8,30E-05	++++++++---+-+-+++++++++	19,3	0,202
rs3787851	t	c	0,165	51257	-3,935	8,32E-05	-+-++---------+--+---+--	0	0,791
rs12452350	a	g	0,833	51257	-3,932	8,41E-05	----------+------+--+--	0	0,601
rs10769092	t	g	0,497	51258	-3,932	8,42E-05	-+--+--+---+---+-+---+-	0	0,555
rs6895949	a	g	0,279	51258	3,93	8,50E-05	+++--+++++-++++-++++-+-	27,2	0,114
rs10101647	a	g	0,160	51258	3,93	8,51E-05	+-+++++--++++++-++++++	0	0,662
rs731428	t	c	0,936	49819	-3,929	8,53E-05	--------+-+------?----+	0	0,560
rs4077278	c	g	0,475	51257	-3,928	8,56E-05	--------+---+---+----+-	0	0,939
rs7317531	t	g	0,457	51256	-3,927	8,59E-05	-+---------+------+----	0	0,804
rs12519063	t	c	0,637	51258	3,926	8,64E-05	+++++-+-++++-+++-+++++	0	0,836
rs2111380	a	t	0,435	51258	3,926	8,65E-05	++++++-++++-+++-+++-++	0	0,908
rs7822661	t	c	0,140	51258	3,925	8,66E-05	+-++++++-++-++++-++++++	0	0,595
rs12209628	t	c	0,800	51258	-3,925	8,67E-05	----+--+++--++-+-------	0	0,970
rs1530303	t	c	0,350	51258	3,925	8,68E-05	++++++-++--++++++-+++-+	0	0,924
rs7620638	a	g	0,958	47020	3,924	8,70E-05	+++++-+-+?+-+--+++?+-+-	0	0,661
rs12205387	t	c	0,200	51258	3,924	8,72E-05	++++-++---++--+-+++++++	0	0,971
rs1501192	t	c	0,701	51258	-3,924	8,72E-05	---------+-----+++-----+	0	0,478
rs9898999	t	c	0,167	51257	3,922	8,80E-05	++++++++-+-++++++-++-++	0	0,598
rs13248919	a	g	0,264	48103	3,918	8,92E-05	-?++--+-++-++++++--+++++	6,7	0,371
rs10742718	c	g	0,525	51258	-3,916	9,00E-05	-+--+--+--------+-+---+-	0,3	0,456
rs10742725	t	c	0,475	50243	3,914	9,07E-05	+-+??++-++++++-+-+++-+	0	0,574
rs926300	a	t	0,828	51258	3,913	9,12E-05	++++++++++++++++-+-++++-	0	0,703
rs6545190	t	g	0,564	51258	-3,91	9,25E-05	------+-----+---+---+--	0	0,915
rs7631883	a	g	0,042	47020	-3,909	9,27E-05	-----+-+-?-+-+----?-+-+	0	0,657
rs2352545	t	c	0,565	51258	-3,908	9,33E-05	------+-----+---+---+--	0	0,912
rs7930681	t	c	0,498	51258	3,904	9,45E-05	+-++-++-+++-+-+-+-+++-+	9,6	0,330
rs2139686	a	c	0,957	47020	3,902	9,53E-05	+++++-+-+?+-+--+++?+-+-	0	0,673
rs2163946	a	g	0,529	48818	-3,901	9,57E-05	-+-??--+---+---+-+--?+-	0	0,464
rs1738819	t	c	0,133	51258	3,901	9,57E-05	-++++++-+-+++-++++-+++++	0	0,738
rs4901754	a	g	0,654	51257	-3,9	9,62E-05	++------+----+------++-	0	0,581
rs1395268	a	c	0,173	51258	3,899	9,66E-05	+-++-++-++++-++++-+++++	0	0,588
rs2836007	t	c	0,165	51257	-3,899	9,68E-05	-+-++--------+------+--	0	0,834
rs7713437	a	g	0,720	51258	-3,898	9,68E-05	---++-----+----+----+-+	25	0,136
rs2762089	a	t	0,355	51257	-3,898	9,69E-05	-----+---+-++++--+-----	31,2	0,078
rs11179688	t	g	0,377	51258	-3,898	9,70E-05	+---+--+--------------+	0	0,846
rs11746102	a	g	0,363	51258	-3,897	9,74E-05	-----+-+-----+---+-----	0	0,837
rs11179690	a	g	0,623	51258	3,897	9,75E-05	-++++-++-++++++++++++-	0	0,843
rs7316126	a	t	0,624	51258	3,893	9,88E-05	-++++-++-++++++++++++-	0	0,834
rs4736893	a	g	0,231	51258	-3,893	9,91E-05	------+------+-+-----+-	0	0,942
rs7728789	t	c	0,637	51258	3,891	9,97E-05	+++++-+-+++++-+++-+++++	0	0,835

Table S4 Replication of top SNPs (p-value <10^{-5}) from previous genome-wide association studies

				Original study		Current study			
						Discovery set (n = 34,549)		Overall meta-analysis (n = 51,258)	
Study	SNP	Chr	Effective allele	Direction of effect	*P*-value	Direction of effect	*P*-value	Direction of effect	*P*-value
Sullivan 2009	rs2522833[a]	7	C	+	1.2e-06	+	0.26	+	0.83
Wray 2012[b]	rs11579964	1	T	-	4.4e-06	+	0.19	+	0.19
	rs7647854	3	G	+	4.6e-06	+	0.61	+	0.57
	rs12446956	16	C	+	1.1e-06	+	0.94	-	0.77
	rs12457996	18	C	-	5.7e-06	-	0.79	+	0.68
Terracciano 2010[c]	rs12912233	15	T	+	6.3e-07	-	0.85	-	0.37
	rs8070473	17	T	-	1.5e-06	+	0.94	+	0.26
	rs349475	5	T	+	2.4e-06	+	0.72	+	0.28
	rs12420464	11	T	-	3.3e-06	+	0.17	+	0.51
	rs1927745	13	A	-	4.7e-06	-	0.69	+	0.78
	rs10514585	16	A	+	4.9e-06	-	0.053	-	0.011
	rs11009175	10	A	+	5.4e-06	-	0.067	-	0.17
	rs17864092	7	T	-	5.5e-06	-	0.60	-	0.33
	rs1449984	2	A	-	6.6e-06	+	0.90	-	0.76
	rs1924397	13	A	+	7.6e-06	+	0.66	+	0.21
	rs10744304	12	A	-	8.7e-06	+	0.31	-	0.80
	rs2017305	10	A	-	9.0e-06	-	0.99	-	0.63

SNP, single-nucleotide polymorphism. Sullivan 2009, 1738 cases and 1802 controls (1); Wray 2012, 5763 cases and 6901 controls (2); Terracciano 2010, n = 4811 (3).
[a]This SNP was tested for association in the current study as it was replicated previously.
[b]Largest meta-analysis of major depressive disorder.
[c]Meta-analysis of trait depression, only independent top SNPs were tested here.

Supplemental references

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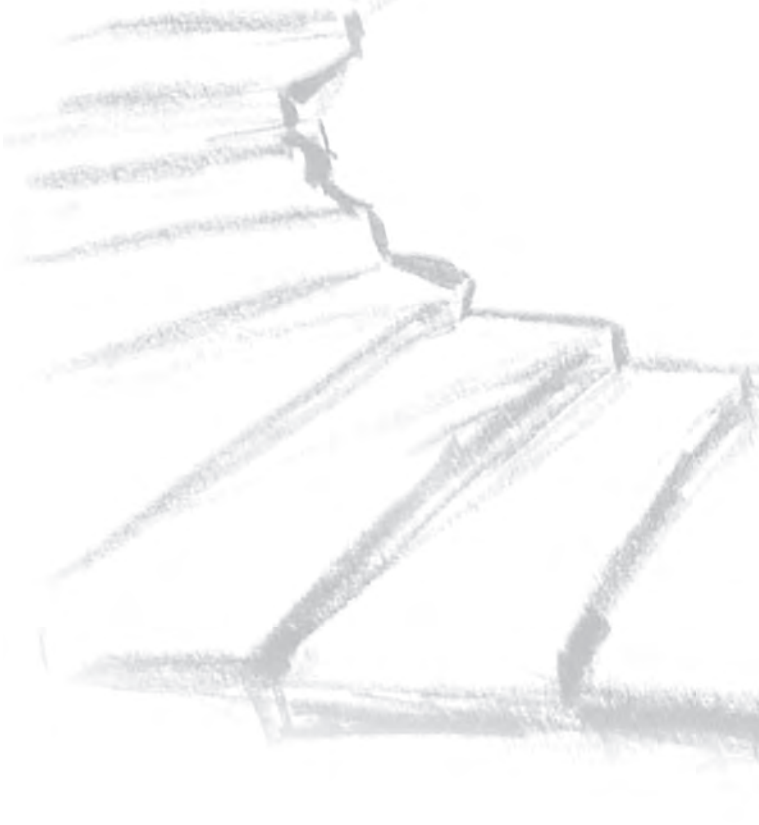
3. Terracciano A, Tanaka T, Sutin AR, Sanna S, Deiana B, Lai S, et al. (2010): Genome-wide association scan of trait depression. Biol Psychiatry 68: 811-817.



Chapter 8

Genetic risk profiles for depression and anxiety in adult and elderly cohorts

Ayşe Demirkan, Brenda W.J.H. Penninx, Karin Hek, Naomi R. Wray, Najaf Amin, Yurii S. Aulchenko, Richard van Dyck, Eco J.C. de Geus, Albert Hofman, André G. Uitterlinden, Jouke-Jan Hottenga, Willem A. Nolen, Ben A. Oostra, Patrick F. Sullivan, Gonneke Willemsen, Frans G. Zitman, Tiemeier, A. Cecile J.W. Janssens, Dorret I. Boomsma, Cornelia M. van Duijn, Christel M. Middeldorp

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Abstract

The first generation of genome-wide association studies (GWA studies) for psychiatric disorders has led to new insights regarding the genetic architecture of these disorders. We now start to realize that a larger number of genes, each with a small contribution, are likely to explain the heritability of psychiatric diseases. The contribution of a large number of genes to complex traits can be analyzed with genome-wide profiling. In a discovery sample, a genetic risk profile for depression was defined based on a GWA study of 1738 adult cases and 1802 controls. The genetic risk scores were tested in two population-based samples of elderly participants. The genetic risk profiles were evaluated for depression and anxiety in the Rotterdam Study cohort and the Erasmus Rucphen Family (ERF) study. The genetic risk scores were significantly associated with different measures of depression and explained up to ~0.7% of the variance in depression in Rotterdam Study and up to ~1% in ERF study. The genetic score for depression was also significantly associated with anxiety explaining up to 2.1% in Rotterdam study. These findings suggest the presence of many genetic loci of small effect that influence both depression and anxiety. Remarkably, the predictive value of these profiles was as large in the sample of elderly participants as in the middle-aged samples.

Introduction

Genetic factors have an important role in the susceptibility to depression. A meta-analysis of twin studies on major depressive disorder (MDD) estimated the heritability at 37% (1). However, the success of studies aiming to find genes underlying the vulnerability for depression has been limited. An overview of promising results of linkage studies on MDD and neuroticism, a related personality trait, shows some overlap in regions of interest, but, so far, no single locus has been identified (2). Candidate gene studies, mostly focusing on genes involved in neurotransmitter circuits or in reactions to stress, have also not been able to unambiguously identify a genetic variant explaining differences in the vulnerability for depression (3-4).

An important issue in research on the etiological factors of MDD has been the frequent comorbidity with anxiety disorders. In the National Comorbidity Survey Replication, 59% of the subjects with a lifetime diagnosis of MDD also fulfilled the criteria for a lifetime anxiety disorder diagnosis (5). A review of twin and family studies indicated that this comorbidity might be explained by shared, mostly genetic factors (6). Still, an overview of promising results of linkage studies of anxiety only showed one overlapping region of interest with MDD and neuroticism (2).

The recent success of genome-wide association (GWA) studies has fueled expectations on finding genes for MDD. One of the first GWA studies of depression showed evidence for the role of the presynaptic protein piccolo (PCLO) gene (7-8).Recently, this result was replicated with a *P*-value of 2×10^{-9} in a meta-analysis of the results in three population-based samples, but not when five clinical samples were also included (7,9). However, the first GWA studies of MDD as well as those of other psychiatric phenotypes have also shown that genome-wide significant findings are rare and explain a small part of heritability (7,10-11). This might be due to a lack of power. The Genetic Association Information Network (GAIN)-MDD GWA study, for example, including ~1700 cases and 1800 controls, had 80% statistical power to detect relative risks of 1.59, 1.40 and 1.35 with a *P*-value of 1×10^{-7}, for minor allele frequencies of 0.10, 0.25 and 0.40. This is well comparable to other first generation GWA studies. However, the first results of GWA studies suggest that the strongest odds ratios may be < 1.2 (12). Another explanation for the scarce genome-wide significant findings could be that there is not a distinct number of genes for MDD with moderate-to-small risks but rather a large number of variations spread over the genome, each with small effects. Such a polygenic model predicts that the more markers are used, the better the disease is predicted and it implies that everybody carries risk variants but patients carry more than non-diseased people. We examined if a polygenetic component influences MDD implying that a large number of genetic variants are involved in explaining its heritability.

The evidence for a polygenic origin has recently been examined for schizophrenia and

the hypothesis of a polygenic component was directly tested using GWA data (13). In this approach, the joint effect of multiple single-nucleotide polymorphisms (SNPs) is tested rather than the effects of individual SNPs. These individual SNPs are not required to reach a genome-wide significance level by themselves. This approach aims to test whether the genetic disease liability reflects, at least in part, the additive effect of a large number of variants spread across the genome whose joint action may be captured in a genome-wide genetic risk score. To obtain this risk score, a discovery set is used to select SNPs based on specific *P*-value thresholds (for example, 0.0001, 0.001, 0.01 and so on) from a genome-wide scan for the disease of interest. In the target samples, genetic risk scores are calculated for each individual for each set of SNPs. The selected SNPs will contain false positives but if they are enriched with true-associated variants with low effect size then the genetic risk score might still be significantly associated with the disease in an independent sample. The problem is to distinguish truly associated SNPs from the false positives, which occur massively around liberal *P*-value thresholds. In the schizophrenia study, the genetic risk scores based on the multiple SNPs in the discovery sample were associated to schizophrenia in three independent samples. The variance explained by the risk scores increased as more SNPs were included, that is, risk scores based on SNPs that had a *P*-value below 0.5 in the discovery sample explained more variance in the replication sample than the risk scores based on SNPs selected at *P*-value below 0.1 (13). Moreover, the genetic risk scores for schizophrenia were also significantly associated with bipolar disorder assessed in two samples suggesting that the genes influencing schizophrenia and bipolar disorder partly overlap.

This study applied the risk score approach to investigate whether a polygenic component can be detected for depression and whether this polygenic component also influences anxiety. As the study samples differ in age, differences in the effect of the polygenic component may indicate that the genetic factors influencing depression and/or anxiety differ across the lifespan. Twin studies have already shown that the relative influence of genetic factors for depression decreases with age (14-16), but that the genes influencing depression remain the same across the years (17). This can be investigated directly in this study.

The discovery set consisted of the GAIN-MDD sample, including 1738 cases and 1802 controls (7,18) with over 400,000 SNPs genotyped. The target sets consisted of two independent Dutch samples. The first sample was based on the Rotterdam Study cohort and consisted of 178 depressive disorder patients diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) and 915 controls at low liability of depression as well as 222 cases for anxiety and 290 controls at low liability for anxiety. The second target sample was the Erasmus Rucphen Family (ERF) study in which symptoms of anxiety and depression were measured in 1886 participants. The

subjects in the GAIN-MDD sample and the ERF sample were around 45 years of age. The Rotterdam sample was an elderly sample with a mean age of around 70. Height and intraocular pressure (IOP), phenotypes unrelated to psychiatric disorders, were additionally investigated to examine if the association with the genetic risk scores was specific to depression and anxiety.

Material and methods

Discovery sample

The discovery sample consisted of subjects from two large-scale longitudinal studies: the Netherlands Study of Depression and Anxiety (19) and the Netherlands Twin Registry (20). The chances of success of genetic risk score analyses depend primarily on the size of the discovery or training set. If the sample size is too small, the risk profiles will be based on random noise and are not expected to explain variance in the target set. To increase the chances of success, the power of the discovery set should therefore be maximized (21). The size of the GAIN-MDD study made it more suitable to be used as the discovery set than the Rotterdam and ERF studies, which thus supplied the target samples.

The Netherlands Study of Depression and Anxiety and the Netherlands Twin Registry were approved by the medical ethics committee of all participating institutes. Collection of the phenotype data and quality control of the genotype data as well as the statistical methods are described in detail elsewhere (7,18). In brief, inclusion criteria for MDD cases were a lifetime diagnosis of DSM-IV MDD (22) assessed with the Composite International Diagnostic Interview (23), age of 18-65 years and self-reported western European ancestry. Persons who were not fluent in Dutch and those with a primary diagnosis of schizophrenia or schizoaffective disorders, obsessive-compulsive disorder, bipolar disorder or severe substance use dependence were excluded. Inclusion criteria for control subjects were availability of biological samples and survey data with assessments of depression, anxiety and neuroticism, no report of MDD at any measurement occasion and low genetic liability for MDD based on the survey data. In addition, controls and their parents were required to have been born in the Netherlands or Western Europe. Only one control per family was selected. The cases and controls were carefully matched on age and sex.

The genotypic data used in the discovery sample were part of one of the six initial GAIN studies sponsored by the Foundation for the National Institutes of Health (NIH) (20). Individual genotyping was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays. The SNPs on these arrays were selected to tag common variation in the HapMap European and

Asian panels. Of the 3820 samples sent to Perlegen, genotypes were delivered for 3761 samples (98.5%) of which 3540 subjects passed quality controls and were available in the final analysis data set including 1738 MDD cases and 1802 controls. The SNP quality control process and the precautions against population stratification are detailed in Sullivan *et al.* (7). A total of 427,037 SNPs on chromosome 1 to chromosome 22 met all selection criteria and were included in the final association analyses, which were performed in PLINK (24).

Target samples

Rotterdam Study

The Rotterdam Study is a prospective cohort study in the district Ommoord of Rotterdam (25). In 1990, all inhabitants aged 55 years and over were invited and 7983 persons agreed to participate. The medical ethics committee of the Erasmus MC, Rotterdam, approved the study. Written informed consent was obtained from all participants.

