Genetic Determinants of Depression

Sandra López León

The work presented in this thesis was conducted at the Genetic Epidemiology Unit, Departments of Epidemiology & Biostatistics and Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands. The Rotterdam Study is supported by the Erasmus University Medical Center and the Erasmus University Rotterdam, the Netherlands Organisation for Scientific Research (NWO), the Netherlands Organisation for Health Reseach and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII), the Municipality of Rotterdam, and the Center for Medical Systems Biology (CMSB). The ERF Study is supported by the Center for Medical Systems Biology (CMSB), the Netherlands Organization for Scientific Research (NWO), the Dutch Kidney Foundation, the Dutch Heart Foundation, the Dutch Brain Foundation, Internationale Stichting Alzheimer Onderzoek (ISAO), and the Dutch Diabetes Foundation. Special thanks to the general practitioners and pharmacists in the Ommord district for their contributions to the Rotterdam Study and to the general practitioners in the ERF region for their contributions to the ERF Study. The printing of this thesis was supported by the Department of Epidemiology & Biostatistics, the Department of Clinical Genetics, the Department of Public Health, the Erasmus University Rotterdam, AstraZeneca, Biogen International BV, J.E. Jurriaanse Stichting, Lundbeck BV, Servier, and Wyeth.

ISBN 978-90-9022993-5
Genetic Determinants of Depression. S López León
Thesis Erasmus University Rotterdam

Cover: Melencolia by Albrecht Durer. Reproduced with permission © Wake Forest University Print Collection
Printed by: PrintPartners Ipskamp, Enschede, the Netherlands, www.ppi.nl
Layout: Legatron Electronic Publishing, Rotterdam

Genetic Determinants of Depression

Genetische Determinanten van Depressie

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus

Prof. dr. S.W.J. Lamberts

en volgens besluit van het College voor Promoties.
De openbare verdediging zal plaatsvinden op

woensdag 14 mei 2008 om 13.45 uur

door

Sandra López León

geboren te Mexico Stad, Mexico.

Promotiecommissie

Promotoren:
Prof. dr. ir. C.M. van Duijn
Prof. dr. B.A. Oostra

Overige leden:
Prof. dr. D.I. Boomsma
Prof. dr. J. Del-Favero
Prof. dr. F.C. Verhulst

Copromotor:
Dr. A.C.J.W. Janssens

Dedicated to Albrecht

Contents

Publications/manuscripts based on the studies described in this thesis

López-León S, Janssens ACJW, González-Zuloeta Ladd AM, Del-Favero J, Claes S, Oostra BA, van Duijn CM. Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2008.

López-León S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM. The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005;57:999-1003.

López-León S, Janssens ACJW, Hofman A, Claes S, Breteler MMB, Tiemeier H, van Duijn CM. No association between the angiotensin-converting enzyme gene and major depression: a case-control study and meta-analysis. Psychiatr Genet 2006;16:225-6.

López-Léon S, Janssens ACJW, Tiemeier H, Hofman A, Aulchenko YS, Snijders PJLM, Claes S, Oostra BA, van Duijn CM. *A*ngiotensinogen M235T polymorphism and symptoms of depression in a population-based study and a family-based study. Psychiatr Genet 2008.

López-León S, Choy WC, Aulchenko YS, Claes S, Oostra BA, Mackenbach JP, van Duijn CM, Janssens ACJW. Genetic factors influence the clustering of depression among individuals with lower socioeconomic status. *Submitted.*

López-León S, Choy WC, Aulchenko YS, Claes S, Oostra BA, Mackenbach JP, van Duijn CM, Janssens ACJW. Shared genetic factors in the co-occurrence of symptoms of depression and cardiovascular risk factors. *Submitted.*

Choy WC, López-León S, Zillikens C, Aulchenko YS, Mackenbach JP, van Duijn CM, Janssens ACJW. Evaluating the evidence of shared etiology of symptoms of depression with fat and muscle mass. *Submitted.*

Introduction to the thesis

"There is mere anger and grief and sad dejection of mind.....those affected with melancholy are not every one of them affected according to one particular form but they are suspicious of poisoning or flee to the desert from misanthropy or turn superstitious or contract a hatred of life. The patients are dull or stern, dejected or unreasonably torpid.....they also become peevish, dispirited and start up from a disturbed sleep."

Arateus (AD 150)

1

Introduction

Depression is a mental condition marked by ongoing feelings of sadness, despair, loss of energy, and difficulty dealing with normal daily life. The impact of depression on personal life can be devastating, as it can result in increased suffering, disability, loss of productivity, deliberate self-harm and it increases the risk of suicide.[1,2]

When used to describe a mood, depression refers to what may be feelings of sadness, despair, and discouragement, but depression is also used to describe the clinical diagnosis of major depressive disorder (MDD).[2] According to the diagnostic and statistical manual of mental disorders (DSM-IV), a person suffers from MDD when depressed mood and/or loss of interest or pleasure are present two or more weeks. MDD is characterized by the presence of five or more of the following nine symptoms: depressed mood, diminished interest or pleasure, significant weight loss, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive or inappropriate guilt, diminished ability to think or concentrate and recurrent thoughts of death.[2]

Though MDD is the outcome of clinical interest, psychiatric research is focusing more and more on quantitative outcomes or endophenotypes.[3] The endophenotypes are also heritable and are probably determined more directly by genes. Another advantage of using quantitative outcomes is that criteria to distinguish affected from non-affected are often arbitrary.[4] Studies of quantitative traits may be more powerful as the information on the total distribution of a trait is used. It should be noted however, that the relation between a quantitative trait such as symptoms of depression

and MDD is not unique. Symptoms of depression may be part of MDD but can also be indicative of normal grief, bipolar disorder, anxiety, substance abuse, dysphoria associated with other psychiatric disorders, and a variety of underlying medical illnesses. Symptoms of depression, which may or may not be due to MDD, is used in a similar way as the phenotype of blood pressure. Blood pressure is studied as a determinant of multiple outcomes such as cardiovascular disease, stroke, dementia, or retinopathology; even though moderate increases in blood pressure often do not lead to clinical pathology. Studies of blood pressure have increased our knowledge about determinants of cardiovascular disease, likewise studies of symptoms of depression may be relevant to our understanding of MDD. The work presented in this thesis focuses for a large part on symptoms of depression.

Epidemiology

Depression is the most frequently occurring psychiatric disease with a life-time incidence of around 25%.[5] It is the leading cause of disability among individuals between 15 to 44 years of age, and is expected to become the second leading source of disability across all ages by 2020.[6] The prevalence of symptoms of depression is higher in women, in singles, in individuals with severe alcohol use, those exposed to life stressors, negative early childhood experiences or those with a socioeconomic disadvantage.[7] It is clear that genetic factors play a significant role.[8] Genetic epidemiological studies in families, twins and adopted individuals consistently show evidence for a genetic basis for depression.[9] Heritability estimates, which denote the proportion of variability in a trait that can be attributed to additive genetic factors and shared early environmental factors, range from 0.17 to 0.78, depending on the population investigated.[10] Further, several studies have shown that first-degree relatives of depressed patients have more than a three-fold increase in their risk of developing a depressive disorder compared with the general population.[11]

Etiology

The etiology of depression is still far from being understood. It is known that it is multifactorial,[7] and most likely a result of many genetic and environmental factors.[10] Serotoninergic and norepinephrine systems have been the main focus of research in depression.[12] Serotonin is involved in the regulatory control of affect, aggression, sleep, and appetite, among others.[13] Norepinephrine is involved in many functions that are profoundly disturbed in depression, including mood, arousal, appetite, reward, and drives.[13] The hypothesis that these neurotransmitters are involved in the etiology of depression derive furthermore from pharmacological experience, as these neurotransmitters have been the basis of treatment for the last fifty years.[12]

There are many other proposed mechanisms involved in depression.[15] For example the dopamine system is especially important for pleasure, sex, and psychomotor activity.[15] The hypothalamic pituitary adrenal axis (HPA) controls reactions to stress and regulates mood, sexuality, and energy usage.[16] The hypersecretion of corticotropin-releasing factor is thought to mediate sleep, appetite disturbances, reduced libido, and psychomotor changes.[17] The renin angiotensin system directly modulates the HPA axis by stimulating corticotropin hormones.[18,19] Cortisol inhibits neurogenesis leading to hippocampal volume loss that may mediate cognitive symptoms of depression.[20] Cholinergic neurons, secreting acetylcholine at their dendritic terminals, are generally antagonistic to the function of serotonin and noradrenalin. Besides, there is increasing interest in second messenger systems, phosphorylation G proteins, signal transduction, deoxyribonucleic acid (DNA) transcription, and messenger ribonucleic acid (RNA) translation.[15] Genetic factors obviously play a role in these biochemical mechanisms,[15] and major efforts are being made to identify the corresponding genes which are associated to depression. The identification of these genes may help to develop new classes of antidepressants with more selective action on specific neurotransmitter receptors.

Genetic risk factors

During the past 30 years, many association studies of depression have been performed, but so far most findings have been inconclusive. Meta-analyses for MDD have been conducted for five polymorphisms in four genes.[21-26] These meta-analyses addressed the serotonin 5-HT-2A receptor (*HTR2A*), the methylenetetrahydrofolate reductase (*MTHFR),* the serotonin transporter (*SLC6A4*), and the tyrosine hydroxylase (*TH*) genes.[21-26] The *SLC6A4* 44bp Ins/Del polymorphism is the most frequently studied to date and has been evaluated in three previous meta-analyses.[21,24,26] The first meta-analysis, including only 275 patients, showed a statistically significant increased MDD risk for carriers of the S allele (OR, 1.23; CI, 1.01-1.42),[24] but two subsequent larger meta-analyses were unable to confirm this finding.[21,26] The *MTHFR* gene was also found to be significantly associated to depression (OR, 1.36; CI, 1.11-1.67).[25]

In recent years, genome-wide linkage screens have been conducted in families with multiple affected relatives.[27-29] Despite differences in ascertainment, clinical assessment, phenotype definition and analysis technique, an increasing number of replications of findings have been observed. Seven regions were found to be replicated within 20 cM in at least two studies: 4q, 5q, 6q, 8p, 11q, 15q and 18q, although no region was confirmed in all studies.[27-30] From these studies, the largest genome-wide linkage screen which was performed in 656 families, found genome

wide significant evidence for linkage to MDD in chromosome 15q25-q26, 17p12 and 8p22-p21.3.[28] Further fine mapping of the chromosome 15q25-q26 region suggested that several genes in this region increase susceptibility to MDD, but no single gene could be pinpointed.[31] The authors refrained from speculating on possible candidate genes in the region. Another region implicated in MDD is the 12q22-23 region,[32,33] which overlaps with a region of interest of other psychiatric phenotypes such as bipolar disorder,[33] and anxiety.[34] Further research is warranted to identify genes in these regions that are associated with risk of depression.

Aim of this study

Genetic epidemiological studies may enable us to unravel the etiology of depression through the identification of genes and therewith the proteins involved. The aim of this genetic epidemiological thesis is to study candidate genes that may play a role in the etiology of depression, and to obtain new insights about biological pathways that may be involved in the etiology of depression by identifying other disorders that share etiological pathways and hence may share the same underlying genes with depression.

Chapters 2 to 5 focus on the first aim. Chapter 2 describes a review of all genetic studies on major depressive disorder and meta-analyses for polymorphisms that had been investigated in at least three studies. In Chapter 3 the role of the *DRD4* 48bp polymorphism in mood disorders is re-evaluated with a meta-analysis. This polymorphism has been consistently associated with attention deficit hyperactivity disorder , schizophrenia, and novelty-seeking.[35-37] It has also been studied in relation to depression in past studies. However the findings were not consistent.[38-41] In Chapter 4 the role of the angiotensin I converting enzyme gene (*ACE* I/D) in MDD is re-evaluated by conducting a case-control study and a meta-analysis. ACE is a component of the renin angiotensin system (RAS), a system that is assumed to be involved in depression. Evidence for this relationship comes from research showing that ACE inhibitors have antidepressant effects,[42] and that the RAS modulates the HPA axis by stimulating corticotrophin hormones.[19] In Chapter 5 the association between the *AGT* M235T polymorphism and symptoms of depression is examined in two independent populations.

Chapters 6 to 8 focus on co-morbidity and the identification of biological pathways that may be involved in the etiology of depression. In Chapter 6 the role of shared genetic factors in the co-occurrence of symptoms of depression and socio-economic status is investigated. In Chapter 7 the impact of shared genetic factors in the co-occurrence of symptoms of depression and cardiovascular risk factors such

as blood pressure, glucose levels and lipid parameters is examined. In Chapter 8 the relationship of shared genetic and environmental factors in the co-occurrence of body composition and symptoms of depression is studied. Finally, the results of the study are discussed in Chapter 9.

References

1. **Lorant V, Deliege D, Eaton W, Robert A, Philippot P, Ansseau M.** Socioeconomic inequalities in depression: a meta-analysis. Am J Epidemiol 2003;157:98-112.
2. **American Psychiatric Association.** Diagnostic and Statistical Manual of Mental Disorders-IV. Washington DC, 1994.
3. **Faraone SV, Santangelo, S.** Research designs and methods in psychiatry. Amsterdam: Ed. Elsevier, 1992.
4. **Carey G GI.** Defining cases by genetic criteria. In What is a Case? London: Grant McIntyre, 1981.
5. **Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al.** The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003;289:3095-105.
6. **Murray CJ, López AD.** Evidence-based health policy-lessons from the Global Burden of Disease Study. Science 1996; 274: 740–743
7. **Blazer DG, 2nd, Hybels CF.** Origins of depression in later life. Psychol Med 2005;35:1241-52.
8. **Kendler KS.** Major depression and the environment: a psychiatric genetic perspective. Pharmacopsychiatry 1998;31:5-9.
9. **Craddock N, Forty L.** Genetics of affective (mood) disorders. Eur J Hum Genet 2006;14:660-8.
10. **Sullivan PF, Neale MC, Kendler KS.** Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157:1552-62.
11. **Weissman MM, Gershon ES, Kidd KK, Prusoff BA, Leckman JF, Dibble E, et al.** Psychiatric disorders in the relatives of probands with affective disorders. The Yale University – National Institute of Mental Health Collaborative Study. Arch Gen Psychiatry 1984;41:13-21.
12. **Hardman JG, Limbird LE, Molinoff PB, Ruddon RW.** Goodman & Gilman, The Pharmacological Basis of Therapeutics. New York: A. McGraw-Hill, 1996.
13. **Maes M MH.** The serotonin hypothesis of major depression. In Psychopharmacology: The Fourth Generation of Progress. New York: Raven Press, 1995.
14. **Schatzberg AF SJ.** Recent studies on norepinephrine systems in mood disorders. In Psychopharmacology: The Fourth Generation of Progress. New York: Raven Press, 1995.
15. **Kaplan HI, Sadock BJ.** Sypnosis of Psychiatry 8th Edition. Baltimore, USA.: Williams & Wilkins, 2005.
16. **Engelmann M, Landgraf R, Wotjak CT.** The hypothalamic-neurohypophysial system regulates the hypothalamic-pituitary-adrenal axis under stress: an old concept revisited. Front Neuroendocrinol 2004;25:132-49.
17. **Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB.** The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 1999;160:1-12.
18. **Jezova D, Ochedalski T, Kiss A, Aguilera G.** Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. J Neuroendocrinol 1998;10: 67-72.
19. **Plotsky PM, Owens MJ, Nemeroff CB.** Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. Psychiatr Clin North Am 1998;21:293-307.
20. **Sapolsky RM.** Depression, antidepressants, and the shrinking hippocampus. Proc Natl Acad Sci U S A 2001;98:12320-2.
21. **Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT.** Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiatr Genet 2005;133:110-5.

22. **Zintzaras E.** C677T and A1298C methylenetetrahydrofolate reductase gene polymorphisms in schizophrenia, bipolar disorder and depression: a meta-analysis of genetic association studies. Psychiatr Genet 2006;16:105-15.
23. **Furlong RA, Rubinsztein JS, Ho L, Walsh C, Coleman TA, Muir WJ, et al.** Analysis and metaanalysis of two polymorphisms within the tyrosine hydroxylase gene in bipolar and unipolar affective disorders. Am J Med Genet 1999;88:88-94.
24. **Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES, et al.** Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. Am J Med Genet 1998;81:58-63.
25. **Lewis SJ, Lawlor DA, Davey Smith G, Araya R, Timpson N, Day IN, et al.** The thermolabile variant of MTHFR is associated with depression in the British Women's Heart and Health Study and a meta-analysis. Mol Psychiatry 2006;11:352-60.
26. **Anguelova M, Benkelfat C, Turecki G.** A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry 2003;8:574-91.
27. **Zubenko GS, Maher B, Hughes HB, 3rd, Zubenko WN, Stiffler JS, Kaplan BB, et al.** Genome-wide linkage survey for genetic loci that influence the development of depressive disorders in families with recurrent, early-onset, major depression. Am J Med Genet B Neuropsychiatr Genet 2003;123:1-18.
28. **Holmans P, Weissman MM, Zubenko GS, Scheftner WA, Crowe RR, Depaulo JR, Jr., et al.** Genetics of recurrent early-onset major depression (GenRED): final genome scan report. Am J Psychiatry 2007;164:248-58.
29. **Camp NJ, Lowry MR, Richards RL, Plenk AM, Carter C, Hensel CH, et al.** Genome-wide linkage analyses of extended Utah pedigrees identifies loci that influence recurrent, early-onset major depression and anxiety disorders. Am J Med Genet B Neuropsychiatr Genet 2005;135:85-93.
30. **Camp NJ, Cannon-Albright LA.** Dissecting the genetic etiology of major depressive disorder using linkage analysis. Trends Mol Med 2005;11:138-44.
31. **Levinson DF, Evgrafov OV, Knowles JA, Potash JB, Weissman MM, Scheftner WA, et al.** Genetics of recurrent early-onset major depression (GenRED): significant linkage on chromosome 15q-5-q26 after fine mapping with single nucleotide polymorphism markers. Am J Psychiatry 2007;164: 259-64.
32. **Abkevich V, Camp NJ, Hensel CH, Neff CD, Russell DL, Hughes DC, et al.** Predisposition locus for major depression at chromosome 12q22-12q23.2. Am J Hum Genet 2003;73:1271-81.
33. **McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, et al.** Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum Mol Genet 2005;14: 3337-45.
34. **Fullerton J, Cubin M, Tiwari H, Wang C, Bomhra A, Davidson S, et al.** Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative-trait loci that influence variation in the human personality trait neuroticism. Am J Hum Genet 2003;72:879-90.
35. **Schinka JA, Letsch EA, Crawford FC**. DRD4 and novelty seeking: results of meta-analyses. Am J Med Genet 2002;114:643-8.
36. **Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, et al.** Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:768-70.
37. **Glatt SJ, Faraone SV, Tsuang MT.** Schizophrenia is not associated with DRD4 48-base-pair-repeat length or individual alleles: results of a meta-analysis. Biol Psychiatry 2003;54:629-35.
38. **Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, et al.** Dopamine D2, D3 and D4 receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 1996;40: 7-13.
39. **Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, et al.** Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry 1999;4:389-92.

40. **Oruc L, Verheyen GR, Furac I, Jakovljevic M, Ivezic S, Raeymaekers P, et al.** Analysis of the tyrosine hydroxylase and dopamine D4 receptor genes in a Croatian sample of bipolar I and unipolar patients. Am J Med Genet 1997;74:176-8.
41. **Serretti A, Macciardi F, Cusin C, Lattuada E, Lilli R, Smeraldi E.** Dopamine receptor D4 gene is associated with delusional symptomatology in mood disorders. Psychiatry Res 1998;80:129-36.
42. **Vuckovic A, Cohen BM, Zubenko GS.** The use of captopril in treatment-resistant depression: an open trial. J Clin Psychopharmacol 1991;11:395-6.

2

META-ANALYSES OF GENETIC STUDIES ON MAJOR DEPRESSIVE DISORDER

Abstract

The genetic basis of major depressive disorder (MDD) has been investigated extensively, but the identification of MDD genes has been hampered by conflicting results from underpowered studies. We review all MDD case-control genetic association studies published before June 2007 and perform meta-analyses for polymorphisms that had been investigated in at least three studies. The study selection and data extraction were performed in duplicate by two independent investigators. The 183 papers that met our criteria studied 393 polymorphisms in 102 genes. Twenty-two polymorphisms (6%) were investigated in at least three studies. Seven polymorphisms had been evaluated in previous meta-analyses, of which for five new were data available. Hence, we performed meta-analyses for 20 polymorphisms in 18 genes. Pooled odds ratios (ORs) with 95% confidence intervals (CI) were calculated. Statistically significant associations were found for the *APOE* ε2 (OR, 0.51), *GNB3* 825T (OR, 1.38), *MTHFR* 677T (OR, 1.20), *SLC6A4* 44bp Ins/Del S (OR, 1.11) alleles and the *SLC6A3* 40bpVNTR 9/10 genotype (OR, 2.06). To date, there is statistically significant evidence for six MDD susceptibility genes (*APOE, DRD4, GNB3, MTHFR, SLC6A3* and *SLC6A4*).

Introduction

Depression is the leading cause of disability for individuals of 15 to 44 years of age and is expected to become the second cause of disability worldwide for individuals of all ages by the year 2020.[1] MDD is caused by a complex interaction of a large number of genetic and non-genetic factors, each with a relatively small contribution to the disorder.[2] The total contribution of genetic factors in the origin of disease, the heritability, is estimated at 37%.[3] First-degree relatives of MDD patients have a two- to threefold increased risk of MDD compared to the general population.[4]

Since the first case-control study on the relationship between polymorphisms and depression in 1978,[5] many genetic association studies have been conducted. Nevertheless, this accumulation of research has not resulted in the identification of many MDD susceptibility genes, because the findings of the association studies have often been inconsistent.[6] A first explanation for these inconsistencies concern methodological differences between the studies, such as differences in study design, study population and MDD diagnostic criteria, which hamper the comparability of the studies. A second explanation is that many studies had small sample sizes and therefore insufficient statistical power to demonstrate statistically significant effects of these low-risk susceptibility genes.[7] The impact of latter problem can be reduced by pooling single studies in meta-analyses.[8]

To date, meta-analyses for MDD have been conducted for seven polymorphisms in six genes (Table 1). These meta-analyses included the genes encoding for angiotensin I-converting enzyme (*ACE*), dopamine receptor D4 (*DRD4*), serotonin 5-HT-2A receptor (*HTR2A*), methylenetetrahydrofolate reductase (*MTHFR),* serotonin transporter (*SLC6A4*) and tyrosine hydroxylase (*TH*).[9-16] The *DRD4* gene was significantly associated to MDD (odds ratio (OR), 1.73; 95% confidence interval (CI), 1.29-2.32),[12] and the *MTHFR* was significantly associated to depression (OR, 1.36; CI, 1.11-1.67).[15] *SLC6A4* 44bp Ins/Del is the most frequently studied polymorphism to date and has been evaluated in three previous meta-analyses.[9,14,16] The first meta-analysis, including only 275 patients, showed a statistically significant increased MDD risk for carriers of the S allele (OR, 1.23),[13] but two subsequent larger meta-analyses were unable to confirm this finding.[9,16]

Many other genes have been studied in relation to MDD. Our aim is to review all case-control studies on the association between genetic polymorphisms and MDD and perform meta-analyses for polymorphisms that had been investigated in at least three studies.

Table 1 Meta-analyses on major depressive disorder published before June 2007

Gene	Polymorphism	Comparison	Cases	Controls	OR	(95% CI)	Year	Reference
ACE	Ins/Del Intron 16	ID vs II	586	5,169	0.85	(0.55-1.30)	2006	[11]
		DD vs II			1.01	(0.71-1.45)		
DRD4	48 bp VNTR Exon 3	2 vs others	319	808	1.73	(1.29-2.32)**	2005	[12]
HTR2A	T102C	C vs T	768	959	0.96	(0.84-1.11)	2003	[16]
MTHFR	C677T	T vs C	291	897	1.15	(0.97-1.36)	2006	[10]
		TT vs CC	1,280	10,429	1.36	(1.11-1.67)*	2006	[15] †
SLC6A4 (SERT)	VNTR Intron 2	9 vs 12	299	772	1.16	(0.55-2.50)	1998	[14]
		10 vs 12			1.01	(0.83-1.23)		
		9 vs 10,12	592	2,094	1.24	(0.72-2.14)	2003	[16]
		10 vs 9,12			0.96	(0.84-1.10)		
		12 vs 9, 10			1.03	(0.89-1.18)		
		Continuous‡	653	1,817	0.99	(0.92-1.06)	2005	[9]
	44bp Ins/Del Promotor	S vs L	275	739	1.23	(1.01-1.42)*	1998	[13]
		S vs L	941	2,110	1.08	(0.96-1.22)	2003	[14]
		S vs L	1,961	3,402	1.05	(0.96-1.14)	2005	[16]
TH	Tetranucleotide repeat	2 vs 1	204	359	0.86	(0.59-1.26)	1999	[13]
		3 vs 1			0.88	(0.57-1.34)		
		4 vs 1			0.99	(0.68-1.42)		
		5 vs 1			0.76	(0.55-1.05)		

*$p \leq 0.05$, **$p \leq 0.001$

†Also includes studies that identified cases by questionnaires for assessment of symptoms of depression.

‡ Odds ratio obtained from logistic regression analyses considering allele length as a continuous variable (9, 10 and 12).

Materials and Methods

Data Sources

We searched PubMed for genetic case-control association studies on MDD published before June 2007. The search strategy was based on the keywords *unipolar depression*, *major depression, depressive disorder* or *affective disorder* in combination with *chromosome, gene, allele* or *polymorphism*. A second search was carried out using the name of the genes identified in the first search in combination with a broader keyword search: *unipolar, depression*, *depressive* or *affective*. In addition, we searched the PubMed, HuGeNet, ISI Web of Science databases, the Genetic Association Database, and the reference lists of the retrieved articles.

Study Selection

Genetic association studies were selected if they had applied a case-control design and compared adults diagnosed with standard diagnostic criteria for MDD. Studies were excluded if (1) cases were selected by questionnaires assessing symptoms of depression, (2) the study population included bipolar patients, (3) the distribution of genotypes in the control population was not in Hardy-Weinberg Equilibrium, or (4) if the data were re-used in a larger study on the same polymorphism. Study selection and data extraction were performed in duplicate by two independent investigators (S.L.L. and A.G.Z.) and discrepancies were discussed with a third investigator (A.C.J.W.J).

Data Synthesis

Meta-analyses were performed for polymorphisms that had been investigated in at least three studies. Published meta-analyses were updated when new studies were available. ORs were summarized using both random effects and fixed effects meta-analyses with 95% CIs as outlined by DerSimonian and Laird.[17] The degree of heterogeneity between the study results was assessed with the I^2 statistics.[18] Forest plots were obtained for meta-analyses with statistically significant results, presenting random effect meta-analyses if there was evidence of heterogeneity (I^2>50),[19] and fixed effects meta-analyses if there is no heterogeneity. Sources of heterogeneity were explored in sensitivity analyses by removing one study at a time and calculating pooled ORs on the remaining studies. Sensitivity analysis also examines the extent to which pooled ORs were determined by significant effects of the first published study.[7] Publication bias was evaluated by visual inspection of the funnel plots.[20] Cochrane Review Manager Version 4.2 was used for all statistical analyses. P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

Of the 215 articles that met our inclusion criteria, 32 were excluded. Five studies selected cases by questionnaires assessing symptoms of depression,[21-25] nine studied MDD and bipolar depression without reporting separate genotype frequencies for the subtypes,[26-34] eight had genotype frequencies in controls that were not in Hardy-Weinberg Equilibrium,[35-42] and ten reported data that had later been re-used in a larger study for the same polymorphism.[43-52] The remaining 183 articles studied 393 different polymorphisms in 102 genes (Figure 1). Of the 393 polymorphisms, 371 were investigated in one or two studies (Table 2). Only 22 (6%) polymorphisms in 20 (19%) genes were investigated in more than 3 studies.

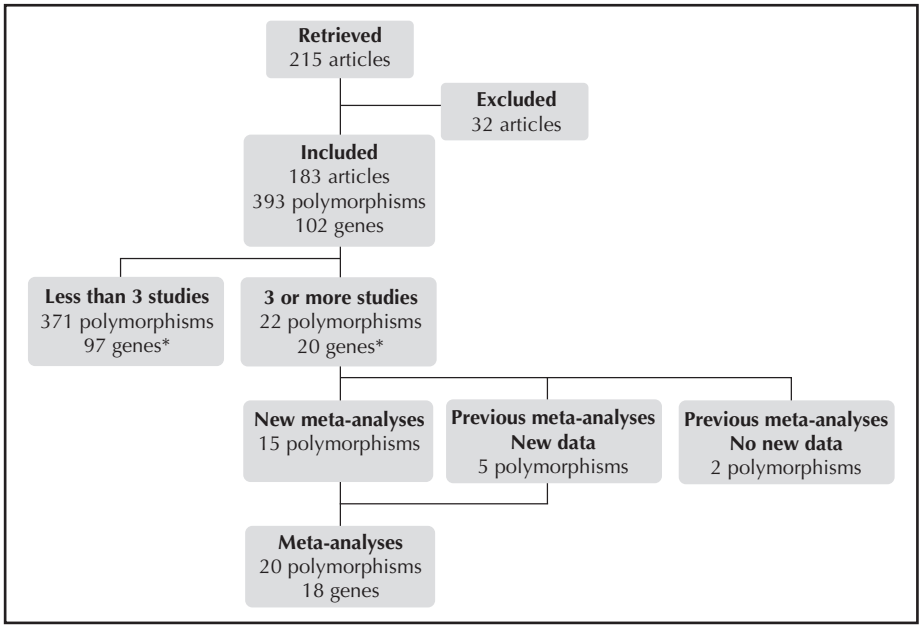

Figure 1 Selection of studies for the meta-analyses

* The number of genes does not add up to 102, because 8 genes were investigated for multiple polymorphisms of which some were addressed in more than three studies.

Of the 22 polymorphisms, 7 had been reviewed in previous meta-analyses, of which for 5 polymorphisms (*ACE* I/D, *HTR2A* T102C, *MTHFR* C677T, *SLC6A4* VNTR and *SLC6A4* 44bp Ins/Del) new data was available. Hence, we performed meta-analyses for 20 polymorphisms in 18 genes (Table 3). Five polymorphisms showed statistically significant association to MDD: apolipoprotein E (*APOE*), guanine nucleotide-binding protein (*GNB3*) 825T, *MTHFR* 677T, dopamine transporter (*SLC6A3*) 40bp

VNTR, and the *SLC6A4* 44bp Ins/Del (Figure 2). Figure 3 shows the funnel plots for the statistically significant meta-analysis.

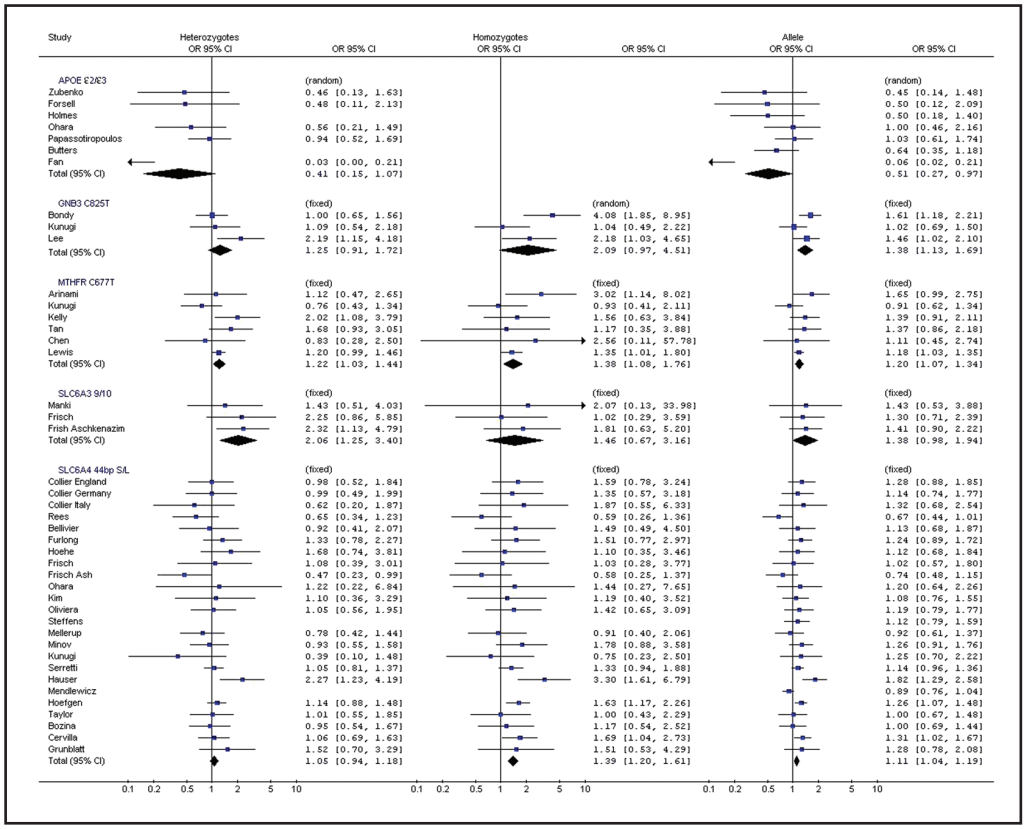

Figure 2 Forest plots for statistically significant meta-analyses

APOE

The *APOE* ε2 allele was examined in 7 studies including a total of 827 cases and 1,616 controls.[53-59] One study found a very strong protective effect for the ε2 allele (OR, 0.06; CI, 0.02-0.21),[57] while the others showed moderate protective effects (OR varying from 0.45 to 1; Figure 2). The pooled OR of the ε2 allele compared to the ε3 allele was 0.51 (CI, 0.27-0.97). The per allele meta-analysis of studies in Caucasian populations also showed a statistically significant pooled OR (0.72; CI, 0.51-1.00) with no evidence for between-study heterogeneity.[54-58] There were not enough studies to perform a meta-analysis of only studies from Asian origin. The pooled OR

of the ε2 allele was not statistically significant when the first published study was removed from the analysis (OR, 0.51; CI, 0.25-1.05).[57] The funnel plot show that the three smaller studies had lower ORs than the larger studies (Figure 3), but that there was no convincing evidence for publication bias.