Ascertainment of depressive symptoms and incident depressive disorders was described previously (26). Depression and anxiety symptoms were assessed with the Center for Epidemiologic Studies Depression Scale (CES-D) and Hospital Anxiety and Depression Scale (HADS). The CES-D scale consists of 20 items with scores ranging from 0 to 60. A score of 16 or higher on the CES-D is considered indicative of a depressive disorder. The HADS-Depression (HADS-D) and HADS-Anxiety (HADS-A) scales each consist of seven items with scores ranging from 0 to 21 with higher scores indicating more symptoms of depression. These questionnaires are valid and reliable self report measures of symptoms of depression (27-28) .The HADS was assessed during the second visit in a randomly selected subgroup of individuals (n = 2231). Depression was measured with the CES-D 3 times during the follow-up.

Among 7983 subjects who agreed to participate, 5974 were successfully genotyped, 524 died before depression screening and 747 did not participate in depression screening. In the remaining sample, 587 persons scoring higher than 16 on the CES-D in the third or fourth visit were invited for a semi-structured interview with the Present State Examination (29) by a clinician. In addition, general practitioner records and specialist letters were surveyed actively for the occurrence of depression. Furthermore, physicians conducted repeated interviews to assess self-reported depression in the interval period. This effort identified 178 persons with current DSM-IV defined depressive disorder (145 MDD, 15 dysthymia and 18 depression-not otherwise specified cases) and eligible genotype data. The control group consisted of 915 persons, who scored in the lowest quartile (CES-D = 0) on CES-D in the third visit (n = 3879) and who did not report any depressive symptoms during the follow up.

Anxiety disorders were assessed during the fourth visit in the total sample by trained

lay-interviewers who conducted a slightly adapted version of the Munich Composite International Diagnostic Interview to obtain DSM-IV diagnoses of generalized anxiety disorder, panic disorder, agoraphobia, social phobia and specific phobia. Obsessive-compulsive disorder and post-traumatic stress disorder were not included. The current sample is selected from the 2779 persons who had valid Munich Composite International Diagnostic Interview assessment and genotype data. Out of 2779, 222 persons were anxiety disorder cases. The control group consists of 290 persons who did not have any anxiety disorder and scored in the lowest quartile (HADS-A = 0) on the HADS-A measured in 1322 persons of the interviewed and sample with eligible genotype data during the second visit.

Genome-wide SNP data were available from the Illumina HumanHap550K (Illumina, Inc., San Diego, CA, USA) array for all cases and controls. Data were excluded based on call rate < 97.5%, sex mismatch, excess autosomal heterozygosity and outliers identified by the clustering analysis. MACH 1.0 software (v1.0.16) (30-31) was used to impute to ~2.54 million SNPs based on the HapMap CEU phased haplotypes (release 22). SNPs included in imputation met thresholds of minor allele frequency ≥ 1%, Hardy Weinberg Equilibrium (HWE) *P*-value ≥ 10^{-6} and SNP call rate ≥ 98.0%. GWA analysis of MDD was performed with Mach2Dat (logistic regression on allele dosage) using the GRIMP interface (30,32). Age and sex were included as covariates in the analysis.

ERF Study

The ERF study is part of the Genetic Research in Isolated Population program. The study population essentially consists of one extended family of descendents from 20 related couples who lived in the isolate between 1850 and 1900 and had at least six children baptized in the community church. The detailed information regarding ERF isolate can be found elsewhere (33-35). The medical ethical committee of the Erasmus MC, Rotterdam approved the study and informed consent was obtained from all participants.

Symptoms of depression and anxiety were assessed using the HADS and the CES-D (27-28) in 2385 participants who also underwent an extensive medical examination.

Data on height and IOP were collected during the medical examination. The height of participants was determined using a stadiometer and bilateral IOP measurements were performed using Goldmann applanation tonometry (36).

Among 2385 persons with phenotypes, high-density genotype data were available for 1886 subjects. The genotype data were available for this population on four different genotyping platforms which were Illumina 6k, Illumina 318K, Illumina 370K and Affymetrix 250K (Affymetrix, Inc., Santa Clara, CA, USA), which were merged and ~2.54×10^6 SNPs were imputed using MACH 1.0 software (v1.0.16) (30-31), using build 36 HapMap (release 22) CEU population as reference. Within each genotyping batch,

only SNPs showing a call rate > 98%, MAF > 1% and HWE *P*-value > 10^{-6} were used for imputations.

As the ERF study included related individuals, GWA analyses were performed using a mixed model by 'mmscore' option in GenABEL software (37), which combines the Family Based Score Test for Association (FASTA) method of Abecasis *et al.* (38), and kinship matrix estimated from genotyped SNPs (39).

Risk score profiling

The score profiling method tests the association of a genetic score variable that reflects a combined effect of a number of selected SNPs with a trait. For a more detailed description of the method, we refer to Purcell *et al.* (13). In brief, SNPs were selected using the results from the GAIN-MDD GWA study (7) (the 'discovery sample'). These sets of SNPs were used to calculate the genetic scores in the target samples. SNPs were selected on the basis of their nominal *P*-value ($P_{discovery}$) for association with MDD in the discovery sample. Genetic risk scores were calculated for $P_{discovery}$ thresholds ranging from 0.00001 to 1.0.

Only those SNPs were included that were directly genotyped in the discovery set (n = 427,049 SNPs). To avoid ambiguity A/T – G/C SNPs were excluded. As an A to T or G to C change will result in the same nucleotides on the opposite strands, this change might be missed during the genotype analysis. SNPs for which the quality of imputation had an $R^2 < 0.95$ in target samples were also excluded. After all quality checks and exclusions, a total of 181,582 SNPs that were available in both ERF and Rotterdam Study samples were selected for calculations of genetic risk scores.

For each individual in the two independent target samples, the genetic score was calculated by multiplying the number of risk alleles per SNP (0, 1 or 2) with the log odds ratio, summed over all SNPs in the considered set of SNPs (40). We calculated individual scores for each set of SNPs using the PLINK (v1.06) software (24).

Logistic regression models were used to test the association of the individual genetic risk scores for depressive and anxiety disorders. Linear regression models were used to test the association between genetic risk scores and the total CES-D, HADS-D and HADS-A scores as well as for height and IOP. Sex and age were used as covariates. As an alternative control for false positivity, 10% of the non-associated cluster of SNPs with $P_{discovery} > 0.9$ (n = 1569 SNPs) in the discovery set was selected and used for computing the risk profile in both target samples.

For the Rotterdam Study regression analyses were performed in SPSS 15.0 for Windows (SPSS, Chicago, IL, USA). As the ERF sample includes relatives, data are not independent, which can lead to biased estimates of standard errors and test statistics if this dependency between measures is not taken into account (41). Association analysis

of genetic risk score and the traits in ERF population were performed in Sequential Oligogenic Linkage Analysis Routines 4.1.5 software package (Southwest Foundation for Biomedical Research, San Antonio, TX, USA) (42) using the 'polygenic' option to adjust for pedigree kinship. Among 1886 people both genotyped and phenotyped, 1697 were clustered into pedigrees (using the pedigree splitting algorithm PedCut (43)) and included in the family-based analysis. The remaining persons were not included in the analysis because they were also (distantly) related. The difference of the explained variance in the null and alternative model was considered as the variance explained by the genetic score. A genetic risk score with a *P*-value < 0.05 in the model was considered as significantly associated with the trait.

Results

Table 1 shows the descriptive data for the case-control studies of GAIN-MDD and the Rotterdam Study, and Table 2 for the ERF study. As in the Rotterdam study subjects were ascertained on the basis of age 55 or more, the mean age was 74 years. This was higher than in the GAIN-MDD and ERF study in which the mean ages were around 45 years. Level of education was higher in the GAIN-MDD sample than in the other two samples. In the target samples, subjects diagnosed with a depressive disorder or an anxiety disorder were more often women and were older. In the discovery sample, cases and controls were matched based on age and sex.

In the ERF study, CES-D, HADS-D and HADS-A scores were highly correlated ($r \sim 0.7$ pair wise for all three). Figure 1a shows the variance explained by the genetic risk scores in the logistic regression analyses performed in the Rotterdam Study using depressive disorder as dependent variable. The genetic score based on the first cluster of six SNPs ($P_{discovery} < 0.00001$) significantly explained 0.66% of the variance in depressive disorder in the Rotterdam Study (*P*-value = 0.03). This association is explained in large part by a cluster of three SNPs (rs2715148, rs2522833, rs2522840) in the *PCLO* gene as after removing the PCLO SNPs in linkage disequilibrium (LD), the risk score was not significantly associated with depression in the target sample anymore. More importantly, the scores based on SNPs with $P_{discovery} < 0.1$ to $P_{discovery} < 0.4$ were associated with depressive disorder in the Rotterdam Study explaining up to 0.65% of the variance, with a *P*-value < 0.05. As shown in Figure 1b, the Rotterdam Study anxiety disorder case-control sample analysis yielded the highest percentage of variance explained with the genetic risk scores from GAIN–MDD study. The risk scores based on SNPs with $P_{discovery} < 0.1$ to $P_{discovery} < 1.0$ significantly explained up to 2.1% of the variance (*P*-value = 0.0025). For $P_{discovery}$ values of 0.1, 0.2 and 0.3, the percentage of variance increased from 1 to 2% when a higher number of SNPs were included in risk scoring.

Table 1 Descriptive data of case-control samples

	GAIN-MDD		Rotterdam Study DD		Rotterdam Study AD	
	Cases (n = 1738)	Controls (n = 1802)	Cases (n = 178)	Controls (n = 915)	Cases (n = 222)	Controls (n = 290)
Age, mean (sd)	42.6 (12.6)	45.1 (14.1)	67.7 (6.8)[a]	64.8 (6.5)[a]	75.4 (5.82)	74.6 (5.32)
Women (%)	69.6	62	75.7	43.5	78	43
Education (%)						
Elementary	7.8	5.7	41.8	22.7	37.2	19.4
Intermediate	62	56.3	53.7	64.5	56.4	64.6
Higher	32.2	38.1	4.5	12.8	6.4	16.0
Antidepressant medication (%)	34.5	0	12.4[a]	0[a]	12.1	2
Comorbid AD/MDD (%)	69.9	0	35.8[b]	3.6[c]	10.8	0.7

AD, anxiety disorder; DD, depressive disorder; GAIN, Genetic Association Information Network; MDD, major depressive disorder; sd, standard deviation.
[a]Recorded at baseline. [b]Data available in 108 out of 178 cases. [c]Data available in 701 of 915 controls.

Table 2 Descriptive data of ERF study

	Mean (sd)
CES-D	10.6 (9.6)
HADS-D	5.9 (4.3)
HADS-A	6.7(4.5)
Age	48.2 (14.7)
Women (%)	57.4
Education (%)	
Elementary	30.8
Intermediate	63.8
Higher	5.4
Antidepressant medication (%)	5.0

CES-D, Center for Epidemiologic Studies Depression Scale; ERF, Erasmus Rucphen Family; HADS-A, Hospital Anxiety and Depression Scale-Anxiety subscale; HADS-D, Hospital Anxiety and Depression Scale-Depression subscale; sd, standard deviation.

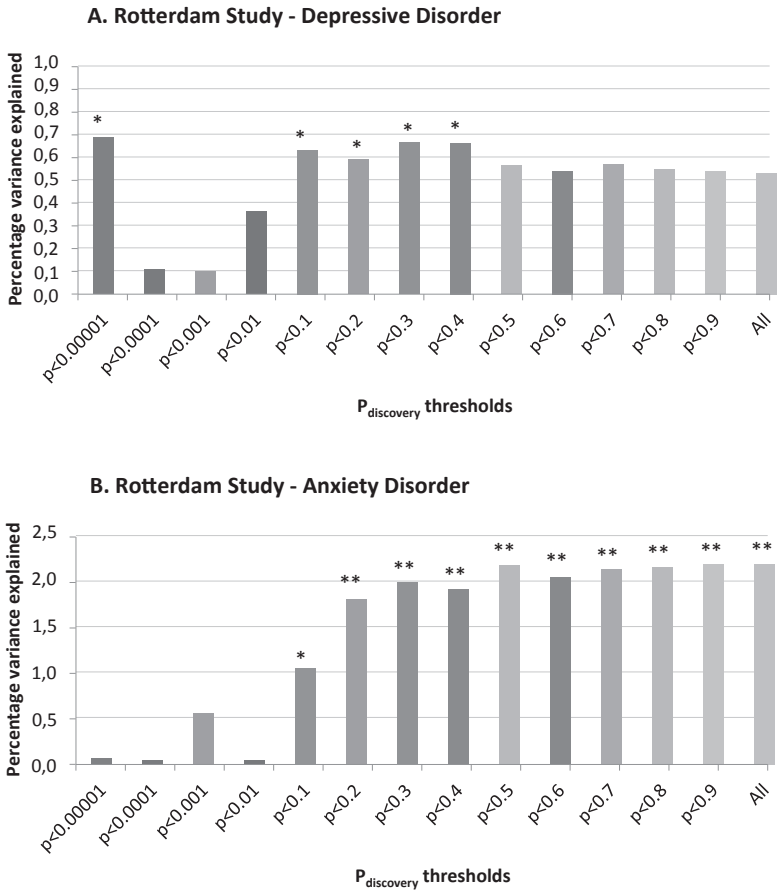

Figure 1 Percentage of variance explained by genetic risk scores in Rotterdam Study. Percentage of variance represented as difference in Nagelkerke R^2 after adjustment for age and sex. **(a)** Analyses based on comparison of MDD persons (n = 178) to persons scoring in the lowest quartile of CES-D (CES-D = 0) scale and who did not report any depressive complaints during the follow-up (n = 915). **(b)** Analyses based on comparison of persons with anxiety disorder (n = 222) to persons scoring in the lowest quartile of HADS-A (HADS-A = 0) scale and who did not report any depression or anxiety symptoms during the follow-up (n = 290). **P*-value < 0.05, ***P*-value < 0.01.

Figures 2a-c show the linear regression results for the analysis using the continuous scores on the CES-D, HADS-D and HADS-A in the ERF study. For CES-D, the scores based on SNPs with $P_{discovery}$ values < 0.01 and 0.1 explained ~0.5% of the variance (*P*-value = 0.007 and *P*-value = 0.008). For the HADS-D, the score based on SNPs with $P_{discovery}$ < 0.01 significantly explained 0.3% of the variance (*P*-value = 0.03). The MDD-based genetic score was also significantly associated with anxiety measured with the HADS-A explaining up to 0.5% of the variance (*P*-value = 0.01).

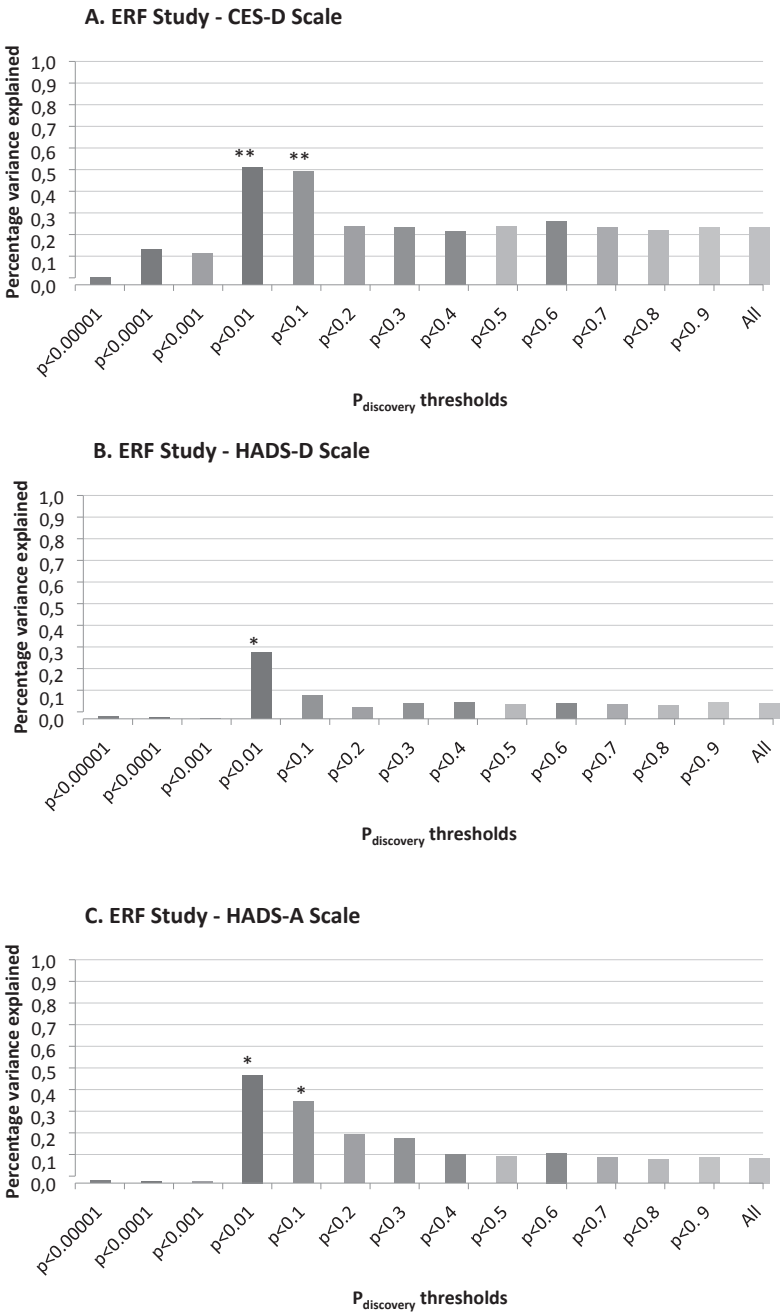

Figure 2 Percentage of variance explained in depression and anxiety symptoms in ERF study by the genetic risk scores. Percentage of variance represented as difference in R^2 after adjusting for age, sex and family relations. **(a-c),** analyses of continuous scales. **P*-value < 0.05, ***P*-value < 0.01.

To examine whether these results were due to chance, we tested whether the MDD-based genetic risk score predict also variation in height and IOP measured in ERF. Heritability of IOP was 35% and 86% for height (36,44) and none of the traits was correlated to depression or anxiety in the ERF study. The genetic risk score for MDD failed to predict IOP and height (Figure 3a and b) suggesting that this relation is specific to depression and anxiety. Moreover, a genetic score of SNPs with $P_{discovery} < 0.9$ in the discovery set did not show significant association with any of the phenotypes in the target samples (data not shown).