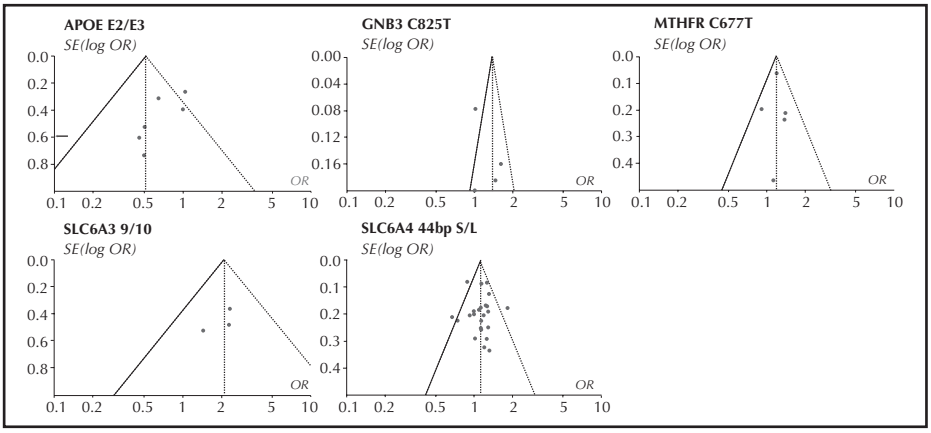

Figure 3 Funnel plots for statistically significant meta-analyses
Each dot represents one paper. Se(log OR) = standard error of the log Odds Ratio.

GNB3

The association between the *GNB3* C825T polymorphism and MDD was investigated in 3 studies with a total of 375 cases and 492 controls.[45,60,61] All ORs in the individual studies were greater than 1. The per allele meta-analysis, testing for a trend by the number of T alleles, showed a significant effect of the T allele (OR, 1.38; CI, 1.13-1.69; Figure 2).

MTHFR

Six studies investigated the *MTHFR* C677T polymorphism in a total of 875 cases and 3,859 controls. [15,62-66] Five studies showed an increased MDD risk for homozygous carriers of the T allele, but this association was only statistically significant in 1 study (Figure 2).[62] The combined OR was 1.20 (CI, 1.07-1.34) for the T allele, 1.38 (CI, 1.08-1.76) for homozygous carriers, and 1.22 (CI, 1.03-1.44) for heterozygous carriers. These associations were still statistically significant when the first published study was removed (OR_{TvsC}, 1.18; CI, 1.05-1.32, OR_{TCvsCC}, 1.22; CI, 1.03-1.44, $OR_{T-TvsCC}$, 1.31; CI, 1.02-1.69).[62]

Table 2 Genes investigated in relation to major depressive disorder in less than three studies

AACT [92]	0/1	*CHRM2* [93]	1/1	*DRD1* [94]	1/7	*GABRA5* [95]	1/1	*HTR3A* [94,96]	0/18	*NOS3* [97]	0/3	*PDE11A* [98]	1/1
ACE [99,100]	2/38	*CHRNA7* [101]	1/1	*DRD2* [67,94,102]	1/12	*GABRA6* [103]	0/1	*HTR3B* [96]	1/12	*NPY* [104,105]	2/2	*PENK* [94]	0/2
ADCY9 [106]	0/2	*CLOCK* [107]	0/1	*DRD3* [94]	0/2	*GADI* [108]	0/3	*HTR5A* [109,110]	2/2	*OASL* [111]	0/5	*POMC* [94]	0/6
ADORA2A [112]	0/1	*CNR1* [113]	0/1	*DRD4* [94]	0/6	*GCCR* [114,115]	4/6	*HTR6* [116]	0/1	*OPRD1* [94]	0/2	*PLA2G2A* [117,118]	2/2
ADRA2A [119]	0/1	*CNTF* [120,121]	0/2	*DRD5* [94]	0/1	*GMIP* [122]	4/4	*IL1B* [123]	0/1	*OPRK1* [94]	0/7	*SLC6A2* [124,125]	0/1
ADRB1 [126]	0/1	*COMT* [127,128]	0/1	*DTNBP1* [129]	0/5	*GNAL* [130]	0/2	*IL6* [131]	0/1	*OPRM1* [94]	0/4	*SLC6A4* [94]	0/3
AR [132]	1/1	*CRHBP* [133]	2/7	*DUSP6* [134]	0/1	*GNAS* [130]	0/1	*IL10* [135]	0/1	*P2RX4* [111]	0/6	*TAC1* [99]	0/5
AVPR1B [136]	1/5	*CRHR1* [137]	1/3	*DXS7* [138]	1/1	*GPR50* [139]	2/3	*LBP* [140]	1/1	*P2RX7* [111]	1/16	*TACR1* [99]	0/5
BDNF [121, 41]	0/2	*CRHR2* [142]	1/5	*ESR1* [132,143]	1/1	*GYPA* [144,145]	0/1	*LRP* [140]	0/1	*PAM* [99]	0/5	*TH* [13]	1/1
CAMKK2 [111,146]	0/2	*CTLA4* [147]	0/1	*ESR2* [132]	1/1	*HP* [148,149]	0/1	*M6PR* [150]	1/1	*PDE2A* [98]	0/1	*TPH1* [94,151]	0/3
CCK [94,152]	0/4	*CYP2C9* [153]	1/1	*FACL4* [154]	1/1	*HTR1A* [94]	0/1	*MAOA* [155,156]	0/3	*PDE5A* [98]	1/1	*TPH2* [157-162]	7/34
CCKAR [94]	1/7	*D2S2944* [163-165]*	1/1	*FZD3* [166]	0/4	*HTR1B* [94]	0/9	*MAOB* [155]	0/1	*PDE6C* [98]	0/2	*TNF* [167]	1/1
CCKBR [94]	0/4	*DDC* [168,169]	0/2	*G72* [170]	1/2	*HTR2A* [94]	0/2	*NGFR* [171]	1/1	*PDE9A* [98]	1/1	*WFS1* [172-174]	3/9
CCL2 [175]	1/1	*DISC1* [176]	1/13	*GABRA1* [103,177]	0/5	*HTR2C* [94]	0/1	*NOS1* [178]	0/1	*PDE10A* [98]	0/3		

Numbers indicate the number of polymorphisms that were significantly associated to MDD out of the total number of different polymorphisms studied.
Genes that were also included in the meta-analyses are here listed for other polymorphisms.
*Concerns a genetic marker.

SLC6A3

The *SLC6A3* 40bp VNTR polymorphism was investigated in 3 studies including a total of 151 cases and 272 controls.[67,68] Each study showed an increased MDD risk for carriers of the 9/10 genotype compared to the 10/10 genotype, but only 1 study was statistically significant.[68] The pooled OR for the 9/10 genotype compared to the 10/10 genotype was 2.06 (CI, 1.25-3.40).

SLC6A4

Twenty-four studies investigated *SLC6A4* 44bp Ins/Del short/long (S/L) polymorphism including a total of 3,752 cases and 5,707 controls. [14,42,52,69-77] Twenty of the individual studies reported an OR higher than 1 for the S allele versus the L allele, but only in 3 studies was this association statistically significant (Figure 2).[78-80] The pooled OR for the S allele was 1.11 (CI, 1.04-1.19) and the pooled OR for homozygous carriers was 1.39 (CI, 1.20-1.61). When the first published study was removed, these ORs remained statistically significant (OR_{SvsL}, 1.11; CI, 1.01-1.22, OR_{SSvsLL}, 1.37; CI, 1.16-1.61).[81] The funnel plot shows asymmetry as 17 out of the 24 studies show a higher OR than the pooled estimate, but there was no evidence for publication bias towards studies with higher OR (Figure 3).

Other Genes

The remaining meta-analyses were not significant, their odds ratios ranged from 0.74 to 0.99 for protective effects and from 1.09 to 1.63 for risk alleles/genotypes. Figure 4 shows the ORs in relation to the total number of cases and controls that were included in the meta-analyses. Fifteen out of 20 meta-analyses included less than 3,000 subjects in total, of which 12 had less than 1,000 cases. For example, the meta-analysis of *DRD3* Ser9Gly polymorphism included 541 cases and 606 controls. The OR of the homozygous carriers was 1.71 and was not significant. In contrast, the meta-analysis of the *SLC6A4* 44bp Ins/Del polymorphism totaled more than 9,000 persons and had a statistically significant OR of 1.11 for the S allele.

Table 3 Meta-analyses of genetic association studies on major depressive disorder

				Heterozygotes		
Gene	**Variant**	**Studies**	**Analysis**	**Comparison**	**OR (95% CI)**	**I^2**
ACE	Ins/Del	8	Fixed	ID vs II	0.94 (0.78-1.13)	27
	-Intron 16		Random		0.90 (0.71-1.13)	
APOE	ε2/ε3/ε4	5	Fixed	ε2/ε3 vs ε3/ε3	0.42 (0.28-0.62)***	72*
			Random		0.41 (0.15-1.07)	
		5	Fixed	ε3/ε4 vs ε3/ε3	1.02 (0.78-1.35)	0
			Random		1.02 (0.78-1.35)	
BDNF	Val66Met	8	Fixed	Val/Met vs Val/Val	0.98 (0.89-1.09)	0
			Random		0.98 (0.89-1.09)	
COMT	Val158Met	6	Fixed	Val/Met vs Val/Val	1.14 (0.86-1.52)	29
			Random		1.13 (0.81-1.59)	
DRD3	Ser9Gly	4	Fixed	Ser/Gly vs Ser/Ser	0.92 (0.67-1.26)	33
			Random		0.96 (0.64-1.45)	
GABRA3	CA repeat	6	Fixed	*/1 vs 1/1	0.74 (0.49-1.12)	46
	-Intron 8		Random		0.63 (0.32-1.25)	
GNB3	C825T	3	Fixed	CT vs CC	1.25 (0.91-1.72)	50
			Random		1.30 (0.81-2.09)	
HTR1A	C-1019G	4	Fixed	CG vs CC	0.98 (0.72-1.33)	18
			Random		0.98 (0.69-1.38)	
HTR1B	G861C	3	Fixed	GC vs GG	0.99 (0.74-1.33)	42
			Random		0.98 (0.66-1.44)	
HTR2A	A-1438G	4	Fixed	AG vs AA	1.23 (0.88-1.73)	62*
			Random		1.15 (0.65-2.03)	
	T102C	8	Fixed	TC vs TT	1.00 (0.79-1.27)	0
			Random		1.00 (0.79-1.27)	

Homozygotes				Per allele			
Comparison	**OR (95% CI)**	**I^2**	**Studies**	**Comparison**	**OR (95% CI)**	**I^2**	**References**
DD vs II	1.15 (0.94-1.42)	26	8	D vs I	1.08 (0.97-1.20)	0	11,100,179-183
	1.11 (0.85-1.45)				1.05 (0.91-1.21)		
ε2/ε2 vs ε3/ε3	NA		7	ε2 vs ε3	0.51 (0.39-0.68)***	74***	53-59
	NA				0.51 (0.27-0.97)*		
ε4/ε4 vs ε3/ε3	1.02 (0.44-2.37)	32	7	ε4 vs ε3	0.93 (0.76-1.14)	4	53-59,140
	0.91 (0.25-3.33)				0.93 (0.75-1.15)		
Met/Met vs Val/Val	1.05 (0.84-1.32)	53*	8	Met vs Val	1.01 (0.93-1.09)	55*	141,184-188‡
	1.09 (0.72-1.65)				1.04 (0.90-1.21)		
Met/Met vs Val/Val	0.95 (0.67-1.33)	12	8	Met vs Val	0.98 (0.86-1.13)	43	68,127,128,189-192
	0.95 (0.65-1.39)				1.00 (0.83-1.21)		
Gly/Gly vs Ser/Ser	1.31 (0.80-2.14)	72*	4	Gly vs Ser	1.06 (0.85-1.34)	73**	67,94,127,193‡
	1.71 (0.57-5.13)				1.19 (0.74-1.89)		
/ vs 1/1	0.92 (0.40-2.11)	10	6	Others vs 1	0.91 (0.68-1.20)	49	194,195
	0.97 (0.36-2.62)				0.88 (0.55-1.40)		
TT vs CC	2.13 (1.39-3.28)**	67*	4	T vs C	1.38 (1.13-1.69)**	41	45,60, 61
	2.09 (0.97-4.51)				1.36 (1.04-1.77)*		
GG vs CC	1.33 (0.95-1.87)	79**	4	G vs C	1.16 (0.98-1.38)	77**	94,196-198‡
	1.63 (0.72-3.70)				1.25 (0.85-1.84)		
CC vs GG	0.81 (0.46-1.41)	25	3	C vs G	0.96 (0.77-1.20)	48	94, 199, 200‡
	0.79 (0.41-1.53)				0.94 (0.69-1.29)		
GG vs AA	1.06 (0.73-1.55)	75**	4	G vs A	1.01 (0.85-1.21)	73**	94, 201-203‡
	0.98 (0.45-2.14)				0.96 (0.67-1.36)		
CC vs TT	0.92 (0.71-1.21)	17	8	C vs T	0.96 (0.84-1.09)	26	68, 94, 204-208‡
	0.92 (0.68-1.25)				0.96 (0.82-1.12)		

				Heterozygotes		
Gene	**Variant**	**Studies**	**Analysis**	**Comparison**	**OR (95% CI)**	**I^2**
HTR2C	Cys23Ser	2	Fixed	Cys/Ser vs Cys/Cys	NA	
			Random		NA	
MAOA	VNTR†	4	Fixed	12 vs 22	1.34 (0.95-1.89)	17
	-Promotor		Random		1.29 (0.87-1.91)	
MTHFR	C677T	6	Fixed	CT vs CC	1.22 (1.03-1.44)*	26
			Random		1.23 (0.95-1.58)	
SLC6A2	T-182C	3	Fixed	TC vs TT	1.21 (0.91-1.61)	52
			Random		1.23 (0.81-1.85)	
SLC6A3	40bp VNTR	3	Fixed	9/10 vs 10/10	2.06 (1.25-3.40)**	0
(DAT1)	-31-UTR region		Random		2.05 (1.24-3.40)**	
SLC6A4	44bp Ins/Del	22	Fixed	LS vs LL	1.05 (0.94-1.18)	0
(SERT)	-Promotor		Random		1.05 (0.93-1.18)	
	VNTR	8	Fixed	9/12 vs 12/12	1.25 (0.61-2.55)	0
	Intron 2		Random		1.22 (0.58-2.56)	
		8	Fixed	10/12 vs 12/12	0.94 (0.74-1.20)	24
			Random		0.98 (0.73-1.32)	
TPH1	A218C	9	Fixed	AC vs AA	1.10 (0.91-1.34)	49
			Random		1.14 (0.85-1.52)	

I^2 = Heterogeneity, in percentage; OR = odds ratio, CI = confidence interval NA = Not applicable, if data were available on less than three studies.

*$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$

† Allele 1, 30 bp-repeat sequence present in 3 copies associated with lower transcription activity; allele 2, present in 3.5, 4 and 5 copies of 30 bp-repeat sequence associated with higher transcription activity.

Note: It was not possible to perform a meta-analyses for the *TPH2* G1463A polymorphism, because 4 out of 5 papers did not find the 1463A allele in cases nor in controls.

‡ References 17 to 19 did not present complete information on genotype frequencies. Authors were contacted and all provided genotype data.

Homozygotes				Per allele			
Comparison	**OR (95% CI)**	**I^2**	**Studies**	**Comparison**	**OR (95% CI)**	**I^2**	**References**
Ser/Ser vs Cys/Cys	NA		13	Ser vs Cys	1.03 (0.85-1.25	50*	68, 94, 209 ‡
	NA				0.96 (0.73-1.28)		
11 vs 22	0.71 (0.49-1.02)	0	6	1 vs 2	0.86 (0.74-1.01)	65*	210-215
	0.71 (0.49-1.03)				0.86 (0.65-1.13)		
TT vs CC	1.38 (1.08-1.76)*	0	6	T vs C	1.20 (1.07-1.34)**	0	15,62-66
	1.38 (1.08-1.77)*				1.20 (1.07-1.34)**		
CC vs TT	0.72 (0.45-1.14)	64	3	C vs T	0.97 (0.80-1.18)	68*	125,216,217
	0.75 (0.33-1.67)				0.99 (0.70-1.40)		
9/9 vs 10/10	1.46 (0.67-3.16)	0	3	9 vs 10	1.38 (0.98-1.94)	0	67, 68
	1.47 (0.67-3.19)				1.38 (0.98-1.94)		
SS vs LL	1.39 (1.20-1.61)**	0	24	S vs L	1.11 (1.04-1.19)**	30	(32,45,61-70,72-80)‡
	1.39 (1.20-1.61)**				1.12 (1.03-1.22)**		
9/9 vs 12/12	NA	0	11	9 vs 12	1.33 (0.78-2.27)*	0	14,42,52,69-77
	NA				1.24 (0.69-2.23)		
10/10 vs 12/12	1.17 (0.82-1.68)		11	10 vs 12	1.02 (0.89-1.17)	0	(14,35,190-199)
	1.18 (0.82-1.70)				1.04 (0.91-1.20)		
CC vs AA	0.88 (0.71-1.09)	35	9	C vs A	0.94 (0.85-1.04)	6	68,94,146,151,218-221‡
	0.86 (0.65-1.14)				0.94 (0.84-1.05)		

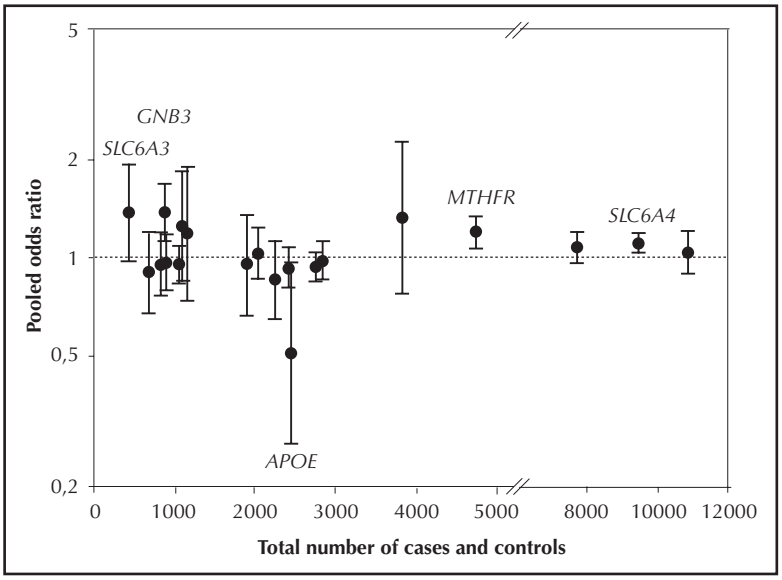

Figure 4 Pooled odds ratios in relation to the total number of cases and controls included in the meta-analyses.
Odds ratios are reported for the per allele analyses.

Discussion

In this review we were able to perform meta-analyses for 18 out of 102 genes studied. We were not able to perform meta-analyses for 371 polymorphisms in 97 genes because there were less than three studies assessing the same polymorphism. We found significant evidence for five MDD susceptibility genes (*APOE, GNB3, MTHFR, SLC6A3* and *SLC6A4*).

The identified genes are involved in known biological pathways. The dopamine transporter (*SLC6A3*) mediates the active reuptake of dopamine from the synapse and is a principal regulator of dopaminergic neurotransmission. All antidepressants, in one way or the other, affect dopamine in the frontal area of the brain and in the nucleus accumbens.[82-84] The protein encoded by the *SLC6A4* gene is the drug target of serotonin reuptake inhibitors (SRIs) such as fluoxetine.[85] The 44bp Ins/Del polymorphism is localized in the promotor region and may affect expression of the protein.[86] *GNB3* encodes for the beta subunit of G proteins, which is a target for antidepressant medication such as tricycles and MAO inhibitors.[87] *MTHFR* is a plausible candidate since the MTHFR enzyme metabolizes folate, and lower folate levels have been found associated with depression.[88] Furthermore, recovery from depression

may be enhanced by folate supplements.[89] The most strongly associated gene in terms of the OR is *APOE*. APOE is recognized a major determinant in lipoprotein metabolism, cardiovascular disease, Alzheimer's disease, cognitive function and immunoregulation,[90] and as each of these disorders may be implicated in MDD, *APOE* remains at least a conceivable candidate gene to be studied further.

While these five genes were significantly associated to MDD in the meta-analyses, this does not mean the others may not be related. First, for many meta-analyses the total number of cases and controls studied was not large enough to detect moderate association to genes (OR ~1.2-1.5). For example, the pooled ORs of *DRD3, HTR1A* and *SLC6A4* VNTR in intron 2 were 1.33-1.71, but not statistically significant. In terms of genetic effects for complex diseases, these are not small effects. In addition to small sample size, other reasons for the absence of statistical significance are the low frequencies of the risk variant and heterogeneity between studies.

Second, most studies have investigated only one single polymorphism per gene. Even if this single polymorphism appears not to be associated, there may still be other variants of the gene that are associated to MDD. The present meta-analyses only included two genes for which two polymorphisms were investigated (*HTR2A* and *SLC6A4*). The 44bp Ins/Del polymorphism of the *SLC6A4* gene was found to be associated with MDD, while the VNTR in intron 2 of *SLC6A4* was not. The latter shows that a negative meta-analysis of one polymorphism does not rule out significant associations of other polymorphisms in the same gene.

Most research on the genetic origin of MDD has focused on a limited number of polymorphisms. Meta-analyses could only be performed for 22 of the 393 (6%) polymorphisms that had been investigated in relation to MDD. There were 371 polymorphisms that were addressed in only one or two studies, while 13 studies investigating the *HTR2C* Cys23Ser polymorphism and 24 studies investigated the *SLC6A4* 44bp Ins/Del polymorphism. *SLC6A4* has been reviewed in three previous meta-analyses,[9,14,16] two showing no evidence for association with MDD. Differences between the meta-analyses may be explained by the inclusion of different studies, by heterogeneity between the results of individual studies, or by underestimation of effect sizes due to bi-allelic (S/L) instead of tri-allelic ($S/L_G/L$) grouping of the genotypes. The tri allelic grouping distinguishes two long alleles (L_G/L), while the L_G expresses similar to the S allele.[86] The high numbers of studies of these polymorphisms are exceptions. For most polymorphisms reviewed in the meta-analyses the number of available studies was still small, and most meta-analyses did not include enough studies to investigate sources of heterogeneity in a meta-regression, or perform formal tests of publication bias. Meta-regression analysis would be useful to

investigate whether the asymmetric funnel plots of the *SLC6A4* 44bp Ins/Del and *APOE* ε2 are suggestive of publication bias or indicate true heterogeneity between populations. This review clearly demonstrates that more MDD genetic association studies are needed.

We excluded studies if they used questionnaires for the identification of cases, if they did not distinguish between MDD and bipolar depression, or if the genotype distributions in controls were not in Hardy-Weinberg equilibrium. Yet, we did not exclude studies or stratify the analyses based on other criteria, e.g., whether controls were selected from the general population or whether they had been screened for the absence of depression, because the number of studies for each polymorphism was small. Such differences between studies may have contributed to the heterogeneity that was found in several of the meta-analyses. To investigate causes of between-study heterogeneity in future meta-analyses, it is essential that individual studies report key characteristics of their populations. To accumulate the evidence in genetic-epidemiological studies harmonizing designs, populations and measurements is deemed important and aimed for by the Network of Investigator Networks of the Human Genome Epidemiology Network.[91]

In conclusion, our meta-analyses found significant evidence for five MDD susceptibility genes (*APOE, GNB3, MTHFR, SLC6A3* and *SLC6A4*). Together with our previously published meta-analysis on *DRD4,*[12] there is evidence for six MDD susceptibility genes. The low coverage of genetic variants of candidate genes makes it impossible to exclude that the other genes studied are not involved in MDD. More research aiming to replicate findings in MDD is needed and efforts are required to standardize the methodology in research of MDD.

References

1. **Murray CJ, López AD.** Evidence-based health policy-lessons from the Global Burden of Disease Study. Science 1996; 274: 740–743.
2. **Ebmeier KP, Donaghey C, Steele JD.** Recent developments and current controversies in depression. Lancet 2006;367:153-67.
3. **Sullivan PF, Neale MC, Kendler KS.** Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157:1552-62.
4. **Weissman MM.** Psychiatric disorders in the relatives of probands with affective disorders: The Yale NIMH collaborative family study. Arch Gen Psychiatry 1984;41:13-21.
5. **Beckman G, Beckman L, Cedergren B, Perris C, Strandman E.** Serum protein and red cell enzyme polymorphisms in affective disorders. Hum Hered 1978;28:41-7.
6. **Levinson DF.** The Genetics of Depression: A Review. Biol Psychiatry 2005;18:18.
7. **Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN.** Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177-82.
8. **Levinson DF.** Meta-analysis in Psychiatric Genetics. Curr Psychiatry Rep 2005;7:143-51.

9. **Lasky-Su JA, Faraone SV, Glatt SJ, Tsuang MT.** Meta-analysis of the association between two polymorphisms in the serotonin transporter gene and affective disorders. Am J Med Genet B Neuropsychiatr Genet 2005;133:110-5.
10. **Zintzaras E.** C677T and A1298C methylenetetrahydrofolate reductase gene polymorphisms in schizophrenia, bipolar disorder and depression: a meta-analysis of genetic association studies. Psychiatr Genet 2006;16:105-15.
11. **López-León S, Janssens ACJW, Hofman A, Claes S, Breteler MM, Tiemeier H, et al.** No association between the angiotensin-converting enzyme gene and major depression: a case-control study and meta-analysis. Psychiatr Genet 2006;16:225-6.
12. **López-León S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM.** The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005;57:999-1003.
13. **Furlong RA, Rubinsztein JS, Ho L, Walsh C, Coleman TA, Muir WJ, et al.** Analysis and metaanalysis of two polymorphisms within the tyrosine hydroxylase gene in bipolar and unipolar affective disorders. Am J Med Genet 1999;88:88-94.
14. **Furlong RA, Ho L, Walsh C, Rubinsztein JS, Jain S, Paykel ES, et al.** Analysis and meta-analysis of two serotonin transporter gene polymorphisms in bipolar and unipolar affective disorders. Am J Med Genet 1998;81:58-63.
15. **Lewis SJ, Lawlor DA, Davey Smith G, Araya R, Timpson N, Day IN, et al.** The thermolabile variant of MTHFR is associated with depression in the British Women's Heart and Health Study and a meta-analysis. Mol Psychiatry 2006;11:352-60.
16. **Anguelova M, Benkelfat C, Turecki G.** A systematic review of association studies investigating genes coding for serotonin receptors and the serotonin transporter: I. Affective disorders. Mol Psychiatry 2003;8:574-91.
17. **DerSimonian R, Laird N.** Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
18. **Higgins JP, Thompson SG, Deeks JJ, Altman DG.** Measuring inconsistency in meta-analyses. BMJ 2003;327:557-60.
19. **Higgins JP, Thompson SG.** Quantifying heterogeneity in a meta-analysis. Stat Med 2002;21:1539-58.
20. **Egger M, Davey Smith G, Schneider M, Minder C.** Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
21. **Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM.** Folate, vitamin B12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. Arch Gen Psychiatry 2003;60:618-26.
22. **Exton MS, Artz M, Siffert W, Schedlowski M.** G protein beta3 subunit 825T allele is associated with depression in young, healthy subjects. Neuroreport 2003;14:531-3.
23. **Schmand B, Hooijer C, Jonker C, Lindeboom J, Havekes LM.** Apolipoprotein E phenotype is not related to late-life depression in a population-based sample. Soc Psychiatry Psychiatr Epidemiol 1998;33:21-6.
24. **Kravitz HM, Janssen I, Lotrich FE, Kado DM, Bromberger JT.** Sex steroid hormone gene polymorphisms and depressive symptoms in women at midlife. Am J Med 2006;119:S87-93.
25. **Almeida OP, Flicker L, Lautenschlager NT, Leedman P, Vasikaran S, van Bockxmeer FM.** Contribution of the MTHFR gene to the causal pathway for depression, anxiety and cognitive impairment in later life. Neurobiol Aging 2005;26:251-7.
26. **Bailer U, Wiesegger G, Leisch F, Fuchs K, Leitner I, Letmaier M, et al.** No association of clock gene T3111C polymorphism and affective disorders. Eur Neuropsychopharmacol 2005;15:51-5.
27. **Khait VD, Huang YY, Zalsman G, Oquendo MA, Brent DA, Harkavy-Friedman JM, et al.** Association of serotonin 5-HT2A receptor binding and the T102C polymorphism in depressed and healthy Caucasian subjects. Neuropsychopharmacology 2005;30:166-72.
28. **Bellivier F, Szoke A, Henry C, Lacoste J, Bottos C, Nosten-Bertrand M, et al.** Possible association between serotonin transporter gene polymorphism and violent suicidal behavior in mood disorders. Biol Psychiatry 2000;48:319-22.

29. **Kessing LV, Jorgensen OS.** Apolipoprotein E-epsilon 4 frequency in affective disorder. Biol Psychiatry 1999;45:430-4.
30. **Tanna VL, Winokur G.** A study of association and linkage of ABO blood types and primary affective disorder. Br J Psychiatry 1968;114:1175-81.
31. **Cusin C, Serretti A, Lattuada E, Lilli R, Lorenzi C, Smeraldi E.** Association study of MAO-A, COMT, 5-HT2A, DRD2, and DRD4 polymorphisms with illness time course in mood disorders. Am J Med Genet 2002;114:380-90.
32. **Lin S, Jiang S, Jiang K.** Genetic association between mood disorder and monoamine oxidase gene. Zhonghua Yi Xue Za Zhi 1999;79:897-9.
33. **Kurumaji A, Nomoto H, Yamada K, Yoshikawa T, Toru M.** No association of two missense variations of the benzodiazepine receptor (peripheral) gene and mood disorders in a Japanese sample. Am J Med Genet 2001;105:172-5.
34. **Qian W, Homma M, Itagaki F, Tachikawa H, Kawanishi Y, Mizukami K, et al.** MDR1 gene polymorphism in Japanese patients with schizophrenia and mood disorders including depression. Biol Pharm Bull 2006;29:2446-50.
35. **Pae CU, Yoon SJ, Patkar A, Kim JJ, Jun TY, Lee C, et al.** Manganese superoxide dismutase (MnSOD: Ala-9Val) gene polymorphism and mood disorders: A preliminary study. Prog Neuropsychopharmacol Biol Psychiatry 2006.
36. **Hong CJ, Huo SJ, Yen FC, Tung CL, Pan GM, Tsai SJ.** Association study of a brain-derived neurotrophic-factor genetic polymorphism and mood disorders, age of onset and suicidal behavior. Neuropsychobiology 2003;48:186-9.
37. **Tsai SJ, Cheng CY, Yu YW, Chen TJ, Hong CJ.** Association study of a brain-derived neurotrophic-factor genetic polymorphism and major depressive disorders, symptomatology, and antidepressant response. Am J Med Genet B Neuropsychiatr Genet 2003;123:19-22.
38. **Du L, Faludi G, Palkovits M, Bakish D, Hrdina PD.** Serotonergic genes and suicidality. Crisis 2001;22:54-60.
39. **Lin CN, Tsai SJ, Hong CJ.** Association analysis of a functional G protein beta3 subunit gene polymorphism (C825T) in mood disorders. Neuropsychobiology 2001;44:118-21.
40. **Du L, Bakish D, Hrdina PD.** Tryptophan hydroxylase gene 218A/C polymorphism is associated with somatic anxiety in major depressive disorder. J Affect Disord 2001;65:37-44.
41. **Tsai SJ, Hong CJ, Wang YC.** Tryptophan hydroxylase gene polymorphism (A218C) and suicidal behaviors. Neuroreport 1999;10:3773-5.
42. **Bellivier F, Henry C, Szoke A, Schurhoff F, Nosten-Bertrand M, Feingold J, et al.** Serotonin transporter gene polymorphisms in patients with unipolar or bipolar depression. Neurosci Lett 1998;255:143-6.
43. **Choi MJ, Kang RH, Ham BJ, Jeong HY, Lee MS.** Serotonin receptor 2A gene polymorphism (-1438A/G) and short-term treatment response to citalopram. Neuropsychobiology 2005;52:155-62.
44. **Jun TY, Pae CU, Chae JH, Bahk WM, Kim KS, Pyo CW, et al.** Tumor necrosis factor-beta gene polymorphism may not be associated with major depressive disorder in the Korean population. Psychiatry Clin Neurosci 2003;57:31-5.
45. **Bondy B, Baghai TC, Zill P, Bottlender R, Jaeger M, Minov C, et al.** Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? Mol Psychiatry 2002;7:1120-6.
46. **Arias B, Gasto C, Catalan R, Gutierrez B, Pintor L, Fananas L.** The 5-HT(2A) receptor gene 102T/C polymorphism is associated with suicidal behavior in depressed patients. Am J Med Genet 2001;105:801-4.
47. **Bondy B, Kuznik J, Baghai T, Schule C, Zwanzger P, Minov C, et al.** Lack of association of serotonin-2A receptor gene polymorphism (T102C) with suicidal ideation and suicide. Am J Med Genet 2000;96:831-5.
48. **Zill P, Baghai TC, Zwanzger P, Schule C, Minov C, Riedel M, et al.** Evidence for an association between a G-protein beta3-gene variant with depression and response to antidepressant treatment. Neuroreport 2000;11:1893-7.