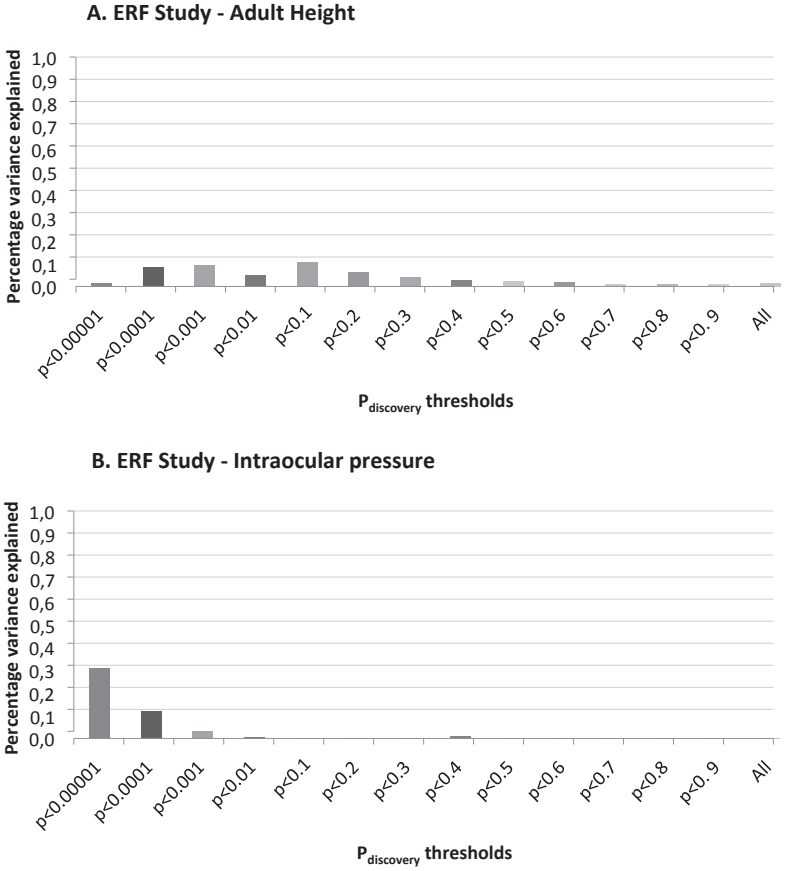

Figure 3 Predicting height **(a)** and intraocular pressure **(b)** in ERF study. Linear regression analysis of height and intraocular pressure. Percentage of variance represented as difference in R^2 after adjusting for age, sex and family relations.

Discussion

The aim of this study was to investigate the genetic architecture of depression and the potential overlap in genetic risk factors with anxiety. Owing to the availability of an elderly cohort, it was also possible to examine whether the genetic factors influencing anxiety and depression change across the life span. Using genetic risk scores derived from the association results of the GAIN-MDD study in two independent target samples, we evaluated the evidence for a genome-wide signature for several measures of depression and anxiety used as outcome variables in the target samples. For depression, either diagnosed according to the DSM-IV or measured with the CES-D or HADS-D, we could explain up to ~1% of the variance with the genetic risk scoring approach. Moreover, the genetic risk scores for depression were also associated with anxiety explaining up to 2.1% of the variance when approximately half of the genome-wide SNP data were included in the score. The explained variance was highest in the elderly sample indicating that the genetic factors influencing anxiety and depression hardly change with age. No significant results were found for the control variables height and IOP, implying that the association of the genetic risk score with depression and anxiety does not reflect chance alone. Overall, these findings suggest the presence of many loci, each with a small effect influencing depression as well as anxiety.

We checked whether our results were only because of SNPs in high LD segregating together. We performed a strict LD pruning (200 SNPs sliding window with $r^2_{snp\text{-}snp}$ threshold of 0.25). Considering the CES-D scale in ERF study, percentage of variance explained by the risk scores based on SNPs with $P_{discovery} < 0.01$ dropped slightly after LD pruning from 0.52 to 0.49 but remained significant (*P*-value = 0.01) whereas a less strict pruning with an $r^2_{snp\text{-}snp}$ threshold of 0.50 improved the percentage of explained variance to 0.62 (*P*-value = 0.0003). Results with HADS-A and HADS-D scales were similar which shows that LD pruning itself does not add a major difference to the method (data not shown). Excluding the SNPs with minor allele frequency < 0.05 did not change the explained variance. This finding suggests the common disease common variant hypothesis is explaining MDD heritability, on the other hand, the power to detect the effect of rare variants in the discovery and target sets was low and such rare variants may be detected by other approaches such as linkage or deep sequencing.

Our results are in agreement with the results from the International Schizophrenia Consortium (13) that pointed out a polygenic component influencing schizophrenia as well as bipolar disorder. There was a somewhat higher amount of explained variance for schizophrenia (3.2% compared with 1%). This may be due to power issues such as differences in sample size (~3300 cases for International Schizophrenia Consortium vs ~1800 cases for GAIN-MDD), MDD being a common disease with clear non-genetic influence because of life events, and lower heritability compared with schizophrenia (~40 vs ~80%).

The percentage of explained variance in anxiety (2.1%), supports the idea of shared genetic background between these disorders. This has already been suggested by twin studies (45) and is confirmed by our results. The trend of increase in R^2 for anxiety with different $P_{discovery}$ thresholds is different from the trend that we observe in depression, pointing out that the effect sizes are different, but the direction of effect is the same. We would like to stress that difference in explained variance between the target samples can evenly well be explained by chance. Moreover, it is important to note that 70% of the GAIN-MDD cases had a comorbid lifetime anxiety diagnosis. However, this high comorbidity is exactly what is expected if two disorders are influenced by similar genes and diagnoses are not mutually exclusive. Future research, preferably with a more balanced proportion of pure depressed and comorbid cases, can shed more light on the overlap in genetic factors influencing anxiety and depression.

A limitation of this study was that there were some differences between the discovery set and the target samples. Different instruments were used to measure depression and anxiety. In the GAIN–MDD study, the Composite International Diagnostic Interview was used to diagnose MDD and anxiety disorders, while in the Rotterdam study, the Present State Examination (PSE) was used. However, both instruments aim to make diagnoses according to the DSM-IV criteria and have adequate agreement for overall syndromes (46). In the ERF population, symptoms of depression and anxiety were measured using the CES-D and HADS. Several validation studies on various types of patients using different diagnostic tools have shown that HADS performs well in assessing the symptom severity and case status of anxiety disorders and depression in both somatic, psychiatric and primary care patients and in the general population (47). The HADS-D subscale has shown high sensitivity (~0.9) and specificity (~0.7) for MDD as diagnosed by DSM-IV in various studies (48). The CES-D scale was found to be satisfactory in a semi-clinical sample of the elderly and in general population (sensitivity = 0.9 and specificity = 0.6) for life-time MDD and also performed excellent for 1 month of prevalence of MDD as diagnosed by DSM-IV (sensitivity = 1.0 and specificity = 0.9) among elderly Dutch (49-51). Considering the HADS-A subscale, the sensitivity and specificity for DSM-IV generalized anxiety disorder was reported to be 0.9 and 0.8, respectively (52). In addition, the discovery set in this study included lifetime MDD cases, whereas the Rotterdam Study recorded depressive disorders during a 9-year follow-up rather than lifetime MDD. Similarly, CES-D and HADS measure depressive and anxious symptoms in the last week. This means that subjects in the control groups in the target samples may be non-current but life-time MDD or anxiety cases. To summarize, although the measurements of anxiety and depression used in the three study samples are definitely related to each other, the fact that they are not entirely similar implies some heterogeneity, biasing the results toward the null hypothesis.

Another point involves the difference in gender ratios between discovery and target samples. In the discovery sample, the cases and controls were carefully matched on age and sex. Meta-analysis of twin studies suggests that genetic factors that influence depression are mostly shared between men and women (1,53-54). Sex was also used as a covariate when predicting depression or anxiety in the target samples. Thus, it seems unlikely that the gender ratio may have a major effect in the replication of the findings. There was also heterogeneity in education level as a measure of socioeconomical status. In spite of these differences, we still found a significant effect of the genetic risk score suggesting that the effects of the risk scores are actually even stronger. In both the International Schizophrenia Consortium study and the current studies, the low variance explained compared with the heritability of the disorders will also reflect that the analyses did not include the X chromosome, that gene-gene or gene-environment interactions are not considered and that the current generation of genotyping platforms do not fully tag genomic variance (55).

This study is the second study showing direct evidence for a polygenic component influencing the susceptibility for a psychiatric disorder as well as overlap in genetic risk factors with another psychiatric condition. In addition, this study suggests that the genetic factors influencing anxiety and depression hardly change with age. The results imply that causal SNPs or the SNPs in LD with such variants do exist, but have lower effect sizes than the first generation of GWA studies on psychiatric disorders was powered to detect. This provides optimism that variants associated at genome-wide levels of significance will be detectable as sample sizes increase in the next generation of GWA studies and their meta/mega analyses. Moreover, it confirms that genome-wide profiling is a useful approach to analyze the genetic architecture of disorders, that is, similarities and differences in genetic factors influencing several disorders or influencing the same disorder across the lifespan.

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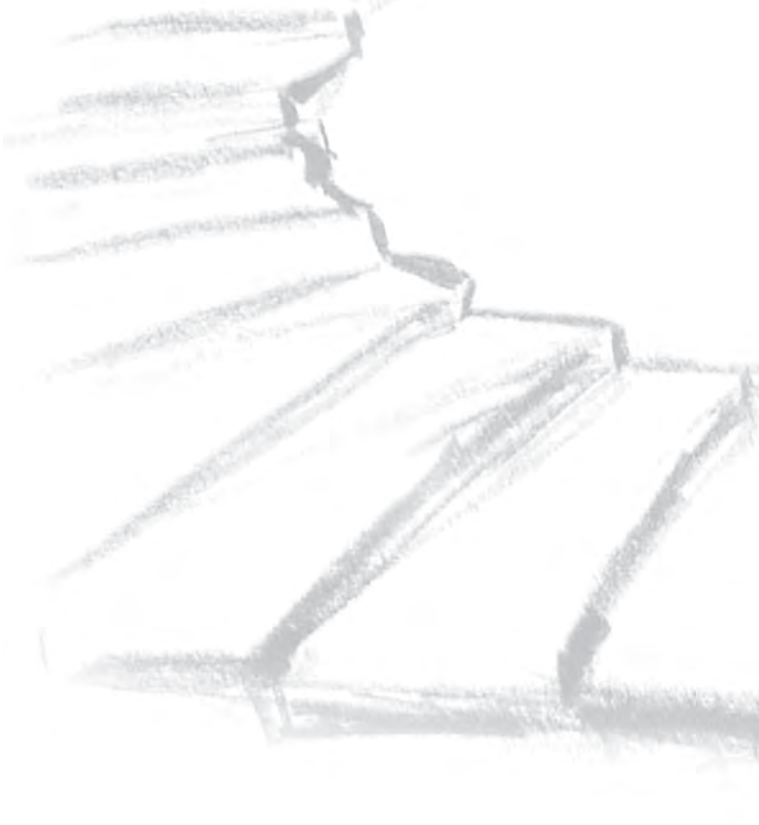

Chapter 9

Obesity and incident depression: findings from conventional and Mendelian randomization analyses

Hendrika J. Luijendijk, Karin Hek, M. Maria Glymour,
Albert Hofman, Henning Tiemeier, Xander Koolman

Abstract

Context: Observational studies show that obesity is associated with increased risk of depressive symptoms. However, this association may be due to unmeasured confounding by health behaviours or past depression, rather than causal effects of obesity on depression. **Objective:** To obtain unconfounded estimates of the effect of obesity on incident depression. **Design, setting and participants:** Data were collected between 1993 and 2005 in a cohort of elderly Rotterdam residents. We performed conventional logistic regression analyses (n = 4841), and Mendelian randomization analyses using genes that influence obesity as natural experiments (n = 4529). **Data assessment methods:** Obesity was defined as a body mass index ≥ 30 kg/m^2 at baseline. Depressions were identified through (1) 4-yearly examinations with Center for Epidemiologic Studies Depression scale and psychiatric interviews, (2) continuous monitoring of medical records, and (3) self-reported past depression. Depressions were categorized as depressive symptoms (Center for Epidemiologic Studies Depression scale score ≥ 16), or clinical depression (physician diagnosed and DSM-IV defined depressions). From previous meta-analyses of genome-wide analyses, we identified 19 single-nucleotide polymorphisms in 16 genes that predict body mass index. **Main Outcome Measure:** Multivariate logistic regression analyses yielded odds ratios of incident depression for obese versus normal weight participants. Probit regression models with continuous endogenous regressors provided Mendelian randomization-adjusted risk differences per 5 units increase in body mass index. **Results:** In conventional analyses, body mass index ≥ 30 was associated with an increased odds of depressive symptoms (OR 1.50; 95% CI, 1.13, 1.98) and clinical depression (OR 1.31; 95% CI, 0.98, 1.77). However, Mendelian randomization analysis indicated that a 5 unit increase of body mass index was associated with a 10% decreased risk of depressive symptoms (RD -0.10; 95% CI, -0.30, 0.01) and clinical depression (RD -0.10; 95% CI, -0.32, -0.01). **Conclusion:** Contrary to conventional analyses, our Mendelian randomization results suggest that obesity does not increase the risk of incident depression. Rather, individuals with a higher body mass index caused by genetic factors may be at lower risk of depression.

Introduction

Over the last three decades, the association between obesity and depression has been studied in numerous observational studies (1-2). Obesity, usually defined as a body mass index (BMI) of 30 kg/m^2 or over, is assumed to cause depression through its negative effects on self-image (3), or through its somatic outcomes, such as diabetes and cardiovascular disease (4-6).

Cross-sectional studies have reported an increased risk of depression in obese patients, with odds ratios (OR) varying between 1.3 and 2.0 (5,7-9). A meta-analysis of 17 studies in general populations showed a somewhat lower but still statistically significant odds of 1.2 (1). Moreover, in one study severe obesity (BMI ≥ 40) was associated with a more than four-fold increased risk of major depression (10). In 2010, a meta-analysis of longitudinal studies confirmed these results (2). The meta-analysis showed that obesity, and to a lesser extent overweight, was associated with an increased risk of depressive symptoms during follow-up (unadjusted OR of 1.6 and 1.3 respectively). For the obese, the risk of clinical depression was even higher (unadjusted OR 2.2).

If, and only if, these associations could be causally interpreted, interventions that effectively prevent or reduce obesity would be expected to diminish the risk of depression. However, bias is always a threat to causal inference in observational studies. For instance, as depression may give rise to obesity through dysregulated stress systems or unhealthy lifestyles, confounding by past depression (reverse causation) may have affected the results of cross-sectional studies (11-12). Most longitudinal studies did not differentiate depression at baseline from depressions occurring during follow-up (2,13), or did not adjust for past depression (14-15). In addition, likely confounders such as socioeconomic status, diet, alcohol intake, exercise, disability, or chronic diseases have often been omitted in observational studies.

Mendelian randomization (MR) is a comparatively new technique intended to eliminate bias due to confounding (16-17). This can be especially useful when the confounders are not all measured or measured with error. MR uses genes as instrumental variables, taking advantage of the fact that genes are randomly passed on from parents to offspring (Figure 1).

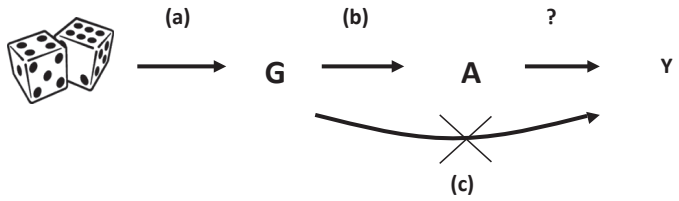

Figure 1 Assumptions of Mendelian randomization. Mendelian randomization requires that gene G is (a) randomly distributed among people, (b) robustly predicts exposure A, and (c) does not predict outcome Y except through exposure A in order to assess the unconfounded effect of exposure A on outcome Y.

Because of this randomization at meiosis, the estimates of the effects of genes on phenotypes such as BMI are unbiased. Similarly, estimates of the effects of genetically determined differences in BMI on outcomes such as depression may also be unbiased. Recently, two studies were published that used MR to assess the effect of obesity on psychological health, showing qualitatively opposite results. In the Whitehall study, genes predicting long-standing obesity predicted higher risk of symptoms of anxiety or depression; this finding is consistent with the majority of evidence from conventional studies (18). However, in a much larger Danish general-population study, genes associated with obesity predicted decreased risk of psychological distress, contradicting previous evidence and suggesting that obesity may be associated with improved mental health (19). As both studies used screening instruments to assess endpoints, it is questionable whether the findings can be generalized to clinical depression.

The aim of the current study was to assess the effect of obesity on incident depression in a general population using both a conventional multivariate analysis and an MR analysis to minimize bias. The Rotterdam Study data were very suitable for these analyses, because they include detailed information on incident Center for Epidemiologic Studies Depression scale (CES-D) and depression diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (20), anthropomorphic characteristics, potential confounders and genetic profiles.

Methods

Setting

This study was embedded in the Rotterdam Study, a prospective study that started in 1990 among 7983 inhabitants of Ommoord, a district of Rotterdam (21). The study focuses on the occurrence and determinants of chronic diseases in the elderly. Participants were 55 years of age or older and 98.5% is Caucasian. The medical ethics committee of Erasmus Medical Center, Rotterdam approved the study and written informed consent was obtained from all participants. So far, five examination rounds have taken place, during which participants underwent an extensive interview and a physical examination, and blood was drawn. In addition, continuous monitoring for major events such as depression took place through automated linkage with the medical files from the general practitioners (GPs) from baseline onwards. The Dutch health care system requires all residents to be registered with a GP, and clinicians report back to the GP. Information on vital status was obtained bimonthly from the municipal authorities in Rotterdam.