49. **Huang YY, Grailhe R, Arango V, Hen R, Mann JJ.** Relationship of psychopathology to the human serotonin1B genotype and receptor binding kinetics in postmortem brain tissue. Neuropsychopharmacology 1999;21:238-46.
50. **Kunugi H, Tatsumi M, Sakai T, Hattori M, Nanko S.** Serotonin transporter gene polymorphism and affective disorder. Lancet 1996;347:1340.
51. **Ogilvie AD, Battersby S, Bubb VJ, Fink G, Harmar AJ, Goodwim GM, et al.** Polymorphism in serotonin transporter gene associated with susceptibility to major depression. Lancet 1996;347:731-3.
52. **Harmar AJ, Ogilvie AD, Battersby S, Smith CA, Blackwood DH, Muir WJ, et al.** The serotonin transporter gene and affective disorder. Cold Spring Harb Symp Quant Biol 1996;61:791-5.
53. **Fan PL, Chen CD, Kao WT, Shu BC, Lung FW.** Protective effect of the apo epsilon2 allele in major depressive disorder in Taiwanese. Acta Psychiatr Scand 2006;113:48-53.
54. **Butters MA, Sweet RA, Mulsant BH, Ilyas Kamboh M, Pollock BG, Begley AE, et al.** APOE is associated with age-of-onset, but not cognitive functioning, in late-life depression. Int J Geriatr Psychiatry 2003;18:1075-81.
55. **Forsell Y, Corder EH, Basun H, Lannfelt L, Viitanen M, Winblad B.** Depression and dementia in relation to apolipoprotein E polymorphism in a population sample age 75+. Biol Psychiatry 1997;42:898-903.
56. **Holmes C, Russ C, Kirov G, Aitchison KJ, Powell JF, Collier DA, et al.** Apolipoprotein E: depressive illness, depressive symptoms, and Alzheimer's disease. Biol Psychiatry 1998;43:159-64.
57. **Zubenko GS, Henderson R, Stiffler JS, Stabler S, Rosen J, Kaplan BB.** Association of the APOE epsilon 4 allele with clinical subtypes of late life depression. Biol Psychiatry 1996;40:1008-16.
58. **Papassotiropoulos A, Bagli M, Jessen F, Rao ML, Schwab SG, Heun R.** Early-onset and late-onset depression are independent of the genetic polymorphism of apolipoprotein E. Dement Geriatr Cogn Disord 1999;10:258-61.
59. **Ohara K, Nagai M, Suzuki Y, Yoshida K, Tsukamoto T.** Apolipoprotein E epsilon 4 allele and Japanese late-onset depressive disorders. Biol Psychiatry 1999;45:308-12.
60. **Kunugi H, Kato T, Fukuda R, Tatsumi M, Sakai T, Nanko S.** Association study of C825T polymorphism of the G-protein b3 subunit gene with schizophrenia and mood disorders. J Neural Transm 2002;109:213-8.
61. **Lee HJ, Cha JH, Ham BJ, Han CS, Kim YK, Lee SH, et al.** Association between a G-protein beta 3 subunit gene polymorphism and the symptomatology and treatment responses of major depressive disorders. Pharmacogenomics J 2004;4:29-33.
62. **Arinami T, Yamada N, Yamakawa-Kobayashi K, Hamaguchi H, Toru M.** Methylenetetrahydrofolate reductase variant and schizophrenia/depression. Am J Med Genet 1997;74:526-8.
63. **Kunugi H, Fukuda R, Hattori M, Kato T, Tatsumi M, Sakai T, et al.** C677T polymorphism in methylenetetrahydrofolate reductase gene and psychoses. Mol Psychiatry 1998;3:435-7.
64. **Tan EC, Chong SA, Lim LC, Chan AO, Teo YY, Tan CH, et al.** Genetic analysis of the thermolabile methylenetetrahydrofolate reductase variant in schizophrenia and mood disorders. Psychiatr Genet 2004;14:227-31.
65. **Chen CS, Tsai JC, Tsang HY, Kuo YT, Lin HF, Chiang IC, et al.** Homocysteine levels, MTHFR C677T genotype, and MRI Hyperintensities in late-onset major depressive disorder. Am J Geriatr Psychiatry 2005;13:869-75.
66. **Kelly CB, McDonnell AP, Johnston TG, Mulholland C, Cooper SJ, McMaster D, et al.** The MTHFR C677T polymorphism is associated with depressive episodes in patients from Northern Ireland. J Psychopharmacol 2004;18:567-71.
67. **Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, et al.** Dopamine D2, D3 and D4 receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 1996;40:7-13.
68. **Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, et al.** Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry 1999;4:389-92.

69. **Battersby S, Ogilvie AD, Smith CA, Blackwood DH, Muir WJ, Quinn JP, et al.** Structure of a variable number tandem repeat of the serotonin transporter gene and association with affective disorder. Psychiatr Genet 1996;6:177-81.
70. **Collier DA, Arranz MJ, Sham P, Battersby S, Vallada H, Gill P, et al.** The serotonin transporter is a potential susceptibility factor for bipolar affective disorder. Neuroreport 1996;7:1675-9.
71. **Stober G, Heils A, Lesch KP.** Serotonin transporter gene polymorphism and affective disorder. Lancet 1996;347:1340-1.
72. **Rees M, Norton N, Jones I, McCandless F, Scourfield J, Holmans P, et al.** Association studies of bipolar disorder at the human serotonin transporter gene (hSERT; 5HTT). Mol Psychiatry 1997;2:398-402.
73. **Hoehe MR, Wendel B, Grunewald I, Chiaroni P, Levy N, Morris-Rosendahl D, et al.** Serotonin transporter (5-HTT) gene polymorphisms are not associated with susceptibility to mood disorders. Am J Med Genet 1998;81:1-3.
74. **Liu W, Gu N, Feng G, Li S, Bai S, Zhang J, et al.** Tentative association of the serotonin transporter with schizophrenia and unipolar depression but not with bipolar disorder in Han Chinese. Pharmacogenetics 1999;9:491-5.
75. **Mellerup E, Bennike B, Bolwig T, Dam H, Hasholt L, Jorgensen MB, et al.** Platelet serotonin transporters and the transporter gene in control subjects, unipolar patients and bipolar patients. Acta Psychiatr Scand 2001;103:229-33.
76. **Yen FC, Hong CJ, Hou SJ, Wang JK, Tsai SJ.** Association study of serotonin transporter gene VNTR polymorphism and mood disorders, onset age and suicide attempts in a Chinese sample. Neuropsychobiology 2003;48:5-9.
77. **Kunugi H, Hattori M, Kato T, Tatsumi M, Sakai T, Sasaki T, et al.** Serotonin transporter gene polymorphisms: ethnic difference and possible association with bipolar affective disorder. Mol Psychiatry 1997;2:457-62.
78. **Hauser J, Leszczynska A, Samochowiec J, Czerski PM, Ostapowicz A, Chlopocka M, et al.** Association analysis of the insertion/deletion polymorphism in serotonin transporter gene in patients with affective disorder. Eur Psychiatry 2003;18:129-32.
79. **Hoefgen B, Schulze TG, Ohlraun S, von Widdern O, Hofels S, Gross M, et al.** The power of sample size and homogenous sampling: association between the 5-HTTLPR serotonin transporter polymorphism and major depressive disorder. Biol Psychiatry 2005;57:247-51.
80. **Cervilla JA, Rivera M, Molina E, Torres-Gonzalez F, Bellon JA, Moreno B, et al.** The 5-HTTLPR s/s genotype at the serotonin transporter gene (SLC6A4) increases the risk for depression in a large cohort of primary care attendees: The PREDICT-gene study. Am J Med Genet B Neuropsychiatr Genet 2006;141B:912-917.
81. **Collier DA, Stober G, Li T, Heils A, Catalano M, Di Bella D, et al.** A novel functional polymorphism within the promoter of the serotonin transporter gene: possible role in susceptibility to affective disorders. Mol Psychiatry 1996;1:453-60.
82. **D'Aquila PS, Collu M, Gessa GL, Serra G.** The role of dopamine in the mechanism of action of antidepressant drugs. Eur J Pharmacol 2000;405:365-73.
83. T**remblay LK, Naranjo CA, Cardenas L, Herrmann N, Busto UE.** Probing brain reward system function in major depressive disorder: altered response to dextroamphetamine. Arch Gen Psychiatry 2002;59:409-16.
84. **Ainsworth K, Smith SE, Sharp T.** Repeated administration of fluoxetine, desipramine and tranylcypromine increases dopamine D2-like but not D1-like receptor function in the rat. J Psychopharmacol 1998;12:252-7.
85. **Routledge C, Middlemiss DN.** The 5-HT hypothesis of depression revisited. Mol Psychiatry 1996;1:437.
86. **Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, et al.** Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. Am J Hum Genet 2006;78:815-26.
87. **Rasenick MM, Chaney KA, Chen J.** G protein-mediated signal transduction as a target of antidepressant and antibipolar drug action: evidence from model systems. J Clin Psychiatry 1996;57 Suppl 13:49-55; discussion 56-8.

88. **Crellin R, Bottiglieri T, Reynolds EH.** Folates and psychiatric disorders. Clinical potential. Drugs 1993;45:623-36.
89. **Paul RT, McDonnell AP, Kelly CB.** Folic acid: neurochemistry, metabolism and relationship to depression. Hum Psychopharmacol 2004;19:477-88.
90. **Mahley RW, Rall SC, Jr.** Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000;1:507-37.
91. **Ioannidis JP, Gwinn M, Little J, Higgins JP, Bernstein JL, Boffetta P, et al.** A road map for efficient and reliable human genome epidemiology. Nat Genet 2006;38:3-5.
92. **Ohara K, Suzuki Y, Yoshida K, Terada H, Ohara K.** Lack of association between alpha1-antichymotrypsin polymorphism and late-onset depressive disorder. Psychiatry Res 1999;88:221-6.
93. **Comings DE, Wu S, Rostamkhani M, McGue M, Iacono WG, MacMurray JP.** Association of the muscarinic cholinergic 2 receptor (CHRM2) gene with major depression in women. Am J Med Genet 2002;114:527-9.
94. **Koks S, Nikopensius T, Koido K, Maron E, Altmae S, Heinaste E, et al.** Analysis of SNP profiles in patients with major depressive disorder. Int J Neuropsychopharmacol 2006;9:167-74.
95. **Oruc L, Verheyen GR, Furac I, Ivezic S, Jakovljevic M, Raeymaekers P, et al.** Positive association between the GABRA5 gene and unipolar recurrent major depression. Neuropsychobiology 1997;36:62-4.
96. **Yamada K, Hattori E, Iwayama Y, Ohnishi T, Ohba H, Toyota T, et al.** Distinguishable Haplotype Blocks in the HTR3A and HTR3B Region in the Japanese Reveal Evidence of Association of HTR3B with Female Major Depression. Biol Psychiatry 2006; 60: 192–201.
97. **Reif A, Strobel A, Jacob CP, Herterich S, Freitag CM, Topner T, et al.** A NOS-III haplotype that includes functional polymorphisms is associated with bipolar disorder. Int J Neuropsychopharmacol 2006;9:13-20.
98. **Wong ML, Whelan F, Deloukas P, Whittaker P, Delgado M, Cantor RM, et al.** Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. Proc Natl Acad Sci U S A 2006.
99. **Mendlewicz J, Oswald P, Claes S, Massat I, Souery D, Van Broeckhoven C, et al.** Patient-control association study of substance P-related genes in unipolar and bipolar affective disorders. Int J Neuropsychopharmacol 2005;8:505-13.
100. **Baghai TC, Binder EB, Schule C, Salyakina D, Eser D, Lucae S, et al.** Polymorphisms in the angiotensin-converting enzyme gene are associated with unipolar depression, ACE activity and hypercortisolism. Mol Psychiatry 2006; 11: 1003–1015.
101. **Lai IC, Hong CJ, Tsai SJ.** Association study of nicotinic-receptor variants and major depressive disorder. J Affect Disord 2001;66:79-82.
102. **Furlong RA, Coleman TA, Ho L, Rubinsztein JS, Walsh C, Paykel ES, et al.** No association of a functional polymorphism in the dopamine D2 receptor promoter region with bipolar or unipolar affective disorders. Am J Med Genet 1998;81:385-7.
103. **Yamada K, Watanabe A, Iwayama-Shigeno Y, Yoshikawa T.** Evidence of association between gamma-aminobutyric acid type A receptor genes located on 5q34 and female patients with mood disorders. Neurosci Lett 2003;349:9-12.
104. **Heilig M, Zachrisson O, Thorsell A, Ehnvall A, Mottagui-Tabar S, Sjogren M, et al.** Decreased cerebrospinal fluid neuropeptide Y (NPY) in patients with treatment refractory unipolar major depression: preliminary evidence for association with preproNPY gene polymorphism. J Psychiatr Res 2004;38:113-21.
105. **Lindberg C, Koefoed P, Hansen ES, Bolwig TG, Rehfeld JF, Mellerup E, et al.** No association between the -399 C > T polymorphism of the neuropeptide Y gene and schizophrenia, unipolar depression or panic disorder in a Danish population. Acta Psychiatr Scand 2006;113:54-8.
106. **Toyota T, Hattori E, Meerabux J, Yamada K, Saito K, Shibuya H, et al.** Molecular analysis, mutation screening, and association study of adenylate cyclase type 9 gene (ADCY9) in mood disorders. Am J Med Genet 2002;114:84-92.
107. **Desan PH, Oren DA, Malison R, Price LH, Rosenbaum J, Smoller J, et al.** Genetic polymorphism at the CLOCK gene locus and major depression. Am J Med Genet 2000;96:418-21.

108. **Lappalainen J, Sanacora G, Kranzler HR, Malison R, Hibbard ES, Price LH, et al.** Mutation screen of the glutamate decarboxylase-67 gene and haplotype association to unipolar depression. Am J Med Genet B Neuropsychiatr Genet 2004;124:81-6.
109. **Birkett JT, Arranz MJ, Munro J, Osbourn S, Kerwin RW, Collier DA.** Association analysis of the 5-HT5A gene in depression, psychosis and antipsychotic response. Neuroreport 2000;11:2017-20.
110. **Arias B, Collier DA, Gasto C, Pintor L, Gutierrez B, Valles V, et al.** Genetic variation in the 5-HT5A receptor gene in patients with bipolar disorder and major depression. Neurosci Lett 2001;303:111-4.
111. **Lucae S, Salyakina D, Barden N, Harvey M, Gagne B, Labbe M, et al.** P2RX7, a gene coding for a purinergic ligand-gated ion channel, is associated with major depressive disorder. Hum Mol Genet 2006;15:2438-45.
112. **Tsai SJ, Hong CJ, Hou SJ, Yen FC.** Association study of adenosine A2a receptor (1976C>T) genetic polymorphism and mood disorders and age of onset. Psychiatr Genet 2006;16:185.
113. **Tsai SJ, Wang YC, Hong CJ.** Association study between cannabinoid receptor gene (CNR1) and pathogenesis and psychotic symptoms of mood disorders. Am J Med Genet 2001;105:219-21.
114. **van Rossum EF, Binder EB, Majer M, Koper JW, Ising M, Modell S, et al.** Polymorphisms of the glucocorticoid receptor gene and major depression. Biol Psychiatry 2006;59:681-8.
115. **van West D, Van Den Eede F, Del-Favero J, Souery D, Norrback KF, van Duijn C, et al.** Glucocorticoid Receptor Gene-Based SNP Analysis in Patients with Recurrent Major Depression. Neuropsychopharmacology 2006;31:620-627.
116. **Hong CJ, Tsai SJ, Cheng CY, Liao WY, Song HL, Lai HC.** Association analysis of the 5-HT(6) receptor polymorphism (C267T) in mood disorders. Am J Med Genet 1999;88:601-2.
117. **Papadimitriou GN, Dikeos DG, Souery D, Del-Favero J, Massat I, Avramopoulos D, et al.** Genetic association between the phospholipase A2 gene and unipolar affective disorder: a multicentre case-control study. Psychiatr Genet 2003;13:211-20.
118. **Pae CU, Yu HS, Kim JJ, Lee CU, Lee SJ, Lee KU, et al.** BanI polymorphism of the cytosolic phospholipase A2 gene and mood disorders in the Korean population. Neuropsychobiology 2004;49:185-8.
119. **Ohara K, Nagai M, Tani K, Tsukamoto T, Suzuki Y.** Polymorphism in the promoter region of the alpha 2A adrenergic receptor gene and mood disorders. Neuroreport 1998;9:1291-4.
120. **Gelernter J, Van Dyck C, van Kammen DP, Malison R, Price LH, Cubells JF, et al.** Ciliary neurotrophic factor null allele frequencies in schizophrenia, affective disorders, and Alzheimer's disease. Am J Med Genet 1997;74:497-500.
121. **Grunblatt E, Hupp E, Bambula M, Zehetmayer S, Jungwirth S, Tragl KH, et al.** Association study of BDNF and CNTF polymorphism to depression in non-demented subjects of the "VITA" study. J Affect Disord 2006;96:111-6.
122. **Tadokoro K, Hashimoto R, Tatsumi M, Kosuga A, Kamijima K, Kunugi H.** The Gem interacting protein (GMIP) gene is associated with major depressive disorder. Neurogenetics 2005;6:127-33.
123. **Yu YW, Chen TJ, Hong CJ, Chen HM, Tsai SJ.** Association study of the interleukin-1 beta (C-511T) genetic polymorphism with major depressive disorder, associated symptomatology, and antidepressant response. Neuropsychopharmacology 2003;28:1182-5.
124. **Owen D, Du L, Bakish D, Lapierre YD, Hrdina PD.** Norepinephrine transporter gene polymorphism is not associated with susceptibility to major depression. Psychiatry Res 1999;87:1-5.
125. **Zill P, Engel R, Baghai TC, Juckel G, Frodl T, Muller-Siecheneder F, et al.** Identification of a naturally occurring polymorphism in the promoter region of the norepinephrine transporter and analysis in major depression. Neuropsychopharmacology 2002;26:489-93.
126. **Zill P, Baghai TC, Engel R, Zwanzger P, Schule C, Minov C, et al.** Beta-1-adrenergic receptor gene in major depression: influence on antidepressant treatment response. Am J Med Genet B Neuropsychiatr Genet 2003;120:85-9.
127. **Garriock HA, Delgado P, Kling MA, Carpenter LL, Burke M, Burke WJ, et al.** Number of risk genotypes is a risk factor for major depressive disorder: a case control study. Behav Brain Funct 2006;2:24.
128. **Funke B, Malhotra AK, Finn CT, Plocik AM, Lake SL, Lencz T, et al.** COMT genetic variation confers risk for psychotic and affective disorders: a case control study. Behav Brain Funct 2005;1:19.

129. **Zill P, Baghai TC, Engel R, Zwanzger P, Schule C, Eser D, et al.** The dysbindin gene in major depression: an association study. Am J Med Genet B Neuropsychiatr Genet 2004;129:55-8.
130. **Zill P, Engel R, Baghai TC, Zwanzger P, Schule C, Minov C, et al.** Analysis of polymorphisms in the olfactory G-protein Golf in major depression. Psychiatr Genet 2002;12:17-22.
131. **Hong CJ, Yu YW, Chen TJ, Tsai SJ.** Interleukin-6 genetic polymorphism and Chinese major depression. Neuropsychobiology 2005;52:202-5.
132. **Geng YG, Su QR, Su LY, Chen Q, Ren GY, Shen SQ, et al.** Comparison of the polymorphisms of androgen receptor gene and estrogen alpha and beta gene between adolescent females with first-onset major depressive disorder and controls. Int J Neurosci 2007;117:539-47.
133. **Claes S, Villafuerte S, Forsgren T, Sluijs S, Del-Favero J, Adolfsson R, et al.** The corticotropin-releasing hormone binding protein is associated with major depression in a population from Northern Sweden. Biol Psychiatry 2003;54:867-72.
134. **Toyota T, Watanabe A, Shibuya H, Nankai M, Hattori E, Yamada K, et al.** Association study on the DUSP6 gene, an affective disorder candidate gene on 12q23, performed by using fluorescence resonance energy transfer-based melting curve analysis on the LightCycler. Mol Psychiatry 2000;5:461, 489-94.
135. **Jun TY, Pae CU, Chae JH, Bahk WM, Kim KS, Han H.** Report on IL-10 gene polymorphism at position -819 for major depression and schizophrenia in Korean population. Psychiatry Clin Neurosci 2002;56:177-80.
136. **van West D, Del-Favero J, Aulchenko Y, Oswald P, Souery D, Forsgren T, et al.** A major SNP haplotype of the arginine vasopressin 1B receptor protects against recurrent major depression. Mol Psychiatry 2004;9:287-92.
137. **Liu Z, Zhu F, Wang G, Xiao Z, Wang H, Tang J, et al.** Association of corticotropin-releasing hormone receptor1 gene SNP and haplotype with major depression. Neurosci Lett 2006;404:358-62.
138. **Qian Y, Lin S, Jiang S, Jiang K, Wu X, Tang G, et al.** Studies of the DXS7 polymorphism at the MAO loci in unipolar depression. Am J Med Genet 1999;88:598-600.
139. **Thomson PA, Wray NR, Thomson AM, Dunbar DR, Grassie MA, Condie A, et al.** Sex-specific association between bipolar affective disorder in women and GPR50, an X-linked orphan G protein-coupled receptor. Mol Psychiatry 2005;10:470-8.
140. **Schahab S, Heun R, Schmitz S, Maier W, Kolsch H.** Association of polymorphism in the transcription factor LBP-1c/CP2/LSF gene with Alzheimer's disease and major depression. Dement Geriatr Cogn Disord 2006;22:95-8.
141. **Schumacher J, Jamra RA, Becker T, Ohlraun S, Klopp N, Binder EB, et al.** Evidence for a relationship between genetic variants at the brain-derived neurotrophic factor (BDNF) locus and major depression. Biol Psychiatry 2005;58:307-14.
142. **Villafuerte SM, Del-Favero J, Adolfsson R, Souery D, Massat I, Mendlewicz J, et al.** Gene-based SNP genetic association study of the corticotropin-releasing hormone receptor-2 (CRHR2) in major depression. Am J Med Genet 2002;114:222-6.
143. **Tsai SJ, Wang YC, Hong CJ, Chiu HJ.** Association study of oestrogen receptor alpha gene polymorphism and suicidal behaviours in major depressive disorder. Psychiatr Genet 2003;13:19-22.
144. **Alda M, Grof P, Grof E.** MN blood groups and bipolar disorder: evidence of genotypic association and Hardy-Weinberg disequilibrium. Biol Psychiatry 1998;44:361-3.
145. **Beckman L, Cedergren B, Perris C, Strandman E.** Blood groups and affective disorders. Hum Hered 1978;28:48-55.
146. **Furlong RA, Ho L, Rubinsztein JS, Walsh C, Paykel ES, Rubinsztein DC.** No association of the tryptophan hydroxylase gene with bipolar affective disorder, unipolar affective disorder, or suicidal behaviour in major affective disorder. Am J Med Genet 1998;81:245-7.
147. **Jun TY, Pae CU, Chae JH, Bahk WM, Kim KS.** Polymorphism of CTLA-4 gene for major depression in the Korean population. Psychiatry Clin Neurosci 2001;55:533-7.
148. **Matsuyama SS, Joseph J.** Haptoglobin types and unipolar depression. Hum Hered 1984;34:65-8.
149. **Maes M, Delanghe J, Scharpe S, Meltzer HY, Cosyns P, Suy E, et al.** Haptoglobin phenotypes and gene frequencies in unipolar major depression. Am J Psychiatry 1994;151:112-6.

150. **Kolsch H, Ptok U, Majores M, Schmitz S, Rao ML, Maier W, et al.** Putative association of polymorphism in the mannose 6-phosphate receptor gene with major depression and Alzheimer's disease. Psychiatr Genet 2004;14:97-100.
151. **Tan EC, Chan AO, Tan CH, Mahendran R, Wang A, Chua HC.** Case-control and linkage disequilibrium studies of the tryptophan hydroxylase gene polymorphisms and major depressive disorder. Psychiatr Genet 2003;13:151-4.
152. **Hattori E, Yamada K, Ebihara M, Toyota T, Nankai M, Shibuya H, et al.** Association study of the short tandem repeat in the 5′ upstream region of the cholecystokinin gene with mood disorders in the Japanese population. Am J Med Genet 2002;114:523-6.
153. **Lerena A, Berecz R, Dorado P, Gonzalez AP, Penas LEM, De La Rubia A.** CYP2C9 gene and susceptibility to major depressive disorder. Pharmacogenomics J 2003;3:300-2.
154. **Covault J, Pettinati H, Moak D, Mueller T, Kranzler HR.** Association of a long-chain fatty acid-CoA ligase 4 gene polymorphism with depression and with enhanced niacin-induced dermal erythema. Am J Med Genet B Neuropsychiatr Genet 2004;127:42-7.
155. **Muramatsu T, Matsushita S, Kanba S, Higuchi S, Manki H, Suzuki E, et al.** Monoamine oxidase genes polymorphisms and mood disorder. Am J Med Genet 1997;74:494-6.
156. **Tadic A, Rujescu D, Szegedi A, Giegling I, Singer P, Moller HJ, et al.** Association of a MAOA gene variant with generalized anxiety disorder, but not with panic disorder or major depression. Am J Med Genet B Neuropsychiatr Genet 2003;117:1-6.
157. **Zill P, Baghai TC, Zwanzger P, Schule C, Eser D, Rupprecht R, et al.** SNP and haplotype analysis of a novel tryptophan hydroxylase isoform (TPH2) gene provide evidence for association with major depression. Mol Psychiatry 2004;9:1030-6.
158. **Van Den Bogaert A, Sleegers K, De Zutter S, Heyrman L, Norrback KF, Adolfsson R, et al.** Association of Brain-Specific Tryptophan Hydroxylase, TPH2, With Unipolar and Bipolar Disorder in a Northern Swedish, Isolated Population. Arch Gen Psychiatry 2006;63:1103-10.
159. **Zhou Z, Roy A, Lipsky R, Kuchipudi K, Zhu G, Taubman J, et al.** Haplotype-based linkage of tryptophan hydroxylase 2 to suicide attempt, major depression, and cerebrospinal fluid 5-hydroxyindoleacetic acid in 4 populations. Arch Gen Psychiatry 2005;62:1109-18.
160. **Zhang X, Gainetdinov RR, Beaulieu JM, Sotnikova TD, Burch LH, Williams RB, et al.** Loss-of-function mutation in tryptophan hydroxylase-2 identified in unipolar major depression. Neuron 2005;45:11-6.
161. **Delorme R, Durand CM, Betancur C, Wagner M, Ruhrmann S, Grabe HJ, et al.** No human tryptophan hydroxylase-2 gene R441H mutation in a large cohort of psychiatric patients and control subjects. Biol Psychiatry 2006;60:202-3.
162. **Slow E.** TPH2: an answer for unipolar depression? Clin Genet 2005;67:392-4.
163. **Philibert R, Caspers K, Langbehn D, Troughton EP, Yucuis R, Sandhu HK, et al.** The association of the D2S2944 124 bp allele with recurrent early onset major depressive disorder in women. Am J Med Genet B Neuropsychiatr Genet 2003;121:39-43.
164. **Langbehn DR, Philibert R, Caspers KM, Yucuis R, Cadoret RJ.** Association of a D2S2944 allele with depression specifically among those with substance abuse or antisocial personality. Drug Alcohol Depend 2006; 83: 33–41.
165. **Zubenko GS, Hughes IH, Stiffler JS, Zubenko WN, Kaplan BB.** D2S2944 identifies a likely susceptibility locus for recurrent, early-onset, major depression in women. Mol Psychiatry 2002;7:460-7.
166. **Hashimoto R, Suzuki T, Iwata N, Yamanouchi Y, Kitajima T, Kosuga A, et al.** Association study of the frizzled-3 (FZD3) gene with schizophrenia and mood disorders. J Neural Transm 2005;112:303-7.
167. **Jun TY, Pae CU, Hoon H, Chae JH, Bahk WM, Kim KS, et al.** Possible association between -G308A tumour necrosis factor-alpha gene polymorphism and major depressive disorder in the Korean population. Psychiatr Genet 2003;13:179-81.
168. **Borglum AD, Bruun TG, Kjeldsen TE, Ewald H, Mors O, Kirov G, et al.** Two novel variants in the DOPA decarboxylase gene: association with bipolar affective disorder. Mol Psychiatry 1999;4:545-51.

169. **Jahnes E, Muller DJ, Schulze TG, Windemuth C, Cichon S, Ohlraun S, et al.** Association study between two variants in the DOPA decarboxylase gene in bipolar and unipolar affective disorder. Am J Med Genet 2002;114:519-22.
170. **Hong W, Fang YR, Wang ZW, Qian YP, Zhang QT, Jiang SD, et al.** Association between depression and G72 gene polymorphism. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2006;23:532-5.
171. **Kunugi H, Hashimoto R, Yoshida M, Tatsumi M, Kamijima K.** A missense polymorphism (S205L) of the low-affinity neurotrophin receptor p75NTR gene is associated with depressive disorder and attempted suicide. Am J Med Genet B Neuropsychiatr Genet 2004;129:44-6.
172. **Ohtsuki T, Ishiguro H, Yoshikawa T, Arinami T.** WFS1 gene mutation search in depressive patients: detection of five missense polymorphisms but no association with depression or bipolar affective disorder. J Affect Disord 2000;58:11-7.
173. **Koido K, Koks S, Nikopensius T, Maron E, Altmae S, Heinaste E, et al.** Polymorphisms in wolframin (WFS1) gene are possibly related to increased risk for mood disorders. Int J Neuropsychopharmacol 2005;8:235-44.
174. **Furlong RA, Ho LW, Rubinsztein JS, Michael A, Walsh C, Paykel ES, et al.** A rare coding variant within the wolframin gene in bipolar and unipolar affective disorder cases. Neurosci Lett 1999;277:123-6.
175. **Pae CU, Yu HS, Kim TS, Lee CU, Lee SJ, Jun TY, et al.** Monocyte chemoattractant protein-1 (MCP1) promoter -2518 polymorphism may confer a susceptibility to major depressive disorder in the Korean population. Psychiatry Res 2004;127:279-81.
176. **Hashimoto R, Numakawa T, Ohnishi T, Kumamaru E, Yagasaki Y, Ishimoto T, et al.** Impact of the DISC1 Ser704Cys polymorphism on risk for major depression, brain morphology and ERK signaling. Hum Mol Genet 2006;15:3024-33.
177. **Horiuchi Y, Nakayama J, Ishiguro H, Ohtsuki T, Detera-Wadleigh SD, Toyota T, et al.** Possible association between a haplotype of the GABA-A receptor alpha 1 subunit gene (GABRA1) and mood disorders. Biol Psychiatry 2004;55:40-5.
178. **Yu YW, Chen TJ, Wang YC, Liou YJ, Hong CJ, Tsai SJ.** Association analysis for neuronal nitric oxide synthase gene polymorphism with major depression and fluoxetine response. Neuropsychobiology 2003;47:137-40.
179. **Furlong RA, Keramatipour M, Ho LW, Rubinsztein JS, Michael A, Walsh C, et al.** No association of an insertion/deletion polymorphism in the angiotensin I converting enzyme gene with bipolar or unipolar affective disorders. Am J Med Genet 2000;96:733-5.
180. **Pauls J, Bandelow B, Ruther E, Kornhuber J.** Polymorphism of the gene of angiotensin converting enzyme: lack of association with mood disorder. J Neural Transm 2000;107:1361-6.
181. S**egman RH, Shapira Y, Modai I, Hamdan A, Zislin J, Heresco-Levy U, et al.** Angiotensin converting enzyme gene insertion/deletion polymorphism: case-control association studies in schizophrenia, major affective disorder, and tardive dyskinesia and a family-based association study in schizophrenia. Am J Med Genet 2002;114:310-4.
182. **Arinami T, Li L, Mitsushio H, Itokawa M, Hamaguchi H, Toru M.** An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders. Biol Psychiatry 1996;40:1122-7.
183. **Konuk N, Atik L, Simsekyilmaz O, Dursun A, Acikgoz S.** Association of angiotensin converting enzyme gene polymorphism and affective disorders in Turkish patients. Aust N Z J Psychiatry 2006;40:717-8.
184. **Oswald P, Del-Favero J, Massat I, Souery D, Claes S, Van Broeckhoven C, et al.** No implication of brain-derived neurotrophic factor (BDNF) gene in unipolar affective disorder: evidence from Belgian first and replication patient-control studies. Eur Neuropsychopharmacol 2005;15:491-5.
185. **Hwang JP, Tsai SJ, Hong CJ, Yang CH, Lirng JF, Yang YM.** The Val66Met polymorphism of the brain-derived neurotrophic-factor gene is associated with geriatric depression. Neurobiol Aging 2005; 27: 1834–1837.