Study populations for conventional and MR analyses

During the second examination round of the Rotterdam Study, the baseline for the current analysis, participants were screened for depressive symptoms for the first time. They filled out the validated Dutch version of the CES-D, or the Hospital Anxiety and Depression Scale (22-23). Persons with a score of 16 or higher on the first scale or 9 or higher on the latter were considered screen-positive. Of the 5769 participants screened, we excluded 105 persons with dementia and 9 persons with bipolar disorder at baseline. For the conventional analysis, we additionally excluded persons with depressive symptoms at baseline (549), persons lost to follow-up immediately after screening (2), and persons with BMI < 18.5 (underweight, 33) or without BMI measurement (230), resulting in a sample for analysis of 4841 persons. For the MR study, we included persons with information on depression at baseline (549) and during follow-up (5104), but excluded persons with no information about gene status (1047), no BMI measurement (39), or BMI < 18.5 (38), which resulted in a sample for analysis of 4529 persons.

Assessment of depression

At baseline, participants were screened for depressive symptoms as described above. Assessment of incident depressions has been described in detail before (24). Information was obtained from (1) psychiatric examinations, (2) self-reported past depression, and (3) medical records. The psychiatric examination during 4-yearly examination rounds consisted of a screening with the CES-D. Subsequently, a trained clinician conducted a semi-structured interview (Schedules for Clinical Assessment in Neuropsychiatry) in the screen-positive participants to obtain DSM-IV defined diagnoses (25). Past depressions were solicited during examination rounds with standardized questions about whether and when participants had suffered from a depressive episode, and if so whether they had been treated. Trained research assistants reviewed the GPs' medical records and copied the information about a potential depression. Two research physicians independently assessed this information according to a predefined protocol, and discussed discordant assessments.

Based on these sources, we categorized a depression as (1) depressive symptoms, if a participant scored 16 or higher on the CES-D, or (2) clinical depression, including DSM-IV defined major depressive disorder, dysthymia, and minor depression diagnosed by a psychiatrist or other mental health professional, depressions recorded by a GP, and self-reported depression for which the participant had consulted a GP or mental health professional. Past depressions were assessed with self-reported histories and medical records, and categorized with the same criteria that were used for incident depressions.

Measures of obesity

Our primary analyses are based on obesity defined by BMI, which was calculated as weight in kilos divided by height in meters squared. Height and body weight were measured with participants standing in light clothes, without shoes, and with emptied pockets. In addition, we performed secondary analyses in which obesity was defined as waist circumference (WC). WC is often a more accurate predictor of cardiovascular outcomes than BMI (26-27), and seemed to be more associated with depressive symptoms in men than measures of general body fat (13). WC was measured in centimeters at the level midway between the lower rib margin and the iliac crest with the participant standing and breathing out gently.

Genotyping and selection of single-nucleotide polymorphisms for analysis

We identified genes that predict either BMI or WC in Caucasian adults from meta-analyses of genome-wide-analyses presented on the continuously updated digital knowledge base Huge Navigator (28-29). We found 26 single-nucleotide polymorphisms (SNPs) on 16 different loci including FTO and MC4R that predicted BMI or WC in Caucasian populations (30-43) (Table 1). The data for 24 of these SNPs (19 for BMI and 5 for WC) were available for the participants of the Rotterdam Study.

Genotyping was performed on DNA isolated from the blood taken during the baseline examination round. Genotypes were determined with the Infinium HumanHap550K Genotyping Bead Chip version 3 (Illumina, Inc. San Diego, CA, USA). MACH 1.0 software (v1.0.16) was used to impute to ~2.5 million autosomal SNPs based on HapMap CEU release 22 (44). All SNPs had good imputation quality (observed to expected variance ratio > 0.90).

Covariates

The following baseline characteristics were considered as potential confounders: age, sex, socioeconomic status, past depression, smoking, and alcohol use. We did not include disability, hypertension, history of ischemic cardiovascular disease, heart failure, and stroke, because these factors, although related to obesity at baseline, are not risk factors but most likely consequences of obesity in the elderly (45-46). Socioeconomic status was determined in terms of highest education attained and net income (47). Self-reported smoking and alcohol use were categorized into never, former, and current.

Statistical analyses

First, we performed conventional analyses using logistic regression models for the outcomes incident depressive symptoms and incident clinical depression. In line with previous studies, the exposure variable BMI was entered as a categorical variable: $18.5 \leq \text{BMI} < 25$ kg/m^2 (normal weight), $25 \leq \text{BMI} < 30$ kg/m^2 (overweight), $\text{BMI} \geq 30$ kg/m^2 (obese). Unadjusted

and multivariate models including the confounders mentioned above were fitted for each outcome. In all analyses, participants were followed up until incidence of depression, dementia, death, loss-to-follow-up, or the end of the study on October 1, 2005.

We also performed MR analyses to assess the unconfounded effect of obesity on the risk of depression. Under three assumptions, genetic alleles with known effects on the exposure can be used to estimate unconfounded exposure–outcome associations (Figure 1). A Mendelian randomization analysis requires the identification of a genetic variant that is (a) randomly distributed among people, that is, does not share a common cause with the outcome, (b) has a robust causal effect on the exposure of interest, and (c) does not affect the outcome except through this exposure (16-17,48-49). It is generally assumed that genetic alleles are randomly assigned from the parents to the offspring at the time of gamete formation (Mendel's Second Law) (50).

Table 1 Single-nucleotide polymorphisms that predict body mass index and waist circumference in meta-analyses of genome-wide association studies

Locus	SNP	Phenotype	Availability	Study
FTO	rs9939609	BMI	Y	Frayling 2007 (30)
	rs9930506	BMI	Y	Scuteri 2007 (31)
	rs10146997	BMI	Y	Willer 2009 (32)
	rs1558902	WC	Y	Heard-Costa 2009 (33)
MC4R	rs2229616	BMI	Y	Geller 2004 (34), Heid 2005 (35), Stutzmann 2007 (36), Young 2007 (37)
	rs17782313	BMI	Y	Loos 2008 (38), Grant 2009 (39), Willer 2009 (32)
	1251L	BMI	N	Stutzmann 2007 (36)
	rs489693	WC	Y	Heard-Costa 2009 (33)
TNF	rs1800629	BMI	N	Sookoian 2005 (40)
SH2B1	rs9931989	BMI	Y	Willer 2009 (32)
	rs7498665	BMI	Y	Willer 2009 (32)
NEGR1	rs2815752	BMI	Y	Willer 2009 (32)
MTCH2	rs4752856	BMI	Y	Willer 2009 (32)
	rs10838738	BMI	Y	Willer 2009 (32)
TMEM18	rs6548238	BMI	Y	Willer 2009 (32)
GNPDA2	rs10938397	BMI	Y	Willer 2009 (32)
KCTD15	rs415237	BMI	Y	Willer 2009 (32)
	rs11084753	BMI	Y	Willer 2009 (32)
CTNNBL1	rs6013029	BMI	Y	Willer 2009 (32)
	rs6020846	BMI	Y	Willer 2009 (32)
SDCCAG8	rs12145833	BMI	Y	Scherag 2010 (41)
LP	rs4988235	BMI	Y	Kettunen 2010 (42)
NRXN3	rs10146997	WC	Y	Heard-Costa 2009 (33)
TFAP2B	rs987237	WC	Y	Lindgren 2009 (43)
LYPLAL1	rs2605100[a]	WC	Y	Lindgren 2009 (43)
MSRA	rs7826222	WC	N	Lindgren 2009 (43)

BMI, body mass index; WC, waist circumference; SNP, single-nucleotide polymorphism.
[a]This SNP predicts waist circumference in females only.

We selected SNPs that were shown to robustly predict BMI in previously published genome-wide association (GWA) meta-analyses (assumption b). To test whether these SNPs predicted BMI in our study population, we performed a linear regression of BMI on the selected SNPs. We tested the joint significance of the genes using an F-test. Individual gene significance was not tested because our sample is much smaller than that used in the original meta-analyses and consequently lacks the required statistical power. We also checked whether the direction of the effects of the SNPs on BMI were similar to those in the meta-analyses. Next, we tested whether these combined SNPs predicted depression only through BMI (assumption c). To test this, we evaluated whether the combined SNPs predicted the outcome, first without BMI and then with BMI included in the model. Finally, we tested whether the SNPs were associated with income and education. Participants' income and education are likely to correlate with parents' income and education, which are potential confounders of the association between the selected SNPs and depression (assumption a). Associations were tested pairwise and weak instrument bias was assessed using first stage F-tests and by comparing results from our default model with a model based on a selection of SNPs with the highest combined F-statistic.

In the MR analysis, any depression, whether it had occurred before, at or after baseline can be and has been included without introducing confounding by past depression (51). Again, we used the outcomes depressive symptoms and clinical depression. We applied a control function approach with a first stage linear regression for continuous BMI on SNPs, and a second stage probit regression for depression measures on BMI and the residual from the linear equation (51). Results are presented in risk differences (RD) for increments of 5 BMI-points, computed as the average predicted difference of actual BMI +2.5 and actual BMI -2.5 using the empirical distribution of the sample. Statistical significance and confidence intervals were based on non-parametric bias-corrected bootstrap confidence intervals with 10,000 replications of the estimation procedure (52). Allele dosage was used in the analyses to take into account imputation uncertainty. The conventional and MR analyses were repeated in the subsample of persons with WC measurement (n = 4504 and n = 4241 respectively). WC was entered as a categorical variable in the conventional analyses: WC for males < 94 cm and females < 80 cm (normal weight), 94 ≥ WC for males < 102 cm and 80 ≥ WC for females > 88 cm (overweight), and WC male ≥ 102 cm and female ≥ 88 cm (obese). RDs were calculated in MR analyses for every 5 centimeter increase in WC. Two-sided *P*-values of < 0.05 were considered statistically significant. Statistical analyses were programmed in Stata 11.2.

Results

The baseline characteristics of the samples for the conventional and MR analysis are shown in Table 2. In the conventional sample, the mean age was 70 years and almost 57% of participants were female. At baseline, 32% of participants had had a past depression, 47% were overweight and 16% obese. The characteristics of the sample for the MR analysis were almost identical.

Table 2 Baseline characteristics of study samples for the conventional and Mendelian randomization (MR) analysis

Characteristic	Conventional study (n = 4841)	MR study (n = 4529)
Age, mean (sd)	69.7 (8.2)	69.7 (8.1)
Female sex, no (%)	2771 (57.2)	2612 (57.7)
Education, primary school only, no (%)	866 (17.9)	840 (18.5)
Income, median (range)	2706 (750-7000)	2670 (750-7000)
Disability, mean (range)	1.29 (1.00-4.00)	1.32 (1.00-4.00)
Past depression, no (%)	1562 (32.3)	1324 (32.2)
Smoking, no (%)		
never	1537 (31.8)	1421 (31.4)
past	2502 (51.7)	2334 (51.5)
current	802 (16.6)	774 (17.1)
Alcohol use, no (%)		
never	744 (15.4)	721 (15.9)
past	473 (9.8)	429 (9.5)
current	3624 (74.9)	3379 (74.6)
Hypertension, no (%)	1077 (22.3)	991 (21.9)
History of ischemic cardiovascular disease[a], no (%)	965 (19.9)	938 (20.7)
Heart failure, no (%)	195 (4.0)	184 (4.1)
Stroke, no (%)	133 (2.8)	122 (2.7)
Body mass index, mean (sd)	26.5 (3.6)	26.6 (3.6)
Body mass index, no (%)		
<25 (normal weight)	1765 (36.5)	1633 (36.1)
25-30 (overweight)	2296 (47.4)	2148 (47.4)
≥ 30 (obese)	780 (16.1)	748 (16.5)
Waist circumference, mean (sd)	90.8 (11.0)	90.9 (11.0)
Waist circumference, no (%)		
< 80 for women, < 94 for men (normal weight)	1511 (33.8)	1410 (33.6)
80-88 for women, 94-102 for men (overweight)	1448 (32.4)	1345 (32.0)
> 88 for women, > 102 for men (obese)	1504 (33.7)	1446 (34.4)

[a]This includes angina pectoris, claudicatio intermittens, history of myocardial infarction, coronary artery bypass graft, percutaneous transluminal coronary angioplasty and peripheral artery bypass graft.

In the 4841 persons free of depression at baseline, 397 clinical depressions occurred during an average follow-up period of 8 years (for WC study sample 375 clinical depressions). During the first follow-up visit, 7.3% of normal weight and 9.4% of obese

persons had a positive CES-D score (37.4 % and 38.2% DSM-IV defined depression respectively), and during the second follow-up visit, 11.9% of normal weight and 17.9% of obese persons (41.7% and 30.6% DSM-IV defined depression respectively). Table 3 presents the results of the conventional analyses. According to the unadjusted model, obesity (BMI ≥ 30) increased the odds of depressive symptoms (1.68; 95% CI 1.28, -2.19), and clinical depression (1.41; 95% CI 1.06, 1.87) during follow-up. With adjustment for confounders, the risk was still increased for depressive symptoms (1.50; 95% CI 1.13, 1.98), and for clinical depression (1.31; 95% CI 0.98, 1.77); a test showed that obesity was significantly associated with clinical depression when compared to the joint category for non-obesity and overweight. The unadjusted models with WC yielded similar odds ratios for both outcomes. However, after adjusting for confounders, the risk estimates were not significant anymore. Overweight, whether defined in terms of BMI or WC, was not associated with incident depression.

Next, we performed the MR analysis. When we tested the underlying assumptions, the SNPs proved jointly significant ($P < .01$) in predicting BMI and the signs for the coefficients of all single SNPs were identical between our first stage regression and the meta-analyses. The SNPs predicted depressive symptoms and clinical depression significantly ($P < .05$), but after adjustment for BMI, the SNPs were not associated with depressive symptoms or clinical depression ($P = .99$); these results are consistent with the assumption that the SNPs affect depressive symptoms exclusively via BMI. None of the SNPs were significantly related to the confounders. The RD of the model using all SNPs was similar to the RD estimate from the model selected to have the highest F-value for the association between the SNPs and BMI (RD: -.15 vs -.19; F-value: 2.2 vs 7.8). We used the model using all SNPs for optimal power. Results for WC showed a qualitatively similar picture.

Table 4 presents the results of the MR analysis. Obesity was negatively, but not statistically significantly, associated with depressive symptoms (RD -0.10; 95% CI -0.30, 0.01). Results for clinical depression were similar and statistically significant (RD -0.10; 95% CI -0.32, -0.01). Thus, every 5-point increase in BMI was associated with a 10% decrease in the risk of clinical depression. Results for the MR analyses using WC to define obesity showed a similar pattern.

Table 3 Obesity and incident depression: unadjusted and multivariate adjusted logistic regression models

	Depressive symptoms[a]				Clinical depression[b]			
	Unadjusted model		Adjusted model[c]		Unadjusted model		Adjusted model[c]	
	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value	OR (95%CI)	*P*-value
BMI in kg/m2 (n=4841)								
18.5 - 25	Reference group		Reference group		Reference group		Reference group	
25 - 30	1.16 (0.93, 1.45)	.19	1.19 (0.95, 1.50)	.12	0.93 (0.73, 1.17)	.52	0.95 (0.75, 1.20)	.66
>= 30	1.68 (1.28, 2.19)	.00	1.50 (1.13, 1.98)	.01	1.41 (1.06, 1.87)	.02	1.31 (0.98, 1.77)	.07
WC in cm (n=4505)								
M: < 94, F: < 80	Reference group		Reference group		Reference group		Reference group	
M: 94-102, F: 80-88	1.28 (0.99, 1.66)	.06	1.06 (0.92, 1.56)	.18	1.12 (0.85, 1.46)	.42	1.07 (0.81, 1.41)	.63
M: >= 102, F: >= 88	1.71 (1.33, 2.18)	.00	1.19 (1.06, 1.80)	.01	1.33 (1.03, 1.73)	.03	1.21 (0.92, 1.60)	.16

BMI, body mass index; WC, waist circumference; M, male; F, female.
[a]Incident positive Center of Epidemiologic Studies Depression scale score as outcome.
[b]Incident DSM-defined depressive disorder or physician-diagnosed depression.
[c]Adjusted for age, sex, socioeconomic status, smoking, alcohol use, and past depression.

Table 4 Obesity and risk of depression using Mendelian randomization-adjusted linear regression models to estimate risk differences (RD)

	Depressive symptoms[a]		Clinical depression[b]	
	RD	(95%CI)	RD	(95%CI)
BMI per increase of 5 kg/m^2 (n=4529)	-0.10	(-0.30, 0.01)	-0.10	(-0.32, -0.01)
WC per increase of 5 cm (n=4241)	-0.03	(-0.09, 0.01)	-0.11	(-0.32, 0.05)

BMI, body mass index; WC, waist circumference.
[a]Positive Center for Epidemiologic Studies Depression scale score at baseline (≥ 16) or during a follow-up examination.
[b]DSM-defined depressive disorder or physician-diagnosed depression before, at or after baseline.

Discussion

This study showed that with multivariate conventional analysis obesity (BMI ≥ 30) is associated with an increased risk of incident depression in the elderly. When adjusted for multiple confounders, the increased risk reduced but remained statistically significant. When applying MR to avoid bias from confounding, the estimated effect of BMI on depression was negative. In other words, individuals with a higher BMI caused by genetic factors were at lower risk of depression, suggesting that obesity was protective. Similar results were found when WC was used to define obesity.

Strengths of our study are the large study population and long follow-up time. In addition, to maximize the detection rate of past, baseline and incident clinical depression, we combined several assessment methods, including continuously monitoring of medical records. We adjusted for most known confounders, although not for diet and physical activity at baseline. To further reduce bias, we used MR analysis. If the underlying assumptions are met, MR analysis eliminates all confounding bias because people are randomly assigned to different genetic alleles at conception (50). For instance, MR rules out confounding by past depression (sometimes referred to as 'reverse causation bias') because genetic status is determined before depression could have developed. The MR analysis uses only the genetically determined variation in BMI to estimate the effect of BMI on depressive symptoms. In addition, as genetic status was used to estimate exposure status, information bias due to measurement error in the ascertainment of weight, height and WC was also circumvented (48). To the extent we were able to evaluate the assumptions underlying MR, we found evidence that the genes we used fulfilled these assumptions: the genes predicted BMI; they were unrelated to depression conditional on BMI; and they were unrelated to potential confounders of the gene-depression association.