186. **Surtees PG, Wainwright NW, Willis-Owen SA, Sandhu MS, Luben R, Day NE, et al.** No association between the BDNF Val66Met polymorphism and mood status in a non-clinical community sample of 7389 older adults. J Psychiatr Res 2006; 41: 404–409.
187. **Jiang X, Xu K, Hoberman J, Tian F, Marko AJ, Waheed JF, et al.** BDNF variation and mood disorders: a novel functional promoter polymorphism and Val66Met are associated with anxiety but have opposing effects. Neuropsychopharmacology 2005;30:1353-61.
188. **Iga JI, Ueno SI, Yamauchi K, Numata S, Tayoshi-Shibuya S, Kinouchi S, et al.** The Val66Met polymorphism of the brain-derived neurotrophic factor gene is associated with psychotic feature and suicidal behavior in Japanese major depressive patients. Am J Med Genet B Neuropsychiatr Genet 2007;144B:1003-1006.
189. **Kunugi H, Vallada HP, Hoda F, Kirov G, Gill M, Aitchison KJ, et al.** No evidence for an association of affective disorders with high- or low-activity allele of catechol-o-methyltransferase gene. Biol Psychiatry 1997;42:282-5.
190. **Ohara K, Nagai M, Suzuki Y.** Low activity allele of catechol-o-methyltransferase gene and Japanese unipolar depression. Neuroreport 1998;9:1305-8.
191. **Massat I, Souery D, Del-Favero J, Nothen M, Blackwood D, Muir W, et al.** Association between COMT (Val158Met) functional polymorphism and early onset in patients with major depressive disorder in a European multicenter genetic association study. Mol Psychiatry 2005;10:598-605.
192. **Serretti A, Rotondo A, Lorenzi C, Smeraldi E, Cassano GB.** Catechol-O-methyltransferase gene variants in mood disorders in the Italian population. Psychiatr Genet 2006;16:181-2.
193. **Dikeos DG, Papadimitriou GN, Avramopoulos D, Karadima G, Daskalopoulou EG, Souery D, et al.** Association between the dopamine D3 receptor gene locus (DRD3) and unipolar affective disorder. Psychiatr Genet 1999;9:189-95.
194. **Henkel V, Baghai TC, Eser D, Zill P, Mergl R, Zwanzger P, et al.** The gamma amino butyric acid (GABA) receptor alpha-3 subunit gene polymorphism in unipolar depressive disorder: a genetic association study. Am J Med Genet B Neuropsychiatr Genet 2004;126:82-7.
195. **Massat I, Souery D, Del-Favero J, Oruc L, Jakovljevic M, Folnegovic V, et al.** Lack of association between GABRA3 and unipolar affective disorder: a multicentre study. Int J Neuropsychopharmacol 2001;4:273-8.
196. **Arias B, Arranz MJ, Gasto C, Catalan R, Pintor L, Gutierrez B, et al.** Analysis of structural polymorphisms and C-1018G promoter variant of the 5-HT(1A) receptor gene as putative risk factors in major depression. Mol Psychiatry 2002;7:930-2.
197. **Lemonde S, Turecki G, Bakish D, Du L, Hrdina PD, Bown CD, et al.** Impaired repression at a 5-hydroxytryptamine 1A receptor gene polymorphism associated with major depression and suicide. J Neurosci 2003;23:8788-99.
198. **Parsey RV, Oquendo MA, Ogden RT, Olvet DM, Simpson N, Huang YY, et al.** Altered serotonin 1A binding in major depression: a [carbonyl-C-11]WAY100635 positron emission tomography study. Biol Psychiatry 2006;59:106-13.
199. **Fehr C, Grintschuk N, Szegedi A, Anghelescu I, Klawe C, Singer P, et al.** The HTR1B 861G>C receptor polymorphism among patients suffering from alcoholism, major depression, anxiety disorders and narcolepsy. Psychiatry Res 2000;97:1-10.
200. **Huang YY, Oquendo MA, Friedman JM, Greenhill LL, Brodsky B, Malone KM, et al.** Substance abuse disorder and major depression are associated with the human 5-HT1B receptor gene (HTR1B) G861C polymorphism. Neuropsychopharmacology 2003;28:163-9.
201. **Ohara K, Nagai M, Tsukamoto T, Tani K, Suzuki Y.** 5-HT2A receptor gene promoter polymorphism--1438G/A and mood disorders. Neuroreport 1998;9:1139-41.
202. **Bonnier B, Gorwood P, Hamon M, Sarfati Y, Boni C, Hardy-Bayle MC.** Association of 5-HT(2A) receptor gene polymorphism with major affective disorders: the case of a subgroup of bipolar disorder with low suicide risk. Biol Psychiatry 2002;51:762-5.
203. **Choi MJ, Lee HJ, Ham BJ, Cha JH, Ryu SH, Lee MS.** Association between major depressive disorder and the -1438A/G polymorphism of the serotonin 2A receptor gene. Neuropsychobiology 2004;49:38-41.

204. **Zhang HY, Ishigaki T, Tani K, Chen K, Shih JC, Miyasato K, et al.** Serotonin2A receptor gene polymorphism in mood disorders. Biol Psychiatry 1997;41:768-73.
205. **Tsai SJ, Hong CJ, Hsu CC, Cheng CY, Liao WY, Song HL, et al.** Serotonin-2A receptor polymorphism (102T/C) in mood disorders. Psychiatry Res 1999;87:233-7.
206. **Arias B, Gutierrez B, Pintor L, Gasto C, Fananas L.** Variability in the 5-HT(2A) receptor gene is associated with seasonal pattern in major depression. Mol Psychiatry 2001;6:239-42.
207. **Minov C, Baghai TC, Schule C, Zwanzger P, Schwarz MJ, Zill P, et al.** Serotonin-2A-receptor and -transporter polymorphisms: lack of association in patients with major depression. Neurosci Lett 2001;303:119-22.
208. **Oswald P, Souery D, Massat I, Del-Favero J, Linotte S, Papadimitriou G, et al.** Lack of association between the 5HT2A receptor polymorphism (T102C) and unipolar affective disorder in a multicentric European study. Eur Neuropsychopharmacol 2003;13:365-8.
209. **Lerer B, Macciardi F, Segman RH, Adolfsson R, Blackwood D, Blairy S, et al.** Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 2001;6:579-85.
210. **Schulze TG, Muller DJ, Krauss H, Scherk H, Ohlraun S, Syagailo YV, et al.** Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder. Am J Med Genet 2000;96:801-3.
211. **Syagailo YV, Stober G, Grassle M, Reimer E, Knapp M, Jungkunz G, et al.** Association analysis of the functional monoamine oxidase A gene promoter polymorphism in psychiatric disorders. Am J Med Genet 2001;105:168-71.
212. **Du L, Bakish D, Ravindran A, Hrdina PD.** MAO-A gene polymorphisms are associated with major depression and sleep disturbance in males. Neuroreport 2004;15:2097-101.
213. **Gutierrez B, Arias B, Gasto C, Catalan R, Papiol S, Pintor L, et al.** Association analysis between a functional polymorphism in the monoamine oxidase A gene promoter and severe mood disorders. Psychiatr Genet 2004;14:203-8.
214. **Kunugi H, Ishida S, Kato T, Tatsumi M, Sakai T, Hattori M, et al.** A functional polymorphism in the promoter region of monoamine oxidase-A gene and mood disorders. Mol Psychiatry 1999;4:393-5.
215. **Yu YW, Tsai SJ, Hong CJ, Chen TJ, Chen MC, Yang CW.** Association study of a monoamine oxidase a gene promoter polymorphism with major depressive disorder and antidepressant response. Neuropsychopharmacology 2005;30:1719-23.
216. **Inoue K, Itoh K, Yoshida K, Shimizu T, Suzuki T.** Positive association between T-182C polymorphism in the norepinephrine transporter gene and susceptibility to major depressive disorder in a japanese population. Neuropsychobiology 2004;50:301-4.
217. **Ryu SH, Lee SH, Lee HJ, Cha JH, Ham BJ, Han CS, et al.** Association between norepinephrine transporter gene polymorphism and major depression. Neuropsychobiology 2004;49:174-7.
218. **Kunugi H, Ishida S, Kato T, Sakai T, Tatsumi M, Hirose T, et al.** No evidence for an association of polymorphisms of the tryptophan hydroxylase gene with affective disorders or attempted suicide among Japanese patients. Am J Psychiatry 1999;156:774-6.
219. **Souery D, Van Gestel S, Massat I, Blairy S, Adolfsson R, Blackwood D, et al.** Tryptophan hydroxylase polymorphism and suicidality in unipolar and bipolar affective disorders: a multicenter association study. Biol Psychiatry 2001;49:405-9.
220. **Ham BJ, Lee MS, Lee HJ, Kang RH, Han CS, Choi MJ, et al.** No association between the tryptophan hydroxylase gene polymorphism and major depressive disorders and antidepressant response in a Korean population. Psychiatr Genet 2005;15:299-301.
221. **R, Zaboli G, Jonsson EG, Asberg M, Leopardi R.** Haplotype Analysis Reveals Tryptophan Hydroxylase (TPH) 1 Gene Variants Associated with Major Depression. Biol Psychiatry 2005; 15;59:295-300

Candidate gene studies

3

The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis

Abstract

Objectives: We conducted a meta-analysis to re-evaluate the role of the dopamine D4 receptor gene 48-base-pair-repeat (*DRD4*) polymorphism in mood disorders. **Methods:** *DRD4* allele frequencies were compared between 917 patients with unipolar (UP) or bipolar affective disorder (BP), and 1,164 controls from twelve samples, using the Cochrane Review Manager. **Results:** An association was found between all mood disorder groups and *DRD4*.2. After correcting for multiple testing, the association between *DRD4*.2 and BP dropped to insignificance, however the evidence of an association between the *DRD4*.2 allele and UP ($p<0.001$), and the combined group ($p<0.001$) remained. There was no evidence for heterogeneity or publication bias. **Conclusions:** These findings suggest that the *DRD4*.2 allele is a risk allele for depression symptomatology.

Introduction

Disturbances in dopamine neurotransmission have been found in mood disorders.[1,2] Animal,[3,4] pharmacological,[5-11] and post-mortem studies,[12] suggest the dopamine D4 receptor gene (*DRD4*) as a candidate gene for mood disorders. The *DRD4* gene is located on chromosome 11p15.5.[13] The gene contains a functional polymorphism consisting of two to ten 48-bp-repeats in the third exon.[14]

The role of the *DRD4* in mood disorders has been researched in a large number of studies, but the findings are not consistent. Significant associations have been reported between *DRD4* alleles with unipolar depressive disorder (UP),[15] and with bipolar depressive disorder (BP).[16,17] However, others did not confirm these findings.[10,18-27] It remains possible that these studies were underpowered due to their small sample size.[28]

The aim of this study is to re-evaluate if there is an association of the dopamine D4 receptor gene 48-base-pair-repeat (*DRD4*) polymorphism with UP or with BP by performing a meta-analysis.

Methods and Materials

Identification of studies

We searched Medline for all publications available up to February 2004 studying the association between the *DRD4* 48-bp-repeat polymorphism and mood disorders with the keywords *mood, affective, unipolar, bipolar, depression, DRD4* and *D4*. References from retrieved publications were checked for any additional studies. Seventeen articles were identified.[10,15-26,29-32] Three articles used a family-based design.[17,18,24]

In order to include the studies with the same methodological and statistical techniques, we limited the analysis to studies using a case-control design and studies that reported allele frequencies. Studies with overlapping cases were excluded,[19,26,29,31,32] to only include the study with the largest number of cases.[25] The included studies are listed in Tables 1 and 2.

We distinguished three groups of cases: UP, BP, and both combined. In total, 318 patients and 814 control individuals from 5 studies were included in the UP meta-analysis.[10,15,20,25] In the BP meta-analysis, seven studies were included.[10,15,16,21,23,25] In total, 599 patients with BP were compared to 992 control individuals. In the combined group, 917 patients and 1,164 control individuals were entered.

Statistical Analysis

Hardy-Weinberg equilibrium was examined in studies where genotype frequencies were included.[15,16,20,21,25] We examined the effects of the three most prevalent alleles:

Table 1 Association studies of *DRD4* 48-bp-repeat polymorphism and unipolar disorders

References	Country/origin	Subject category	N	Allele Frequency, n (%)				p (HWE[1])
				2	4	7	Other	
Frisch et al., 1999	Israel Ashkenazi Jews	Patient	63	12 (9)	86 (68)	24 (19)	4 (3)	0.99
		Control	112	10 (4)	160 (71)	48 (21)	6 (3)	0.99
Frisch et al., 1999	Israel non Ashkenazi Jews	Patient	39	9 (12)	53 (70)	10 (13)	4 (5)	0.00
		Control	60	11 (9)	78 (65)	27 (22)	4 (3)	0.99
Manki et al., 1996	Japan	Patient	49	11 (11.2)	77 (78.6)	0 (0)	10 (10.2)	0.99
		Control	100	12 (6)	179 (89.5)	0 (0)	9 (4.5)	0.00
Oruc et al. 1997	Croatia	Patient	41	13 (15)	50 (58)	16 (19)	7 (8)	0.99
		Control	71	11 (8)	88 (68)	21 (17)	10 (7)	1.00
Serretti et al., 1999	Italy	Patient	126	40 (16)	177 (70)	23 (9)	12 (4)	0.99
		Control	471	98 (10)	655 (70)	143 (15)	46 (6)	0.65

*Genotypes are presented in the paper for all cases combined and controls combined, so both samples are tested together. [1] HWE= Hardy-Weinberg equilibrium

Table 2 Association studies of *DRD4* 48-bp-repeat polymorphism and bipolar disorders

References	Country/origin	Subject category	N	Allele Frequency, n (%)				p (HWE[1])
				2	4	7	Other	
Li et al., 1999	China	Patient	84	35 (20.8)	127 (75.6)	-	6 (3.6)	0.00
		Control	154	55 (17.9)	237 (76.9)	-	16 (5.3)	0.24
Lim et al., 1994	Western Europe	Patient	57	7 (6.1)	74 (64.9)	28 (24.6)	5 (25.5)	0.97*
		Control	59	10 (8.5)	78 (66.1)	16 (13.5)	14 (15.2)	0.00*
Lim et al., 1994	Germany	Patient	90	20 (11.1)	113 (62.8)	33 (18.3)	14 (7.8)	0.97*
		Control	91	19 (10.4)	110 (60.4)	40 (22)	13 (7.2)	0.00*
Manki et al., 1996	Japan	Patient	52	9 (8.7)	87 (83.7)	1 (1)	7 (6.7)	0.53
		Control	100	12 (6)	179 (89.5)	0 (0)	9 (4.5)	0.00
Oruč et al., 1997	Croatia	Patient	42	7 (9)	53 (68)	10 (13)	8 (10)	0.99
		Control	71	11 (8)	88 (68)	21 (17)	10 (7)	1.00
Pérez de Castro et al., 1994	Spain	Patient	64	14 (11)	84 (66)	20 (16)	8 (7)	
		Control	46	12 (13)	60 (66)	10 (11)	9 (10)	
Serretti et al., 1999	Italy	Patient	210	64 (15)	271 (65)	60 (14)	25 (6)	0.01
		Control	471	98 (10)	655 (70)	143 (15)	46 (6)	0.65

*Genotypes are presented in the paper for all cases combined and controls combined, so both samples are tested together. [1] HWE= Hardy-Weinberg equilibrium

two-repeat (*DRD4*.2), four-repeat (*DRD4*.4), and seven-repeat (*DRD4*.7). In addition we examined the association of mood disorders with short (one-repeat to four-repeat) versus long alleles (five-repeat to ten-repeat), a definition commonly used.[21,29] For all the meta-analyses we used the Cochrane Review Manager (RevMan) version 4.2 and analyzed them with RevMan analysis 1.0.1. The method of moments was applied to calculate the odds ratio (OR) in a random-effects model for the pooled data.[33] The p level, adjusted for multiple testing, was considered significant when lower than 0.003 (Bonferroni corrected: p=0.05/15, to justify for the three groups and the five genotypic subgroups tested).

RevMan was also used to produce funnel plots to examine publication bias. The method is based on the fact that precision in the estimation of the true effect increases as sample sizes of the studies increase. OR, representing the relative effects estimated from individual studies (x axis), is plotted against the standard error on a logarithmic scale as a measure of each study's sample size (y axis). This takes into account that the statistical power of a trial is determined both by its total sample size and the number of outcomes. In the absence of bias the plot should resemble a symmetrical inverted funnel, hence the name.[34,35]

Results

All studies used structured diagnostic interviews. One study,[15] used the DSM-III-R criteria,[36] all the other studies applied the RDC.[37,38]

DRD4.4 was the most prevalent allele in all patients and control individuals, while the *DRD4*.7 allele was not present in the Japanese population. The proportion of alleles and genotypes of one control sample in the UP group, and two control samples in the BP group, were not consistent with HWE.[15,16] Therefore we performed the analyses both including and excluding these studies. The exclusion of these samples did not materially change the results. One bipolar study did not include genotype frequencies,[16] therefore it was not possible to test for HWE in this study.