In our conventional analyses, the unadjusted association between obesity (BMI ≥ 30) and new-onset depressive symptoms was 1.7. These results correspond with a meta-analysis of eight longitudinal studies that presented an unadjusted OR of 1.6 (2). When we adjusted for multiple confounders, the risk of depressive symptoms in our population was still significant (OR 1.5). Again, this finding concurs with the results of other prospective studies in elderly populations that all used self-reported depressive symptoms as outcome (13-15,53-55). In the Alameda country study, obesity conferred an odds of 2.1 for new-onset symptoms of major depression at 1-year follow-up (54). This result was reproduced with data from a 5-year follow-up (15). A significant effect was also found in elderly residents from North Carolina: each unit increase in BMI predicted 0.05 more depressive symptoms on the CES-D three years later (55). Like our study, these studies adjusted for multiple confounders, but not for diet and physical activity. Information on both of these confounders was used in an analysis of the Nurses'

Health Study data (14). The study found that obese women aged 54-79 had a slightly increased risk of new-onset depression or anti-depressant use (OR 1.10; CI 1.02-1.20). To our knowledge, only one study, performed in an American elderly population, showed no longitudinal association between BMI, or WC and new-onset self-reported depressive symptoms or anti-depressant use after adjustment for relevant confounders including physical activity (13).

When we focused on clinical depression, we also found that obesity conveyed an unadjusted odds of 1.4, and an adjusted odds of 1.3 compared to non-obesity. This risk estimate is similar to that of the only other study we are aware of that used clinical diagnoses: in elderly Australian men, BMI ≥ 30 had a statistically significant hazard ratio of 1.3 for depression compared to BMI < 30 (53). In the latter, like in our study, WC was not significantly associated with clinical depression. In sum, conventional analyses across different elderly populations, study designs and definitions of depression show that BMI ≥ 30 is associated with an increased risk of depression, although the risk decreases when more narrowly defined definitions of depressions are employed and relevant confounders are taken into account.

MR analyses, however, yield a different picture. We found that every 5-point predicted increase in BMI was associated with a 10% decrease in risk of clinical depression. To date, two MR studies about the effect of obesity on psychological health have been published. In middle-aged male participants of the Whitehall study, in general, obesity was not related with symptoms of depression or anxiety as measured with the General Health Questionnaire (18). An exception was that obesity during the majority of 19 years of follow-up was associated with concurrent symptoms of depression or anxiety. This finding corresponds with a validation study of the General Health Questionnaire showing that persons who screened false positive were likely to be distressed by severe physical illness or loneliness (56). Moreover, as only 2.7% out of the 2981 men in the Whitehall study had long-standing obesity, chance may explain the result (18). The second study involved a community-dwelling population in Denmark aged 20-99. BMI was negatively associated with psychological distress in terms of 'feeling stressed', 'not accomplishing very much', and use of antidepressants or sedatives (19). No association was found between obesity and 'wanting to give up'.

Thus, it seems that the majority of conventional studies have shown an association between obesity and an increased risk of depression, whereas MR studies present no effect of obesity on depression, or a protective effect. What could explain these discrepant findings? One plausible explanation is that important confounders have been missed or misclassified in the conventional analyses. For instance, past depression is a likely confounder because most new-onset depressions in the elderly are recurrent depressions (24). Personality and traumatic childhood experiences may also relate

to comfort eating and overeating as well as to depression in adulthood and late-life. Moreover, past depression, personality factors and life style factors such as diet are difficult to establish without measurement error. For instance, past depressions are underreported (57) and obese people tend to underreport caloric intake (58).

The next question is whether the inverse association between BMI and clinical depression that we found, can be causally inferred. It is appealing, given that a previous population-based MR study found a similar negative relationship. Moreover, even though WC predicts the effect of obesity on somatic disease risk better than BMI does, we also found a negative association between WC and depression (58-59). Possibly, the SNPs that we used induce satisfying but weight increasing behaviours, such as enjoyment of fatty or sugary foods, on the one hand and thereby reduce depression on the other. One implication of our results would be that it is fine for the mental health of skinny people to diet but not for the mental health of obese people.

Nevertheless, the MR results merit careful discussion. First of all, parent gene status or BMI may potentially violate the assumption that there are no common causes of the participant's gene status and risk of depression. It is conceivable that being obese in an 'obese family' makes you less susceptible to negative effects on self-image than being obese in an otherwise 'slim family'. In addition, the use of multiple SNPs to provide more precise estimates might have introduced correlation between the instruments and common causes of BMI and depression (60). However, our tests for weak instrument bias provide no evidence for this type of confounding, and weak instrument bias would have introduced an upward bias in the estimate. Weak instruments bias therefore, does not explain the negative MR estimate.

Secondly, it cannot be ruled out that obesity and clinical depression gave rise to non-participation at baseline or during follow-up visits. The other MR study that reported an inverse relation between BMI and psychological distress had a participation rate of 45% (19,61). To assess this bias, we repeated the MR analysis excluding from the study sample persons from increasingly higher age groups, because the older people are, the higher the chance that somatic and psychiatric obesity-induced disease has manifested itself. In persons aged ≤ 65, RD was -.02 (95% CI -.18, .17), in persons aged ≤ 75 -.13 (95% CI -.23, .001), and in persons aged ≤ 85 -.15 (95% CI -.25, -.05). These results suggest that selection bias might have had a modest effect on our findings. Yet, they do not explain the differences between the conventional and MR findings.

We conclude that obesity does not increase the risk of depression. Instead, individuals with a genetic propensity towards obesity may be at decreased risk of depression. Psychiatric epidemiology could benefit from MR methods to obtain unconfounded associations in observational studies.

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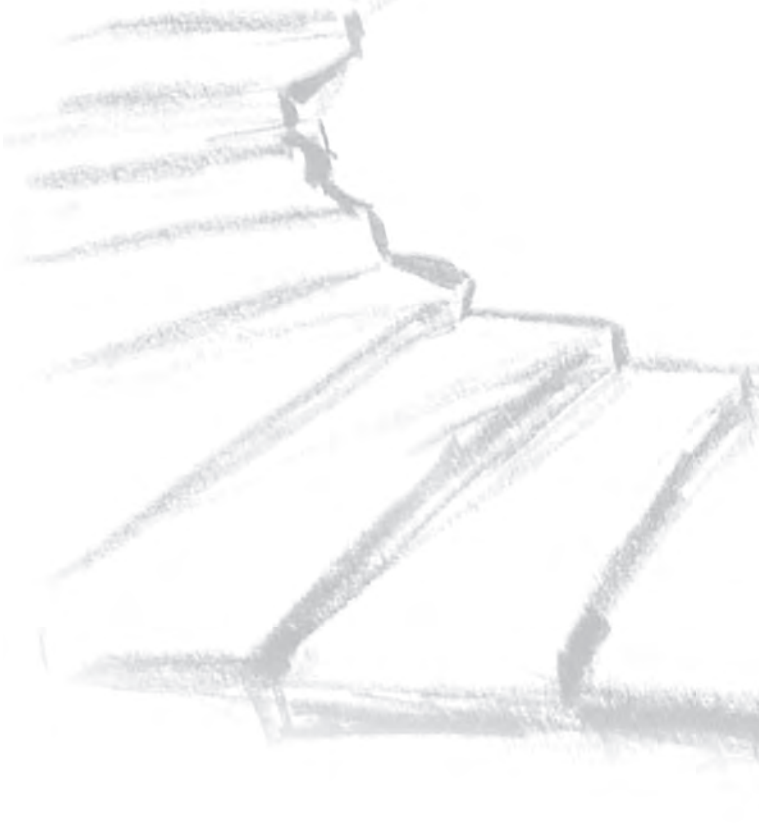

Chapter 10

General discussion

Rationale

Anxiety and depression are the most common mental disorders. The studies presented in this thesis report descriptive, physiological and genetic epidemiologal research on anxiety or depression in older adults from the general population. These studies were aimed to help unravel the etiology of anxiety and depression, which is still largely unclear.

In this chapter we will first discuss the main findings of the studies presented in this thesis. Second, methodological considerations will be discussed and last we will give recommendations for future research.

Main findings

Comorbidity and health care use

The comorbidity between anxiety disorders and depression has been widely studied, also in older adults. However, the reported frequency of comorbidity varies. Inconsistencies in the observed extent of comorbidity may partly be determined by methodological differences between studies. Commonly used instruments to diagnose anxiety and depression, such as the Composite International Diagnostic Interview (CIDI) and the Mini-International Neuropsychiatric Interview (MINI) assess anxiety in parallel to depression. Similar wording of the interview items is used to ascertain the shared and unique symptoms of anxiety and depression. When asked about both disorders during one interview session, respondents may be tempted to give seemingly consistent answers (1). Consequently, comorbidity may be diagnosed more frequently if one assessment tool is used to diagnose anxiety and depression during one session, than if assessed by different instruments or during separate sessions. This "common method variance" could inflate the observed comorbidity between anxiety and depression (for a review see (2)). Therefore, in *Chapter 2* we aimed to assess the extent of comorbidity if anxiety and depression are assessed with different instruments during different sessions. As expected, we found lower comorbidity than many other studies. This suggests an effect of common method variance, but we were not able to estimate the extent of common method variance. In addition, differences in case definition, time frame, sample size, and diagnostic interview could also explain the observed discrepancy. However, we identified one other study of which the design also reduced the effect of common method variance. The ESPRIT study (3) assessed anxiety and depression with the same instrument, but in addition, a team of psychiatrists and psychologists also considered medical history, medication use and a neurological assessment to confirm an anxiety or depression diagnosis. Cross-checking the diagnosis in different sources also reduces common method variance. Notably, this study reported a relatively low comorbidity

between anxiety and depression compared to studies using the same instrument to diagnose anxiety and depression. This suggests that the comorbidity between anxiety and depression is often overestimated.

Yet, even if common method variance is controlled, a high life-time comorbidity of anxiety and depression is observed. This may suggest that these disorders share a vulnerability or that one disorder is a risk factor for the other. To determine whether anxiety is a risk factors for depression or vice versa longitudinal studies are required. These studies require long follow-up times preferably starting in childhood or adolescence. In addition, subtypes of anxiety disorders should be taken into account. For example, generalized anxiety disorder is more comorbid with depression than specific phobia is. Anxiety has often been found to precede depression, however the reverse has also been observed (4-7). Alternatively anxiety and depression might be caused by a shared vulnerability, rather than one of the disorders being a risk factor for the other.

Kendler et al. (8) demonstrated in a twin study that this vulnerability may comprise common genetic factors. Therefore, we performed a genetic study to see if genetic variants associated with depression also explain variance in anxiety. This study will be discussed later on in this chapter. Kendler also suggested that environmental risk factors may then determine which disorder will occur at a specific point in time. Indeed, anxiety and depression have been found to be associated with different risk factors. For example, having a partner with a chronic disease increases the risk for anxiety disorders, while recent widowhood is associated with depression (9). The study of biological factors may also proof useful to determine whether the two disorders are caused by a common vulnerability. An example of such a biological factor potentially conferring a risk to both anxiety and depression is variability in the hypothalamic-pituitary-adrenal (HPA) axis, as measured by variation in levels of the stress-hormone cortisol. We assessed association of cortisol levels with anxiety disorders as discussed later in this chapter.

In *Chapter 3* we described mental health care use in older adults with anxiety, depression or comorbid anxiety and depression. We used multiple registrations in stead of only self-report to provide a more accurate estimation of mental health care use. In addition, we analyzed mental health care use at several care levels, namely at the level of the general practitioner (GP), the pharmacy, and at the level of specialized mental health care institutions. Last, we analysed mental health care use for different types of anxiety disorders and depression.

We found that health care use for anxiety or depression is generally low, depending on the type of disorder. As expected, mental health care use was lowest in those with anxiety disorder, and highest in those with comorbid anxiety and depression. Among the people with an anxiety disorder, mental health care use was highest in those with a panic disorder. This corresponds to the high burden of anxiety symptoms these people

experience. Notably, anti-depressant and anxiolytic medication use was relatively high and the number of people using these medications does not correspond to the low, registered percentage of people visiting the GP for anxiety and depression related symptoms or specialized mental health care services. This suggests that patients with depression and anxiety related symptoms visit the doctor for various reasons and receive anxiolytic or anti-depressant medication. However, the anxiety or depressive disorder is not recognized or is not documented. Because we based our study on registry data we could not determine the mental health care barriers, e.g., whether the patient felt the need to visit the doctor and whether the GP was aware of the patient's anxiety or depressive symptoms although he did not report them.

Given that (life-time) comorbidity between anxiety and depression is high and health care use is low and that both are common, impairing, but treatable disorders, awareness of these disorders should be improved both in caregivers as well as in the general population.

Physiological factors: cortisol and atherosclerosis

The HPA axis is one of the body's main systems that controls response to stress. It acts through the hormone cortisol. While the dysregulation of cortisol has been associated with anxiety disorders, the evidence is inconsistent and only a few studies have assessed this relationship in older adults. In *Chapter 4* we aimed to determine whether in adults aged 65 and over there is a difference in daily cortisol pattern between those with and without anxiety disorders. Therefore we assessed the cortisol awakening response (CAR) and total cortisol secretion over the day. We found that older adults with an anxiety disorder had a lower CAR than those without such a disorder. Our finding contradicted results of the largest study to date on the relation between anxiety and cortisol in adults by Vreeburg et al. (10) who observed a higher cortisol awakening response in people with anxiety disorders. In the study of Vreeburg et al., the high cortisol awakening response was driven by those with a panic disorder with agoraphobia and by those with a comorbid depression. The high CAR may thus be specific to these cases although we did not find an effect of comorbid depression in our population. Moreover, while the CAR may be raised during acute anxiety, the CAR may be lowered after persisting anxiety. This mechanism has also been observed for depression. Oldehinkel et al. observed lower urinary cortisol levels in chronically depressed older adults, but not in more acutely depressed people (11). In our sample of older adults from the general population we could not detect an association of the CAR and the extent of chronicity of anxiety disorders. However, acute and recent onset cases were rare. The median symptoms duration was 40 years and the people in the quartile with the shortest duration of symptoms had an average duration of more than three years.

Hypocortisolism has been observed in several chronic stress-related disorders such as chronic fatigue syndrome, chronic pelvic pain and irritable bowel syndrome (12-13) after a period of prolonged stress accompanied with high cortisol levels (14). Hypocortisolism may be a protective response of the body to counteract the endured increased levels of cortisol (15) and is presumably mediated through increased sensitivity to the negative feedback of circulating cortisol (16). It protects the body from the damaging effects of high cortisol levels, such as diabetes, hypertension and osteoporosis (17) at the cost of chronic stress-related symptoms like fatigue, pain and increased stress sensitivity, as well as immune deficiency. It is known that the CAR of older adults is in general lower than that of younger people. Thus older people with chronic anxiety disorders are at an increased risk of a wide range of hypocortisolism-related disorders and symptoms.

Possibly, altered levels of cortisol increase the vulnerability to have a depression or an anxiety disorder. Vreeburg et al. observed that non-affected family members of people with anxiety and depression had a higher CAR, like people with anxiety and depression (18). The temporal nature of the association between changes in cortisol levels and anxiety is yet unclear. We were not able to test causality of the association between the CAR and anxiety disorders in the current cross-sectional study. Longitudinal studies and genetic studies could shed further light on this.

The vascular depression hypothesis suggests that cerebrovascular disease precedes depression in older adults (19-20). However, studies that showed an association between depression and vascular factors have been largely cross-sectional. From these studies the temporal nature of the association could not be inferred. In addition, depression following overt vascular events such as stroke is sometimes suggested to be psychological rather than physiological.

In *Chapter 5* we performed a longitudinal study to verify the vascular depression hypothesis in older adults. We defined vascular disease as atherosclerosis. Atherosclerosis is an asymptomatic marker of vascular disease and can therefore be used to study whether there is a biological effect of vascular disease on the risk of depression. We found that atherosclerosis does not precede incident depression in older adults. These findings suggest that rather depression contributes to vascular burden or that depression and vascular disease are caused by a common biological substrate, such as inflammation or deregulation of the HPA axis.

It should be noted that we studied peripheral atherosclerosis and not cerebral atherosclerosis. However, peripheral atherosclerosis is associated with cerebral atherosclerosis (21-22) and thus a similar, albeit weaker relation with depression was expected. In a recent study of the same older age population, an association between cerebral hemodynamics and incident depression was observed (23). Undetected or cerebral atherosclerosis in small vessels might explain this finding. Further studies

are needed to assess whether reverse causality, i.e. the long term effects of previous depressive episodes on cerebral hemodynamics, plays a role.
In addition, anxiety disorders have also been associated with vascular factors. Therefore, it is interesting to examine the association of vascular factors with depression and anxiety longitudinally. In addition, it should be investigated whether biological substrates underlying vascular disease also underlie depression and possibly anxiety.

Genetic factors

The study of the genetics of anxiety and depression may help elucidate the pathogenesis of these disorders. In *Chapter 6* we replicated the association of a genetic variant in the PCLO gene with depressive disorders. PCLO encodes the protein piccolo that modulates neurotransmitter release (24-25). A study of piccolo variants in major depressive disorder (MDD) patients and healthy controls suggests that the risk allele modulates amygdala function and alters the processing of fearful stimuli in MDD patients (26). Fine-mapping of the PCLO region did not identify a more strongly associated variant than the variant replicated in our study (27). Interestingly, this variant has been found associated with a blunted cortisol awakening response, which suggests a causal relation between the HPA axis and depression (28). However, the PCLO-depression association was not replicated in a larger genome-wide association study (GWAS) of MDD (29) and this association requires further study.
In *Chapter 7* we then sought to find genetic variants associated with depressive symptoms using a hypothesis-free genome-wide approach. We found evidence for the association of one region with depressive symptoms that reached genome-wide significance in the meta-analysis including both discovery and replication samples. This region resides in a gene desert, with the closest gene, NUDT12, lying more than 1000 kilo bases away and which has not been implicated in psychiatric disorders. So the putative functional variant remains to be found and the association requires further verification. This effort included more than 50,000 individuals and suggests that large sample sizes are necessary to find variants associated with depressive symptoms. The lack of success of the current study is comparable to GWAS of depression, a recent study including 9000 cases and controls in the discovery and another almost 7000 cases in the replication phase did not yield any replicated variants (30).
One reason for the few successes in genetic research of depression phenotypes could be that not single genetic variants, but many variants collectively, each with a very small effect, affect the susceptibility for depression phenotypes. In *Chapter 8* we observed that many variants with small effects combined explain variance of depressive symptoms and depression. In addition, the same variants also explain variance in anxiety disorders. So a genetic vulnerability jointly explaining both variance in anxiety and depression

has been identified, as previously suggested by familiarity of depression and anxiety. This study shed light on the genetic architecture of anxiety and depression. However, specific variants, with a small effect size each, remain to be found. Large samples will be necessary to pinpoint these variants. As shown in *Chapter 7*, a sample of 50,000 people with measures on depressive symptoms was not sufficient.

Last, in *Chapter 9* we studied the relation between obesity and depression. Mixed findings for this relation in observational studies may be explained by reverse causality and residual confounding by e.g., past depression, exercise, and diet. Therefore, we performed a Mendelian randomization analysis that uses genetic variants to assess the relation between obesity and depression. Genetic variants have the advantage that they are passed on randomly from parents to child and are therefore unconfounded. The conventional multivariate analysis we performed showed that obesity is associated with an increased risk of incident depression in older adults. The Mendelian randomization analysis resulted in an opposite finding; a higher body mass index (BMI) was associated with a lower risk of depression. Our results may suggest that observational studies on obesity and depression are indeed hampered by residual confounding. However, the Mendelian randomization results need to be interpreted with caution. Mendelian randomization assumes that the genetic variants only affect depression via BMI. If the genetic variants affected depression risk directly (and not via BMI) or via a factor other than BMI this assumption would be violated and the effect of BMI may be overestimated. This assumption cannot be tested (31-32). And we cannot rule out that genetic variants associated with BMI affect depression risk directly. Furthermore, a recent Mendelian randomization study found that a genetic risk score for BMI was also associated with smoking, and with lower education (33). Both smoking and education have been associated with depression. In addition, this Mendelian randomization study found that BMI was associated with an increased risk of depressive symptoms in adolescents. Our study was set in an older adult population and found the opposite. In conclusion, the relation between obesity and depression requires further study, as does the use of Mendelian randomization for the analysis of complex disorders and complex traits like depression and BMI.

Methodological considerations

Genome-wide association studies test millions of genetic variants with an outcome and meta-analysis of multiple studies is indispensable to achieve a sample size with enough power to survive multiple testing correction. Phenotype definition is essential for the success of GWAS. Misspecification, heterogeneity and lack of precision of the phenotype may result in a loss of power to detect a SNP-phenotype association. In

addition, phenotype definition is important for the validity and comparability of 'ordinary' epidemiological studies. Here we discuss a number of phenotype issues that are relevant for psychiatric genetic research and psychiatric research in general.

Clinical and etiological heterogeneity

The pathogenesis of psychiatric disorders is largely unclear and laboratory tests to confirm a diagnosis are absent. Therefore, diagnosis is based on a set of symptoms that was validated based on clinical observations, outcome and family history (34). In other words, psychiatric diagnoses are consensus based and not evidence based. Diagnoses are listed in the Diagnostic and Statistic Manual of Mental Disorders (DSM), a handbook that is updated regularly, describing all psychiatric disorders. Major depression for example is described in the DSM (4th edition) by five or more symptoms of a list of nine symptoms with at least depressed mood or loss of interest or pleasure that have been present during a two-week period and cause impairment or significant distress. This diagnosis guides clinicians who needs to be treated, what treatment is most suitable and what the prognosis is. However, psychiatric diagnoses often apply to a wide spectrum of patients, so that one patient with MDD may have different symptoms than another patient with MDD. This is called clinical heterogeneity.

Because a psychiatric diagnosis is based on clinical observation and not on pathogenesis, biological and genetic distinction may not be compatible with clinical distinction. For example, patients with major depression respond differently to medication suggesting variation in pathogenesis (etiological heterogeneity).

Nevertheless less common consensus-based diagnoses like schizophrenia and bipolar disorder have a high heritability. Genetic studies for these disorders have been relatively successful at least compared to common psychiatric disorders, like anxiety disorders and depression. Very recently, a GWAS meta-analysis of schizophrenia including more than 40,000 cases and controls was presented that identified 63 independent SNP associations (unpublished results from the Psychiatric GWAS Consortium presented at the world congress of psychiatric genetics, 2012). Psychiatric phenotypes like schizophrenia and bipolar disorder appear to be less hindered by clinical or etiological heterogeneity, as may be the case for anxiety and depression. The heritability estimates of anxiety and depression are lower, the disorders are more common and there is less contrast between cases and controls in the population. Moreover, when studying MDD in families, symptom profiles are alike, suggesting a genetic basis for a specific set of symptoms, not necessarily for all MDD types (35). MDD patients may thus carry nearly indefinite different combinations of MDD risk variants. These may vary by subtype, chronicity, treatment response, etc.

Clinical and etiological heterogeneity reduce statistical power to find an association

between genetic variants and depression as effect sizes will be diluted (30). Clinical heterogeneity is reduced when restricting the analysis to less common subtypes of MDD with a higher heritability, like recurrent early-onset MDD. The higher heritability of these subtypes also suggests reduced etiological heterogeneity. GWAS of MDD subtypes has thus far not been successful (30), but study samples may have been underpowered.
In addition, it is important to improve the taxonomy of the disorders to move from consensus-based diagnosis definitions to evidence-based definitions. Analysing cases using empirically-derived psychiatric phenotypes reduces clinical heterogeneity and hopefully also etiological heterogeneity. For example, latent class analyses give an indication on how to group disorders. This type of analysis has been performed and identified two broad clusters of internalizing and externalizing problems (8). Anxiety and depression grouped together in the internalizing cluster.
The outcomes of latent class analyses or factor score analyses can also be used as outcome for GWAS analyses. We are currently performing a GWAS on a factor score that takes into account different subtypes of anxiety disorders and depression comorbidity. This approach relies on results of a phenotypic study that showed that genetic risk factors predispose to a broad group of internalizing disorders (8).

Phenotype assessment

In psychiatry a diagnosis can be made in different ways. In the clinic, assessment is performed by the clinician who bases his diagnosis on a structured assessment and observation. In psychiatric research, different instruments are available to assess a clinical diagnosis of the disorder, a subthreshold state, or a symptom count. These approaches aim to measure the same underlying construct, but correlation is far from 100%. Therefore, assessment unavoidably introduces some variation (measurement heterogeneity) in the phenotype especially when combining results of different studies. The impact of misclassification of the phenotype depends on the number of misclassified cases or controls relative to the total size of the case or control group.
As mentioned in the paragraph above, besides a DSM-defined clinical diagnosis, a subthreshold diagnosis or a symptom count (a continuous scale) may be relevant phenotypes to study. Depressive symptoms and subthreshold conditions are associated with similar patterns of risk factors as MDD, suggesting shared etiology with varying severity (36). The ability to detect genetic predictors should therefore be improved by analyzing depression quantitatively (37). Furthermore, applying a boundary, like a clinical diagnosis, does not reflect the natural presence of a continuum of symptoms. Indeed anxiety and depression are complex diseases caused by the interplay of many genes and environment. The presence of a continuum is also predicted by the common-trait common-genetic-variant hypothesis underlying GWAS (38). And exploiting the

phenotypic variation within cases and controls by analyzing depression quantitatively has been shown to greatly increase the power to detect genetic variants (38). It is now realized more and more that a continuum may better reflect the nature of a psychiatric disorder than a clinical diagnosis certainly for research purposes. As a result, for the fifth version of the DSM, it is investigated whether continuums can be used as diagnostic system. However, for clinicians a continuum is not straightforward to use. It is unclear how to determine on the basis of a continuum who needs to be treated, how the patient should be treated and what the prognosis is until we have developed prognostic and treatment algorithms for continuous disease measures. Decades of research would need to be repeated to answer these questions.

The use of subthreshold conditions or depressive symptom counts for research also has some drawbacks. In *Chapter 7* we used the CES-D, a scale designed to screen for depression. However, this scale measures depressive symptoms over one week, so when measured once, it does not incorporate who had a history of depression and it will overestimate depression as it detects temporal sadness caused by e.g., loss of a family member. However, depressive symptoms can be ascertained with a five-minutes survey, so large numbers to study can be obtained (see *Chapter 7* in this thesis). In addition, the items used in scales like the CES-D are not empirically derived. For the CES-D for example the major components of depressive symptomatology were derived from clinical literature and factor analytic studies (39). By convention, the CES-D is commonly used in research, even though it has been debated whether for example the four positively worded items (e.g., "I was happy") fit in the continuum of depressive symptoms (40-42). As illustrated above, there are several ways to assess psychiatric phenotypes and each approach has its own advantages and disadvantages. To gain most success it might be necessary to reassess psychiatric phenotypes and design empirically- derived continuous symptom profiles.

Setting

Psychiatric phenotypes may be ascertained from different settings, for example from a primary practice, from a psychiatric hospital or from the general population. Setting is an important determinant of the type of psychiatric disorders ascertained. In clinical settings the disorder is generally more severe, and highly comorbid while in the general population, chronic cases with less severe disease will be found. Cases from clinical studies are also more often recurrent and typically have an earlier onset than cases from population-based studies (43).

Chronicity, comorbidity and severity may associate differently to factors of interest such as cortisol and genetic factors. In *Chapter 4* of this thesis for example we observed a negative association between anxiety and the cortisol awakening response in

the population-based Rotterdam Study. On the contrary, a positive association was observed in a more clinical setting, where a higher percentage of comorbid MDD and a higher percentage of panic disorders was observed (10). In addition, in *Chapter 6* we saw that the association between a genetic variant in the PCLO gene and MDD only replicated in population-based studies, not in clinical settings. Hence, the characteristics of depression observed in clinical studies and population-based studies may not be very similar, and may, in part, have a different genetic etiology. Setting is thus an important factor related to chronicity, severity, comorbidity and other patient characteristics that should be taken into account when designing a research project as well as when comparing results to other studies.

Comorbidity

Psychiatric disorders are often highly comorbid. This was also shown in *Chapter 2* of this thesis, where we observed that anxiety and depression have a high life-time comorbidity. In addition, people with anxiety disorders also benefit from anti-depressant medication. This may point at a common etiology. Indeed, there is cumulative evidence that supports the association of several genes with MDD and anxiety (44). Moreover, in *Chapter 8* of this thesis we found that genetic variants that explain variance in depressive symptoms and depression also explain variance in anxiety disorders. This provides evidence for a common genetic basis for anxiety and depression. As noted before, comorbidity could also result from one disorder increasing the risk to have another disorder. In addition, what was not discussed yet, is that comorbidity could also result from how disorders are defined. The diagnoses of anxiety and depression are such that they share many symptoms. As illustrated in *Chapter 2* this may affect comorbidity estimates. The underlying reason for comorbidity is not entirely clear and comorbidity complicates research. As different studies vary in their way of handling comorbidity (adjustment, exclusion, etc.) it reduces comparability of study results.

There are several approaches to use comorbidity in research. Studies that combine disorders with a high comorbidity, sometimes called cross-disorder studies, have been performed in the field of psychiatry. For example, a GWAS study of bipolar disorder and MDD yielded genome-wide significant results (30). However, the association of this variant with MDD was not replicated in an MDD-only sample (30).

Another way of handling comorbidity is by analysing latent classes underlying the diagnoses. Latent classes are unobserved constructs that are inferred from observed data. They represent patterns of symptoms observed in patients. Instead of analyzing depression, one could analyze the latent class underlying anxiety and depression.

Finally, continuous symptom profiles could also be designed such that they take into accout comorbidity. The CES-D for example, designed to screen for depression,

also detects anxiety disorders, as the symptoms of anxiety and depression overlap. Furthermore, in child psychiatry (research and clinic), the Child Behaviour Checklist measures behavioural problems on a continuum. It assesses internalizing behaviour (i.e., anxious and depressive behaviour) and externalizing behaviour (i.e., aggressive and hyperactive behaviour) and results in profiles of behaviour rather than in diagnoses. A similar checklist could also proof useful for adult psychiatric research and in the clinic.

Recommendations

In this thesis we aimed to help unravel the etiology of anxiety and depression by studying these disorders from different perspectives. Although we did not identify any validated genetic or biological factors causing anxiety and depression, the studies in this thesis did bring up recommendations for future study. Specific recommendations for future study were described in the discussion section of each chapter in this thesis. Here, we will give general recommendations on the use of comorbidity between anxiety and depression in research and on the study of genetics of anxiety and depression.

Comorbidity

Comorbidity is inherent to anxiety and depression and should thus be taken into account when studying these disorders. For genetic research this implies that we should not necessarily exclude people on anxiety from the case group when studying depression and vice versa. In fact, exclusion would reduce the sample size and therefore power. However, it also implies that one should account for anxiety in the control group. As these people have a high risk of having had a depression and may share genetic variants with those in the case group. In non-genetic research, selecting 'pure' disorders is likely an 'illusion' and not a relevant concept as comorbidity is inherent to the disorder. However, current comorbidity should be taken into account, because comorbid prevalent anxiety and depression is more impairing than either disorder alone (45).

Genetics of depression and anxiety

Depression and anxiety are complex disorders, caused by the interplay between genes and environment. Although effect sizes of genetic variants will presumably be very low, the study of genetics has the potential to uncover pieces of the black box that keeps the etiology of anxiety and depression. As these disorders are very common and impairing, every piece of information will prove valuable for future diagnosis and treatment of patients with these disorders. Although genetic studies for these disorders so far have been relatively unsuccessful, there has been enormous evolution over the past years in

this field in phenotype definition, analysis methods and collaboration.

The first GWAS studies analysed major depressive disorder (MDD) cases. Quickly thereafter, specific MDD subtypes, like recurrent MDD were studied. Furthermore, personality, cross-disorders, and depressive symptoms (this thesis *Chapter 7*) were the focus of GWAS studies. Although these attempts have been relatively unsuccessful, they have provided important knowledge on depression genetics. We now know that we are presumably looking for variants with very small effects that require larger sample sizes to detect.

The potential of genetic studies has also been greatly increased by the wealth of methods that is available nowadays. What started from family studies and twin studies, linkage studies, and candidate gene association studies, now is done on whole genome level. In addition, whereas in this thesis we focused on SNPs (single-nucleotide polymorphisms), exome sequencing revealing rare variants, copy number variant analysis and epigenetic analysis is now also underway. Furthermore, methods to analyze the phenotype (e.g., Mendelian randomization analysis, and risk score analysis) have also contributed to an increase in knowledge of disorders. Using a risk score analysis, we found evidence that anxiety and depression share a genetic component in *Chapter 8* of this thesis.

What has probably been the most important gain in the field is the foundation of large collaborations. Collaborations are necessary to reach the large sample sizes that are required to detect small effect sizes. And as a consequence, experts of many fields (e.g., psychiatry, statistics, genetics, epidemiology) collaborate and combine their knowledge to improve research in this field. Although this has not yet led to the finding of genetic variants associated with depression, it will eventually. The first successes for other psychiatric phenotypes like schizophrenia and bipolar disorder were already celebrated.

To conclude: if we are patient and persistent and if we continue to evolve phenotype definitions, methods and collaborations, GWAS successes for anxiety disorders and depression will undoubtedly follow.

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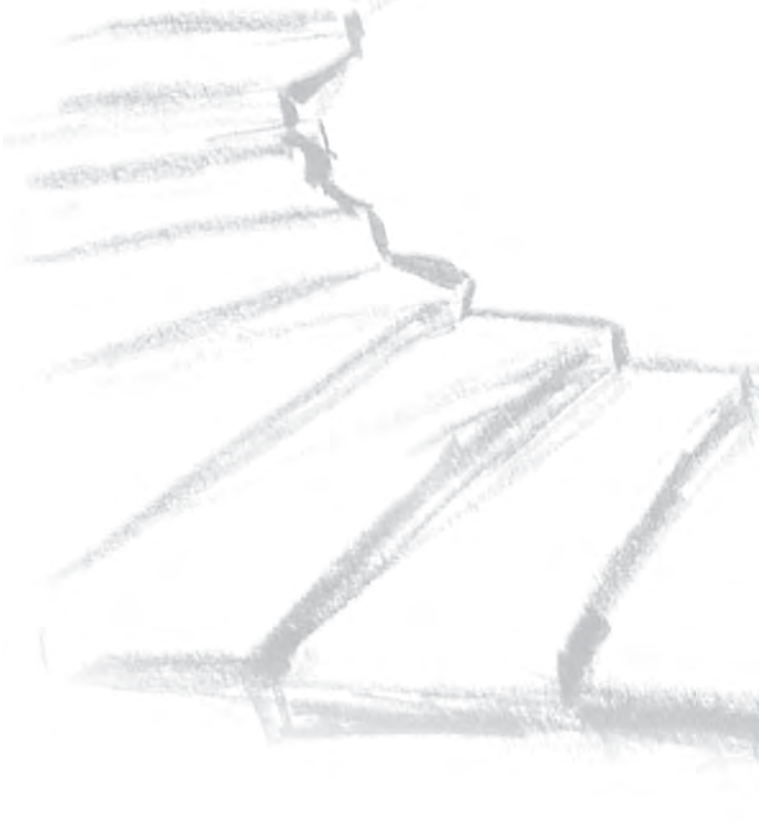
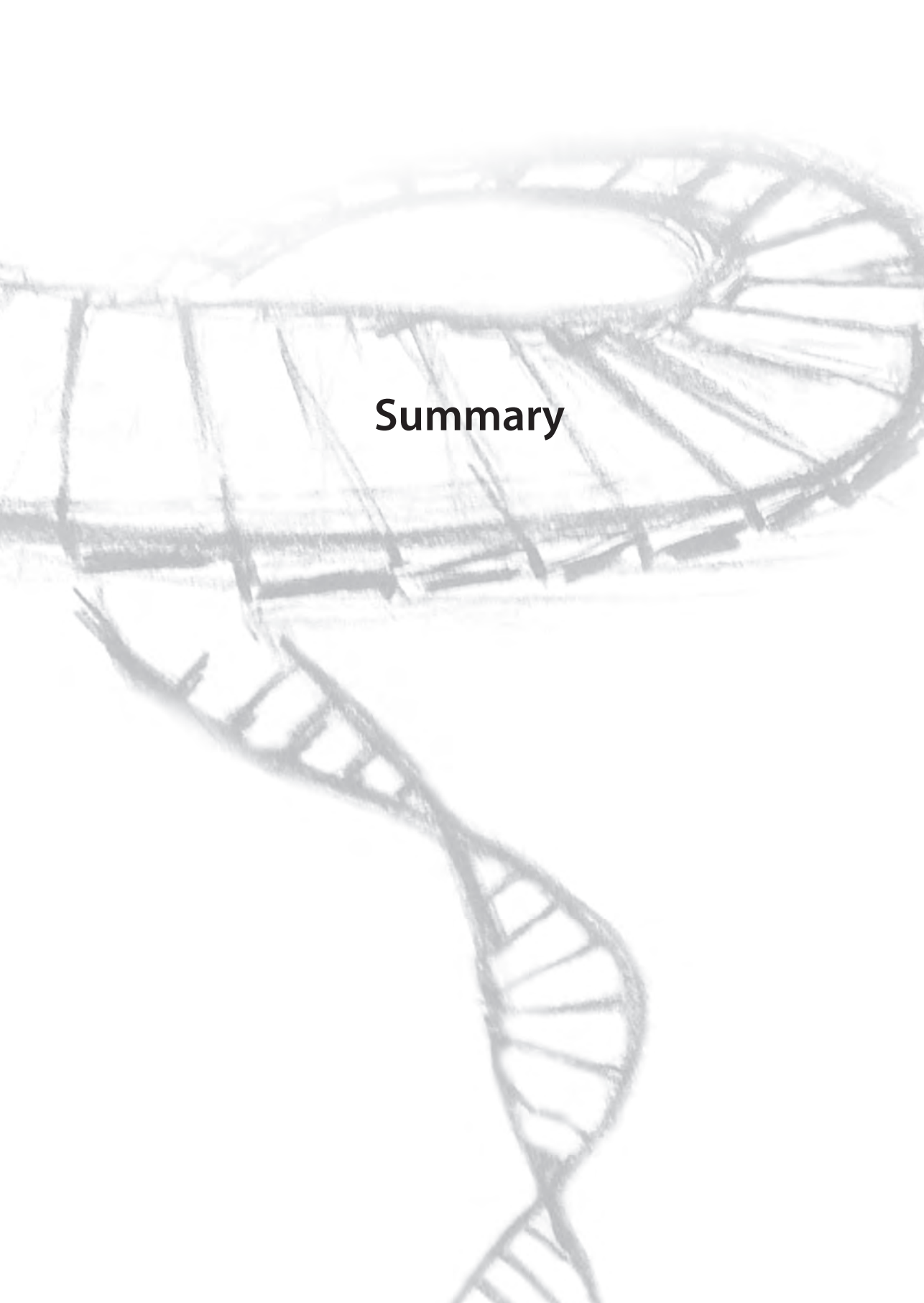




Summary

Background and aim

Anxiety and depression are complex disorders caused by both genetic factors and non-genetic factors. However, their etiology is unknown. Study of the epidemiology of anxiety and depression, i.e. study of the occurrence and the determinants of both disorders, helps unravel their etiology. The aim of this thesis (described in **Chapter 1**) was to help unravel the etiology of anxiety and depression. In particular, comorbidity, health care use, cortisol, atherosclerosis and genetic factors were studied in relation to anxiety and, or depression.

The studies in this thesis were based on data from the Rotterdam Study, a prospective population-based cohort study of older adults ongoing since 1990 in Rotterdam, the Netherlands. Every four years participants undergo an extensive home interview and a physical examination at a research center. Cross-sectional data for anxiety disorders and longitudinal data for depression were available for this thesis.

Comorbidity and health care use

The comorbidity between anxiety and depression has been widely studied, but reported frequencies vary and this may partly be explained by methodological differences. Commonly used instruments to diagnose anxiety and depression assess these disorders in parallel. When asked about both disorders during one interview session, with the same instrument, respondents may be tempted to give consistent answers. Consequently, comorbidity may be diagnosed more frequently if one assessment tool is used to diagnose anxiety and depression during one session, than if assessed by different instruments or in separate sessions. This "common method variance" could inflate the observed comorbidity between anxiety and depression. Therefore, in **Chapter 2** we aimed to assess the extent of comorbidity if anxiety and depression are assessed with different instruments during different sessions. As expected, we found a lower comorbidity rate than many other studies. This suggests an effect of common method variance, but we were not able to estimate the extent of common method variance. What remains when controlling for common method variance is the high life-time comorbidity of anxiety and depression. This may suggest that these disorders share a vulnerability or that one disorder is a risk factor for the other.

In **Chapter 3** we studied mental health care use in older adults with anxiety, depression or comorbid anxiety and depression. Contrary to other studies, we did not depend on self-reported mental health service use, but we used multiple registries to assess mental health care use. We screened general practitioner's records for anxiety and depression-related visits, used pharmacy records to assess anxiety and depression-related medication use, and used a psychiatric case register to assess specialized mental

health care utilization. We confirmed that mental health service use among older adults with anxiety disorders is low and lower than such service use of people with depression or comorbid anxiety and depression. We also found that the majority of those who used mental health services used medication only. Further study is required to determine why mental health service use is low amongst older adults with anxiety and depression.

Physiological factors: cortisol and atherosclerosis

The hypothalamic-pituitary-adrenal (HPA) axis is one of the body's main systems that controls response to stress. It acts through the hormone cortisol. While the dysregulation of cortisol has been associated with anxiety disorders, the evidence is inconsistent and only a few studies have assessed this relationship in older adults. In **Chapter 4** we aimed to determine whether in adults aged 65 and over there was a difference in daily cortisol pattern between those with and without anxiety disorders. Therefore we assessed the cortisol awakening response (CAR) and total cortisol secretion over the day. We found that older adults with an anxiety disorder had a lower CAR than those without such a disorder. Our study sample comprised older adults with chronic anxiety. Acute and recent onset cases were rare. Our finding is consistent with the notion that chronic anxiety may result in downregulation of HPA-axis activity. Older people are known to have a lower CAR and a low CAR has been associated with cardiovascular and auto-immune disorders. Older adults with chronic anxiety disorders may thus have an increased vulnerability to a wide range of disorders.

In **Chapter 5** we described a longitudinal study that aimed to verify the vascular depression hypothesis in older adults. This hypothesis suggests that cerebrovascular disease precedes depression in older adults. However, studies that showed an association have been largely cross-sectional. We defined vascular disease as peripheral atherosclerosis, an asymptomatic marker of vascular disease. Peripheral atherosclerosis is associated with cerebral atherosclerosis. We found that atherosclerosis does not precede incident depression in older adults. These findings do not support the vascular depression hypothesis and rather suggest that depression contributes to vascular burden or that depression and vascular disease are caused by a common biological underpinning.

Genetic factors

In the last part of this thesis we reported genetic epidemiological studies of anxiety and depression. Genome-wide association analysis had revealed a new putative candidate gene for depression: the PCLO gene. In **Chapter 6** we replicated the association of a

genetic variant in the PCLO gene with depressive disorders. No association with a broader syndromal phenotype was observed. In addition, the association was replicated in population-based studies, not in clinical studies. This suggests that depression observed in the clinic and in the population may, in part, have a different genetic susceptibility.

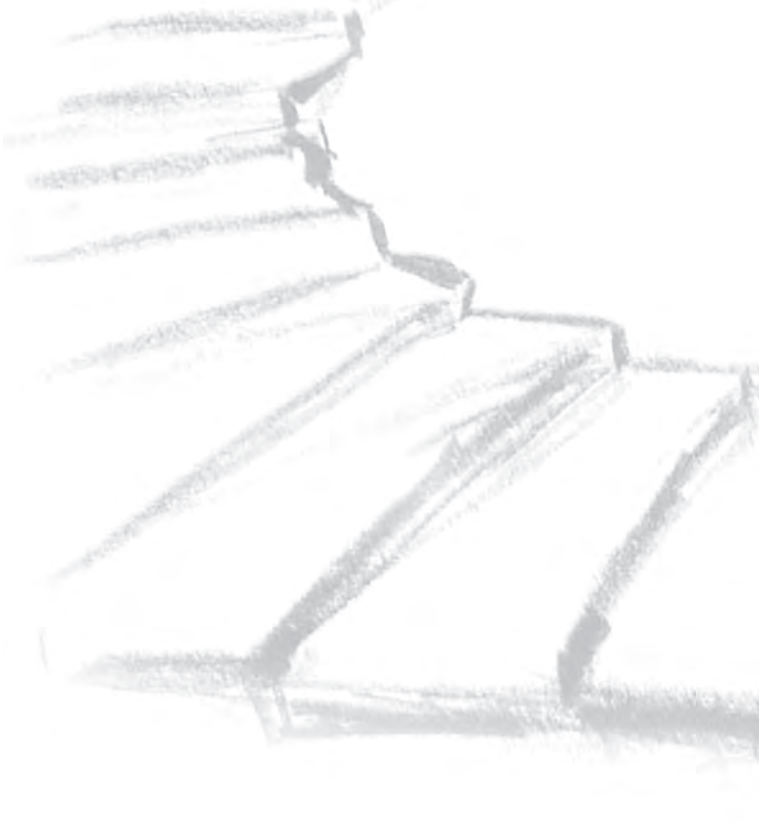
In **Chapter 7** we then sought to find genetic variants associated with depressive symptoms using a hypothesis-free genome-wide approach. We found evidence for the association of one region with depressive symptoms that reached genome-wide significance in a meta-analysis including both discovery and replication samples. This region resides in a gene desert, with the closest gene, NUDT12, lying more than 1000 kilo bases away and which has not been implicated in psychiatric disorders. The putative functional variant remains to be found and the association requires further verification. This effort included more than 50,000 individuals and suggests that large sample sizes are necessary to find variants associated with depressive symptoms.

One reason for the few successes in genetic research of depression phenotypes could be that not single genetic variants, but many variants collectively, each with a very small effect, affect the susceptibility for depression phenotypes. In **Chapter 8** we observed that many variants with small effects combined explain variance of depressive symptoms and depression. In addition, the same variants also explain variance in anxiety disorders. We identified a genetic vulnerability jointly explaining both variance in anxiety and depression. However, specific variants, with a small effect size each, remain to be found.

In **Chapter 9** we studied the relation between obesity and depression. Mixed findings for this relation in observational studies may be explained by reverse causality and, or residual confounding by e.g., past depression, exercise, and diet. Therefore, we performed a Mendelian randomization analysis that uses genetic variants to assess the relation between obesity and depression. Genetic variants have the advantage that they are passed on randomly from parents to child and are therefore unconfounded. The conventional multivariate analysis we performed showed that obesity is associated with an increased risk of incident depression in older adults. The Mendelian randomization analysis resulted in an opposite finding; a higher body mass index (BMI, caused by genetic factors) was associated with a lower risk of depression. Our results may suggest that observational studies on obesity and depression are indeed hampered by residual confounding. However, the Mendelian randomization results need to be interpreted with caution, because this analysis. assumes that the genetic variants only affect depression via BMI. The relation between obesity and depression requires further study, as does the use of Mendelian randomization for the analysis of complex disorders like depression and BMI.

General discussion

Last, in **Chapter 10**, we summarized and discussed our main findings. Then we highlighted methodological considerations of phenotype definition that are relevant for psychiatric research. We discussed clinical and etiological heterogeneity, phenotype assessment, setting, and comorbidity. Finally, we made general recommendations for future research on the use of comorbidity between anxiety and depression in research and on the study of genetics of anxiety and depression. We recommend to always account for comorbidity, as comorbidity is inherent to anxiety and depression. The type of study determines how comorbidity can be best accounted for. In addition, we encourage genetic epidemiologists in the field to continue working on the genetics of anxiety and depression. Evolution in methods, phenotype definition and collaboration will lead to genetic successes for anxiety disorders and depression.



Samenvatting

Achtergrond en doelstelling

Angststoornissen en depressies zijn zeer complexe ziektes die worden veroorzaakt door zowel genetische als niet-genetische factoren. We weten echter nog weinig over welke genetische en niet-genetische factoren ten grondslag liggen aan deze ziektes. Het bestuderen van de epidemiologie van angst en depressie (wie krijgt deze ziektes en welke factoren hangen daarmee samen) kan hierbij helpen. Het doel van dit proefschrift (beschreven in **hoofdstuk 1**) was om oorzaken van angst en depressie te achterhalen door middel van epidemiologische studies. In dit proefschrift hebben we gekeken naar comorbiditeit, zorggebruik, cortisol, atherosclerose en genetische factoren in relatie tot angst en, of depressie.

De studies in dit proefschrift zijn gebaseerd op gegevens van het Erasmus Rotterdam Gezondheid Onderzoek (ERGO, in het Engels the Rotterdam Study). ERGO is een langlopend bevolkingsonderzoek onder 15,000 mensen van 45 jaar en ouder in de Rotterdamse wijk Ommoord. We onderzoeken gezondheidsproblemen die op latere leeftijd veel voorkomen, waaronder angst en depressie. Dankzij dit uitvoerige onderzoek komen we steeds meer te weten over het vóórkomen, het ontstaan en het verloop van ziekte bij ouderen.

Comorbiditeit en zorggebruik

Allereerst hebben we gekeken naar de samenhang (comorbiditeit) van angst en depressie. Hier is al veel onderzoek naar gedaan, maar de gerapporteerde mate van comorbiditeit varieert sterk. Dit zou deels verklaard kunnen worden door verschillen tussen studies in de gehanteerde methode om comorbiditeit te bepalen. Vaak wordt eenzelfde instrument gebruikt om angst en depressie te meten. Wanneer je binnen één interview, met hetzelfde instrument vragen stelt over zowel angst als depressie zijn ondervraagden misschien sneller geneigd om consistent te antwoorden. Dit heeft als gevolg dat er mogelijk meer comorbiditeit tussen angst en depressie wordt gevonden wanneer er in één interview met één instrument angst en depressie wordt gemeten, dan wanneer dit gebeurt in aparte interviews en met verschillende instrumenten. Deze zogenoemde "common method variance" zou de gevonden comorbiditeit tussen angst en depressie dus artificieel kunnen verhogen. In **hoofdstuk 2** hebben we daarom de comorbiditeit tussen angst en depressie bepaald door angst en depressie te meten op verschillende momenten met verschillende instrumenten. Zoals verwacht vonden we een lagere comorbiditeit dan de meeste andere studies. Dit verschil zou verklaard kunnen worden door common method variance, maar we konden niet de grootte van het effect van common method variance bepalen. Wel vonden we een hoge comorbiditeit tussen angst en depressie als je ook de voorgeschiedenis van deelnemers

bekijkt. Dit suggereert dat angst en depressie dezelfde oorzaak hebben, of dat de ene stoornis een risico factor is voor de andere.
In **hoofdstuk 3** hebben we vervolgens het zorggebruik voor angst en depressie bekeken. Waar andere studies afhankelijk waren van vragenlijsten om zorggebruik in kaart te brengen, hebben wij hiervoor gebruik gemaakt van verschillende registers. We hebben dossiers bij de huisarts gescreend op angst- en depressie gerelateerde bezoeken, aflevergegevens van apotheken op angst- en depressie gerelateerde medicatie en we gebruikten het Psychiatrisch Casus Register om inzicht te krijgen in het gebruik van tweedelijns geestelijke gezondheidszorg. Net als eerdere studies vonden wij dat zorggebruik voor angst en depressie onder ouderen met angststoornissen laag is en ook lager dan het zorggebruik door ouderen met depressie of met angst én depressie. We vonden ook dat de meerderheid van de mensen die zorg gebruikten voor hun angst of depressie alleen medicatie gebruikten. Aanvullende studies zijn nodig om te bepalen waarom het zorggebruik voor angst en depressie onder ouderen met deze stoornissen zo laag is.

Fysiologische factoren: cortisol en atherosclerose

De hypothalamus-hypofyse-bijnieras (HPA-as) is één van de systemen in het lichaam die reacties op stress reguleert. De HPA-as werkt via het hormoon cortisol. Sommige studies tonen aan dat angststoornissen samenhangen met een verstoorde HPA-as, maar de resultaten zijn niet consistent en slechts een klein aantal studies onderzocht dit verband bij oudere mensen. De studie beschreven in **hoofdstuk 4** had dan ook als doel om te bepalen of er een verschil was in cortisol patroon gedurende de dag tussen ouderen met en zonder angststoornis. Hiervoor bestudeerden wij de “cortisol awakening response” (CAR, de piek in cortisol levels vlak na het wakker worden) en de totale cortisol afgifte gedurende de dag. We vonden dat ouderen met een angststoornis een lagere CAR hadden dan ouderen zonder angststoornis. Onze studiepopulatie bestond voornamelijk uit oudere mensen met chronische angst. Onze bevinding is dan ook consistent met de hypothese dat chronische angst leidt tot lagere HPA-as activiteit. Het is bekend dat ouderen (ook zonder angststoornis) een lagere CAR hebben dan jongeren en een lagere CAR hangt samen met onder andere een verhoogde kans op hart- en vaatziekten en auto-immuunziekten. Ouderen met een chronische angststoornis hebben dus mogelijk een verhoogde kans op een groot aantal ziekten.
In **hoofdstuk 5** beschreven we een studie die als doel had om ‘de vasculaire depressie hypothese’ te verifiëren. Deze hypothese stelt dat vasculaire depressie bij ouderen een gevolg is van cerebrovasculaire ziekte. Eerdere studies die een verband lieten zien tussen depressie en cerebrovasculaire ziekte waren meestal cross-sectioneel (de relatie werd

niet door de tijd bestudeerd). Wij deden een longitudinale studie en gebruikten perifere atherosclerose als asymptomatische marker voor cerebrovasculaire ziekte. We vonden dat atherosclerose niet samenhangt met het ontstaan van depressie bij ouderen. Onze bevinding weerlegt de vasculaire depressie hypothese en suggereert juist dat depressie leidt tot vasculaire ziekte of dat depressie en vasculaire ziekte dezelfde risicofactoren hebben.

Genetische factoren

In het laatste deel van dit proefschrift rapporteerden we de resultaten van genetisch epidemiologisch onderzoek. We begonnen met een replicatiestudie. Een eerdere genoombrede associatiestudie had een nieuw kandidaatgen voor depressie opgeleverd: het PCLO gen. In **hoofdstuk 6** repliceerden we de associatie tussen het PCLO gen en depressie. We vonden echter geen associatie tussen dit gen en depressieve syndromen. De associatie tussen het PCLO gen en depressie werd alleen gevonden in bevolkingsstudies en niet in klinische studies. Dit suggereert dat depressie in de kliniek en in de algemene bevolking verschillen in hun onderliggende genetische risicofactoren.

In **hoofdstuk 7** gingen we op zoek naar genetische varianten onderliggend aan depressieve symptomen. Hiervoor gebruikten we een hypothesevrije aanpak: de genoombrede associatiestudie. Wanneer we de resultaten van meer dan 50,000 mensen samenvoegden vonden we één regio in het genoom dat samenhing met depressieve symptomen (na correctie voor het aantal testen dat we deden). Het dichtstbijzijnde gen, NUDT12, ligt meer dan 1000 Kb verwijderd van deze regio en was nog niet eerder gevonden in relatie tot een psychiatrische stoornis. De associatie tussen depressieve symptomen en deze regio dient nog geverifieerd te worden en ook de mogelijke functionele genetische variant moet dus nog gevonden worden. Deze studie laat zien dat grote aantallen nodig zijn om genetische varianten te vinden die samenhangen met depressieve symptomen.

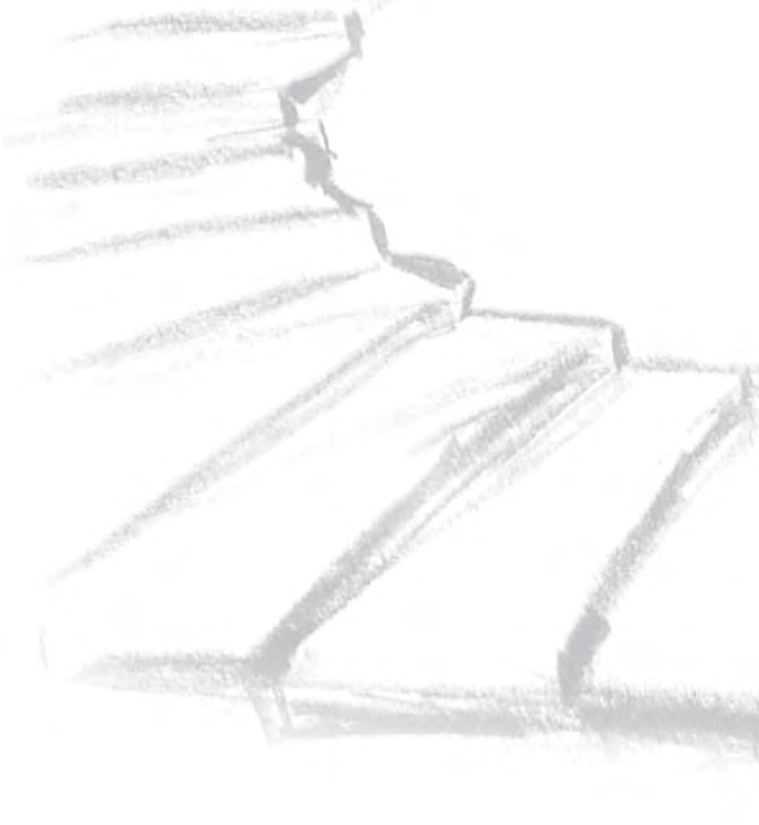
Een reden voor het uitblijven van successen van genetisch onderzoek naar depressie zou kunnen zijn dat niet opzichzelfstaande genetische varianten, maar een combinatie van varianten gezamenlijk, elk met een heel klein effect, het risico op depressie beïnvloedt. In **hoofdstuk 8** zagen we dat genetische varianten met elk heel kleine effecten gezamenlijk een deel van het risico op depressie en depressieve symptomen verklaren. Dezelfde varianten bleken ook deels verklarend voor het hebben van een angststoornis. We identificeerden dus een genetische kwetsbaarheid voor zowel angst als depressie. De volgende uitdaging is om specifieke varianten te identificeren.

In **hoofdstuk 9** bestudeerden we de relatie tussen obesitas en depressie. Tegengestelde

resultaten in observationele studies kunnen verklaard worden door 'reverse causality' en 'residual confounding' doordat er bijvoorbeeld geen informatie is over depressie in de voorgeschiedenis of over dieet en beweging. Daarom hebben wij een Mendeliaanse randomisatie studie uitgevoerd; we gebruikten genetische varianten om de relatie tussen obesitas en depressie te bepalen. Genetische varianten erven willekeurig (random) over van ouder op kind en daardoor is er geen sprake van confounding. In de conventionele analyse vonden we dat obesitas samenhangt met een verhoogd risico op incidente depressie bij ouderen. Uit de Mendeliaanse randomisatie analyse kwam het tegenovergestelde: een hogere body mass index (BMI, gewicht in kg/kwadraat van de lengte in meter) (veroorzaakt door genetische factoren) was geassocieerd met een verlaagd risico op depressie. Dit suggereert dat de resultaten van observationele studies naar obesitas en depressie inderdaad beïnvloed worden door residual confounding. De resultaten van de Mendeliaanse randomisatie analyse moeten wel met enige voorzichtigheid geïnterpreteerd worden. Dit soort analyse veronderstelt namelijk dat de bestudeerde genetische varianten depressie alleen beïnvloeden via BMI. De relatie tussen obesitas en depressie moeten daarom nader bestudeerd worden, net als het toepassen van Mendeliaanse randomisatie studies voor complexe ziektes zoals depressie.

Algemene discussie

Tot slot vatten we in **hoofdstuk 10** de belangrijkste bevindingen samen en bediscussieerden we deze. We bespraken ook een aantal methodologische overwegingen ten aanzien van de definitie van psychiatrische fenotypes, zoals angst en depressie: klinische en etiologische heterogeniteit, fenotype bepaling, setting en comorbiditeit. Daarna deden we aanbevelingen voor toekomstig onderzoek over het gebruik van de comorbiditeit van angst en depressie voor onderzoek en over genetische studies naar angst en depressie. We raadden aan om altijd rekening te houden met comorbiditeit, omdat comorbiditeit een kenmerk is van angst en depressie. Hoe hier het beste rekening mee kan worden gehouden hangt af van het type studie. Ook moedigden we genetisch epidemiologen aan om vooral te blijven werken aan studies naar de genetica van angst en depressie. Vooruitgang in methode, fenotype definitie en samenwerking zullen onvermijdelijk leiden tot succesvolle genetische studies van angst en depressie.



Dankwoord

Het is af! Het was een hele reis (zoiets als met de bus van IJsselstein naar Rotterdam), maar de eindhalte is bereikt en nu is het tijd om iedereen te bedanken die dit proefschrift mede mogelijk heeft gemaakt.

Dit proefschrift is gebaseerd op gegevens van 1000en deelnemers aan de Rotterdam Study. Allereerst daarom mijn grote dank aan hen. Ik wens iedere onderzoeksgroep zulke loyale deelnemers toe!

Dan mijn promotoren. Niels, dank voor de mogelijkheid om mijn promotieonderzoek bij de afdeling Psychiatrie te doen. En dank ook voor de vrijheid die je me daarin gaf. Henning, jij hebt me vanuit de afdeling Epidemiologie de afgelopen jaren bijgestaan. Je gaf snel commentaar als dat nodig was, je submitte ons depressieve symptomen artikel tijdens mijn zwangerschapsverlof en was altijd bereikbaar. Dank hiervoor!

Ook dank aan de leden van de grote en kleine commissie: Professor Hoogendijk, Professor Uitterlinden, Professor Franke, Professor Tendolkar, Professor Verhaak en Professor Kushner, dank voor het lezen van mijn manuscript en voor uw aanwezigheid tijdens de verdediging van mijn proefschrift.

Voor het goed laten verlopen van een onderzoek met zo'n enorme hoeveelheid gegevens is de inzet van een heleboel mensen onmisbaar. In het bijzonder wil ik Nano, Jolande, René en Frank noemen, die altijd enthousiast te hulp schoten bij welke vraag dan ook.

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Dear Jack, it was a pleasure to work with you on the anxiety GWAS. I am sure the work

and the patience will be rewarded with a nice publication and even more important: insight in the genetics of anxiety disorders!

Ik heb met veel plezier bij het Erasmus gewerkt. Dat had zeker ook te maken met mijn kamergenoten. Lieve Astrid, je was een leuke, vrolijke kamergenoot. Dank voor je altijd relativerende woorden. Asia, wij deelden ons buro bij de psychiatrie, zaten ook regelmatig samen op de kamer en ruilden werk uit. Nu zijn we allebei (bijna) klaar, veel succes met afronden! Bij de epidemiologie nam ik de plek over van Julia (dank voor de perfect achtergelaten slaap- en angstsyntaxen) en begon ik met Martina, Marieke en Dika op de kamer. Daarna kwamen Rachel, Maris en Nese. Caroline zat eventjes op de kamer, de slaapgroep (Annemarie en Lisette) volgde daarna. Heidi heeft mijn plekje nu overgenomen. Jullie waren stuk voor stuk leuke, gezellige, betrokken kamergenoten. Dank voor de leuke tijd! Stefan, je wilde geen plekje in deze meidenkamer, maar het was wel altijd gezellig als je langs kwam. Dank voor je hulp bij een heleboel vragen.

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Lieve Raluca en Hille, ik ben blij dat jullie tijdens mijn verdediging achter mij staan. Dank voor de gezellige etentjes, die houden we er in!

Tot slot wil ik ook iedereen van buiten het Erasmus bedanken voor hun interesse in mijn onderzoek en hun steun en motiverende woorden vooral het afgelopen jaar. Ik wil een paar mensen in het bijzonder noemen. Allereerst Suus, Bieb, Marianne, Marjan en Jo, dank voor jullie inbreng in mijn stellingen. Wanneer gaan we weer naar Texel? Marjan, dank voor het lezen van mijn samenvatting tijdens één van je vele diensten! Bieb, dank voor de administratieve (en mentale) ondersteuning vanuit het Erasmus. Suus, jij bent mede-bedenker van de stellingen, al vind je zelf van niet. Dank voor je nuchtere blik op mijn proefschrift. David, jij wilde graag in het dankwoord, bij deze! Dank dat je je tijdens het weekendje Center Parcs rustig hebt gehouden, zodat ik dit dankwoord kon schrijven. Lieve Linda, fijn dat we de afgelopen jaren onze verhalen konden delen, zet em op, voor jou is et einde ook in zicht!

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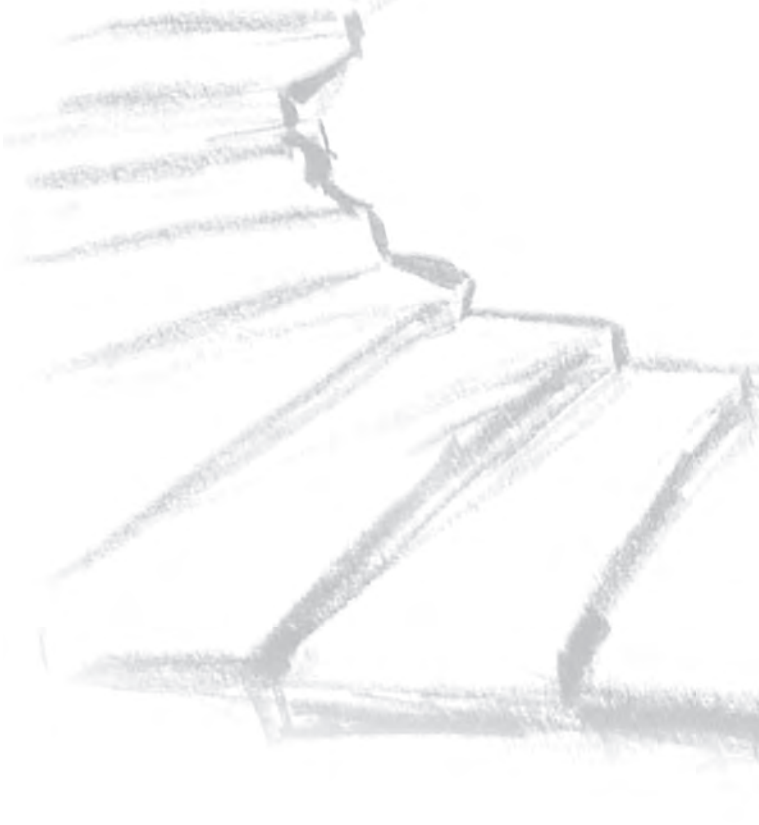
Laten we het de komende jaren iets rustiger houden. Ik ben enorm trots op jullie, lieve zusjes! Lieve Douwe, je bent altijd geïnteresseerd en hebt vaak genoeg geprobeerd om iets van mijn onderzoek te snappen. Vermoedelijk ben je één van de weinigen die dit boekje serieus zal doorbladeren. Je bent m'n leukste, liefste broertje! Lieve pap en mam, jullie hebben me altijd gesteund, dank daarvoor. De afgelopen anderhalf jaar heb ik vaak bij jullie zitten schrijven, terwijl jullie op Hege pasten. Ik kan jullie niet genoeg bedanken voor alles wat jullie voor ons doen.
Lieve Hege, wat zijn we blij met jou! Lieve Jasper, jij werd gek van mijn proefschrift waar ik de afgelopen jaren dag in, dag uit mee bezig was, maar je steunde me. En je verzorgde de randvoorwaarden: het eten stond klaar als ik laat thuis kwam, de boodschappen en de was waren gedaan en het huis was schoon. Mede dankzij jou is het nu af. Laten we nu met z'n drieën gaan genieten van alle vrije tijd die dit oplevert!



About the author

Curriculum Vitae

Karin Hek was born on September 8, 1984 in Nieuwegein, the Netherlands. After finishing her pre-university education at the Anna van Rijn College in Nieuwegein in 2002 (bilingual stream, English as a second language), she started to study Biomedical Sciences at VU University Amsterdam. In 2005 she obtained her Bachelor of Science degree (cum laude). In 2007 she obtained her Master of Science degree in Cancer Genomics and Developmental Biology (minor Communication and Education) from University Medical Center Utrecht. As part of her internships she worked at the Hubrecht Institute in the group of Prof. Dr. H. Clevers and at the Trimbos Institute.

In March 2008 she started the work presented in this thesis at the O3 Research Center, department of Psychiatry and the department of Epidemiology of the Erasmus MC Rotterdam under the supervision of Prof. Dr. C.L. Mulder and Prof. Dr. H. Tiemeier. As part of this PhD-project she obtained a Master of Science degree in Health Sciences (Genetic Epidemiology) in August 2011 from the Netherlands Institute for Health Sciences (NIHES).

In June 2012 she started working as a researcher at the NIVEL (Netherlands Institute for Health Services Research).

PhD Portfolio

Name PhD student:	Karin Hek	PhD period:	2008–2012
Erasmus MC Department:	Psychiatry, Epidemiology	Promotor:	Prof. Dr. C.L. Mulder
Research School:	NIHES		Prof. Dr. H. Tiemeier

1. PhD training	**Year**	**Workload (ECTS)**
General academic skills		
- Biomedical English Writing and Communication	2011	4.0
- InDesign course	2012	0.3
Research skills		
- Principles of Research in Medicine	2008	0.7
- Methods of Clinical Research	2008	0.7
- Methods of Public Health Research	2009	0.7
- Health Economics	2009	0.7
- Conceptual foundation of Epidemiologic Study Design	2009	0.7
- Primary and Secondary Prevention Research	2009	0.7
- History of Epidemiological Ideas	2009	0.7
- Genomics in Molecular Medicine	2008	1.4
- Large-Scale Multicenter Studies	2008	0.4
- Study Design	2008	4.3
- Classical Methods for Data-analysis	2008	5.7
- Modern Statistical Methods		4.3
- Repeated Measurements in Clinical Studies	2009	1.0
- Psychiatric Epidemiology	2009	1.1
- Principles of Genetic Epidemiology	2008	0.7
In-depth courses (e.g., Research school, Medical Training)		
NIHES Master of Genetic Epidemiology		
- Genome-wide association analysis	2008	1.4
- Genetic-Epidemiologic Research Methods	2010	5.7
- SNPs and Human Diseases	2009	1.4
- Advances in Population-based Studies of Complex Genetic Disorders	2009	1.4
- Genetic Linkage Analysis: Model-free Analysis	2009	1.4
- Mendelian Randomization & Bayesian Modelling in Genetic Epidemiology	2011	1.1

Oral presentations			
-	Congress of the International Federation of Psychiatric Epidemiology, Vienna	2009	0.5
-	4th CHARGE meeting, Washington DC	2009	0.5
-	World Congress on Psychiatric genetics, Athens	2010	0.5
-	Studiemiddag divisie ouderen BAVO Europoort/Parnassia	2010	0.2
Other conferences			
-	KNAW conference The role of DNA polymorphisms in complex traits and diseases	2008	0.5
-	Houston 5th CHARGE meeting	2010	0.5
Seminars and workshops			
-	Workshop: workshop geven	2009	0.1
-	Training: omgaan met groepen	2010	0.2
-	CPO minicursus: Methodologie van Patiëntgebonden onderzoek en Voorbereiding van Subsidieaanvragen	2009	0.3
-	RIDE symposium 2009	2009	0.3
-	FEDERA Scientific Program 2009: Aging and the infirmities of old age: new insights and challenges!	2009	0.3
-	Annual CMSB (Center for Medical Systems Biology) Symposium	2009	0.3
-	Regular seminars at the department of psychiatry and the department of epidemiology	2008-2012	4.0
Other			
-	Short term visit at the group of Jack Hettema at the Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, Virginia.	2008	3.0
2. Teaching acitivities			
-	Tutor of 1st year medical students	2010	2.0
-	Supervision genetics research project minor students	2010	0.5

1 ECTS (European Credit Transfer System) equals a workload of 28 hours.

Publications

K Hek, A Demirkan, J Lahti, A Terracciano, A Teumer, MC Cornelis, N Amin, E Bakshis, J Baumert, J Ding, Y Liu, K Marciante, O Meirelles, MA Nalls, YV Sun, N Vogelzangs, L Yu, S Bandinelli, EJ Benjamin, DA Bennett, D Boomsma, A Cannas, LH Coker, E de Geus, PL De Jager, AV Diez-Roux, S Purcell, FB Hu, EB Rimm, DJ Hunter, MK Jensen, G Curhan, K Rice, AD Penman, JI Rotter, N Sotoodehnia, R Emeny, JG Eriksson, DA Evans, L Ferrucci, M Fornage, V Gudnason, A Hofman, T Illig, S Kardia, M Kelly-Hayes, K Koenen, P Kraft, M Kuningas, JM Massaro, D Melzer, A Mulas, CL Mulder, A Murray, BA Oostra, A Palotie, B Penninx, A Petersmann, LC Pilling, B Psaty, R Rawal, EM Reiman, A Schulz, JM Shulman, AB Singleton, AV Smith, AR Sutin, AG Uitterlinden, H Völzke, E Widen, K Yaffe, AB Zonderman, F Cucca, T Harris, K-H Ladwig, DJ Llewellyn, K Räikkönen, T Tanaka, CM van Duijn, HJ Grabe, LJ Launer, KL Lunetta, TH Mosley Jr., AB Newman, H Tiemeier, J Murabito. A genome-wide association study of depressive symptoms. Biological Psychiatry 2013. [in press]

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