Table 3 presents OR and p values for the pooled analyses of the *DRD4* alleles studied. An association was found between all mood disorder groups and *DRD4*.2. After correcting for multiple testing, the association between *DRD4*.2 and BP dropped to insignificance, however the evidence of an association between the *DRD4*.2 allele and UP ($p<0.001$), and the combined group ($p<0.001$) remained. No association was observed with the other alleles tested or when divided into short and long alleles.

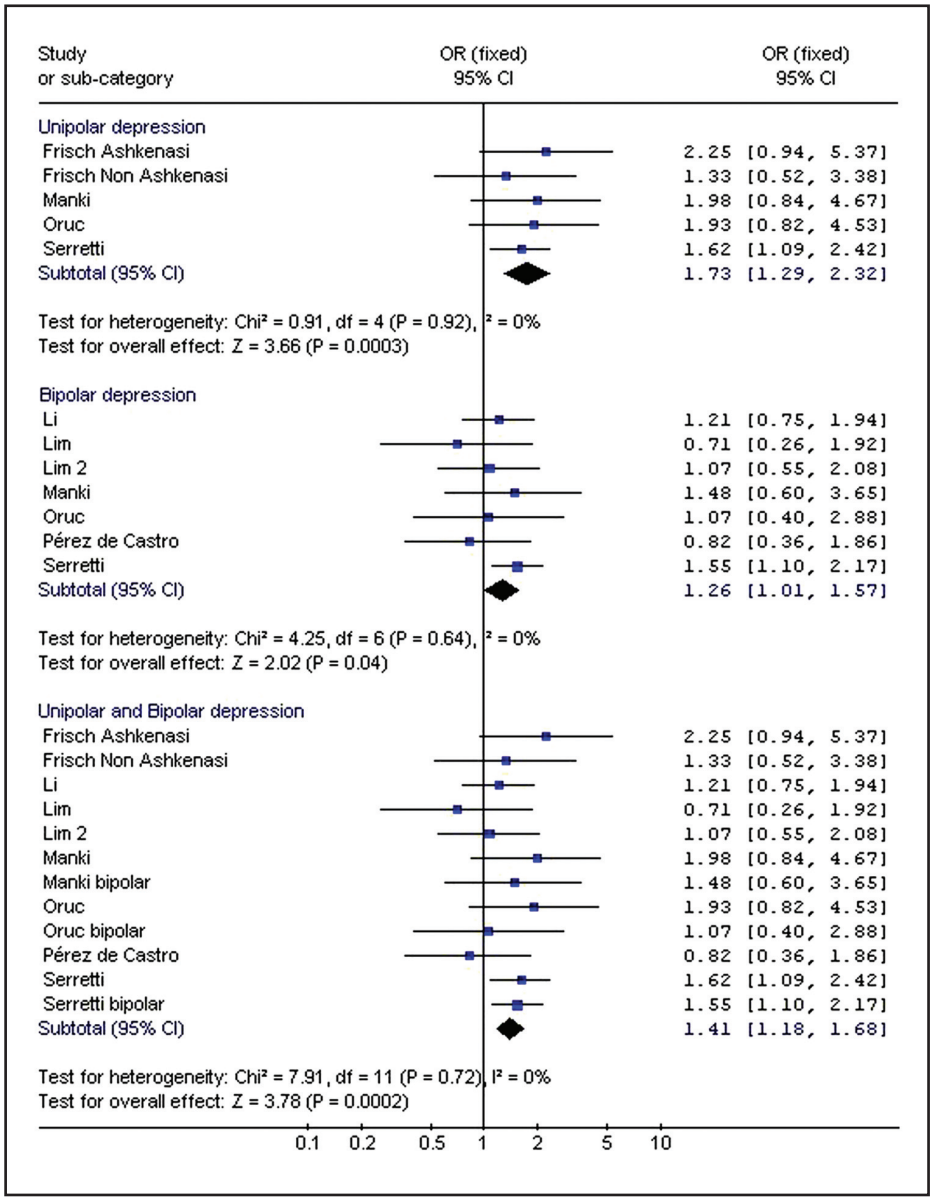

Figure 1 Relation between Unipolar (UP), Bipolar (BP) and the *DRD4*.2 48bp-repeat polymorphism

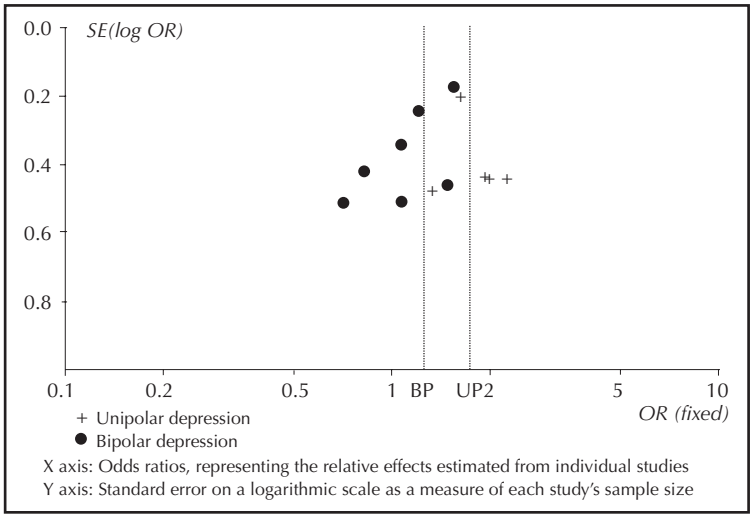

Figure 2 Funnel plots for Unipolar (UP) and Bipolar (BP) groups

Table 3 Relation between Unipolar (UP), Bipolar (BP) and the *DRD4* 48bp-repeat polymorphisms

Allele	Mood Disorder	OR	CI	p value
DRD4.2	Unipolar	1.73	1.29-2.32	0.0003*
	Bipolar	1.26	1.01-1.57	0.04
	Unipolar + Bipolar	1.41	1.18-1.68	0.0002*
DRD4.4	Unipolar	0.88	0.72-1.09	0.24
	Bipolar	0.88	0.75-1.04	0.13
	Unipolar + Bipolar	0.88	0.78-1.00	0.06
DRD4.7	Unipolar	0.70	0.53-0.94	0.02
	Bipolar	1.03	0.82-1.30	0.80
	Unipolar + Bipolar	0.89	0.74-1.06	0.18
Short	Unipolar	1.23	0.89-1.70	0.22
	Bipolar	0.95	0.77-1.17	0.60
	Unipolar + Bipolar	1.02	0.86-1.22	0.80

*significant after Bonferroni correction $p < 0.003$, [1]

Figure 1 presents the OR for the individual studies and the pooled analyses for the *DRD4*.2 allele. Of note is that all UP studies showed a consistent increase in OR while for BP five out of seven studies showed an OR higher than 1. For both UP and BP there was no evidence for heterogeneity in ORs across studies. Figure 2 presents the funnel plots of the *DRD4*.2 allele. The plot for the UP group is quite symmetric, but this is not the case for the BP group. More BP negative results have been published as compared to positive ones.

Discussion

In this study we pooled the data of 917 patients and 1,164 control individuals to re-evaluate the association between the *DRD4* 48-bp-repeat polymorphism and mood disorders. The results of our study showed a significant association between *DRD4*.2 allele in two groups: UP, and UP and BP combined. These findings suggest that the *DRD4*.2 allele is a risk allele for depression symptomatology.

Association studies have been widely used to identify susceptibility genes.[39] There is increasing evidence that small sample sized association studies lack statistical power and have resulted in apparently contradicting past findings.[28] Ioannidis and Lohmueller found that, when combining association studies with contradictory results, approximately 20-30% of genetic association studies were statistically significant.[40,28] The use of meta-analysis is an important step in reconciling previously conducted studies with inconsistent results.

In our study there is no evidence of heterogeneity across studies, even though we included populations of Europe, Israel, China and Japan. Ethnicity was not associated with the *DRD4*.2 allele frequency, making it unlikely that ethnicity was a confounder in this meta-analysis. Both random-effect and fixed-effect models gave the same results, further supporting the absence of heterogeneity.[41] A limitation of meta-analysis is publication bias because the likelihood of publishing a study could be related to the positive results of the study.[35] The funnel plot in the UP group is quite symmetrical, showing no evidence of publication bias. The funnel plot in the BP group shows a small excess of negative studies published. This may have biased our finding towards an OR of 1.

We only included population-based studies and excluded family-based studies because of their different methodology. Another reason is that none of the three family-based studies assessed UP; they focused on BP yielding no significant results.[17,18,24] The exception is the study of Serretti et al 2002, which also included a combined BP and UP group, reporting a p=0.037 for *DRD4*.2. This was not reported as significant since a p value of 0.0125 was set after Bonferroni correction. However

this finding does not exclude the association we observed. All results of the three family-based studies are consistent with our findings.

DRD4 is expressed in many regions of the body, with a high level of expression in the frontal area of the brain and in the nucleus acumbens,[2,42-44] areas associated with anhedonia, lack of motivation, and affective and emotional behaviors.[2] It has been observed that all antidepressants, in one way or the other, affect dopamine in these areas,[3,45-48] and that D4 receptor agonists,[45,49-53] such as methylphenidate,[54-58] have antidepressant properties.

The mechanism by which dopamine D4 receptor expression is regulated is not yet fully understood,[59] and much of the research has focused particularly on *DRD4.7*, since this allele has been consistently associated with ADHD, schizophrenia, and novelty-seeking.[60-62] One of the most studied functions of the D4 receptors is that they inhibit adenylyl cyclase activity and thereby reduce conversion of ATP into cAMP.[63-69] It has been reported that dopamine *DRD4.2* receptors are less potent than the *DRD4.4* or the *DRD4.10* in coupling to adenylyl cyclase, and show a blunted ATP to cAMP conversion.[42,64,65] D4 receptors with a sub-optimal functionality – as seen with *DRD4.2* – may account for symptoms of depression.

Another issue to consider is genetic imprinting, it has been suggested in particular for BP.[70,71] Such a mechanism may explain why the power of the studies is low. However Van Broeckhoven et al 1999, have not found evidence for genetic imprinting in BP and to our knowledge there is no evidence in UP.

In summary, we found evidence of a positive association between the *DRD4*.2 allele in two groups: UP, and UP and BP combined. These results show that meta-analysis can overcome the low power of small sample size studies, which are characteristic of genetic association studies. Thus, our meta-analysis strongly shows that *DRD4* cannot be excluded as a susceptibility gene for mood disorders.

References

1. **Diehl DJ, Gershon S.** The role of dopamine in mood disorders. Compr Psychiatry 1992;33: 115-20.
2. **Emilien G, Maloteaux JM, Geurts M, Hoogenberg K, Cragg S.** Dopamine receptors - physiological understanding to therapeutic intervention potential. Pharmacol Ther 1999;84:133-56.
3. **Ainsworth K, Smith SE, Sharp T.** Repeated administration of fluoxetine, desipramine and tranylcypromine increases dopamine D2-like but not D1-like receptor function in the rat. J Psychopharmacol 1998;12:252-7.
4. **Rubinstein M, Phillips TJ, Bunzow JR, Falzone TL, Dziewczapolski G, Zhang G, et al.** Mice lacking dopamine D4 receptors are supersensitive to ethanol, cocaine, and methamphetamine. Cell 1997;90:991-1001.

5. **Laruelle M, Abi-Dargham A, van Dyck CH, Rosenblatt W, Zea-Ponce Y, Zoghbi SS, et al.** SPECT imaging of striatal dopamine release after amphetamine challenge. J Nucl Med 1995;36:1182-90.
6. **Zarate CA, Jr., Tohen M, Banov MD, Weiss MK, Cole JO.** Is clozapine a mood stabilizer? J Clin Psychiatry 1995;56:108-12.
7. **Calabrese JR, Kimmel SE, Woyshville MJ, Rapport DJ, Faust CJ, Thompson PA, et al.** Clozapine for treatment-refractory mania. Am J Psychiatry 1996;153:759-64.
8. **Barbini B, Scherillo P, Benedetti F, Crespi G, Colombo C, Smeraldi E.** Response to clozapine in acute mania is more rapid than that of chlorpromazine. Int Clin Psychopharmacol 1997;12:109-12.
9. **Tang AH, Franklin SR, Himes CS, Smith MW, Tenbrink RE.** PNU-96415E, a potential antipsychotic agent with clozapine-like pharmacological properties. J Pharmacol Exp Ther 1997;281:440-7.
10. **Oruc L, Verheyen GR, Furac I, Jakovljevic M, Ivezic S, Raeymaekers P, et al.** Analysis of the tyrosine hydroxylase and dopamine D4 receptor genes in a Croatian sample of bipolar I and unipolar patients. Am J Med Genet 1997;74:176-8.
11. **Drevets WC, Gautier C, Price JC, Kupfer DJ, Kinahan PE, Grace AA, et al.** Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. Biol Psychiatry 2001;49:81-96.
12. **Bowden C, Theodorou AE, Cheetham SC, Lowther S, Katona CL, Crompton MR, et al.** Dopamine D1 and D2 receptor binding sites in brain samples from depressed suicides and controls. Brain Res 1997;752:227-33.
13. **Van Tol HH, Bunzow JR, Guan HC, Sunahara RK, Seeman P, Niznik HB, et al.** Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. Nature 1991;350:610-4.
14. **Van Tol HH, Wu CM, Guan HC, Ohara K, Bunzow JR, Civelli O, et al.** Multiple dopamine D4 receptor variants in the human population. Nature 1992;358:149-52.
15. **Manki H, Kanba S, Muramatsu T, Higuchi S, Suzuki E, Matsushita S, et al.** Dopamine D2, D3 and D4 receptor and transporter gene polymorphisms and mood disorders. J Affect Disord 1996;40:7-13.
16. **Lim LC, Nothen MM, Korner J, Rietschel M, Castle D, Hunt N, et al.** No evidence of association between dopamine D4 receptor variants and bipolar affective disorder. Am J Med Genet 1994;54:259-63.
17. **Muglia P, Petronis A, Mundo E, Lander S, Cate T, Kennedy JL.** Dopamine D4 receptor and tyrosine hydroxylase genes in bipolar disorder: evidence for a role of DRD4. Mol Psychiatry 2002;7:860-6.
18. **Bocchetta A, Piccardi MP, Palmas MA, Chillotti C, Oi A, Del Zompo M.** Family-based association study between bipolar disorder and DRD2, DRD4, DAT, and SERT in Sardinia. Am J Med Genet 1999;88:522-6.
19. **Cusin C, Serretti A, Lattuada E, Lilli R, Lorenzi C, Smeraldi E.** Association study of MAO-A, COMT, 5-HT2A, DRD2, and DRD4 polymorphisms with illness time course in mood disorders. Am J Med Genet 2002;114:380-90.
20. **Frisch A, Postilnick D, Rockah R, Michaelovsky E, Postilnick S, Birman E, et al.** Association of unipolar major depressive disorder with genes of the serotonergic and dopaminergic pathways. Mol Psychiatry 1999;4:389-92.
21. **Li T, Liu X, Sham PC, Aitchison KJ, Cai G, Arranz MJ, et al.** Association analysis between dopamine receptor genes and bipolar affective disorder. Psychiatry Res 1999;86:193-201.
22. **Macciardi F, Cavallini MC, Petronis A, Marino C, Verga M, Cauli G, et al.** The D4 receptor gene and Mood Disorders: an association study. Am J Med Genet 1994;55:A336 (Abstract).
23. **Perez De Castro I, Torres P, Fernandez-Piqueras J, Saiz-Ruiz J, Llinares C.** No association between dopamine D4 receptor polymorphism and manic depressive illness. J Med Genet. 1994;31:897-8.
24. **Serretti A, Cristina S, Lilli R, Cusin C, Lattuada E, Lorenzi C, et al.** Family-based association study of 5-HTTLPR, TPH, MAO-A, and DRD4 polymorphisms in mood disorders. Am J Med Genet 2002; 114:361-9.
25. **Serretti A, Lilli R, Di Bella D, Bertelli S, Nobile M, Novelli E, et al.** Dopamine receptor D4 gene is not associated with major psychoses. Am J Med Genet 1999;88:486-91.
26. **Serretti A, Macciardi F, Cusin C, Lattuada E, Lilli R, Smeraldi E.** Dopamine receptor D4 gene is associated with delusional symptomatology in mood disorders. Psychiatry Res 1998;80:129-36.

27. **De Bruyn A, Mendelbaum K, Sandkuijl LA, Delvenne V, Hirsch D, Staner L, et al.** Nonlinkage of bipolar illness to tyrosine hydroxylase, tyrosinase, and D2 and D4 dopamine receptor genes on chromosome 11. Am J Psychiatry 1994;151:102-6.
28. **Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN.** Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177-82.
29. **Serretti A, Lilli R, Lorenzi C, Lattuada E, Smeraldi E.** DRD4 exon 3 variants associated with delusional symptomatology in major psychoses: a study on 2,011 affected subjects. Am J Med Genet 2001;105:283-90.
30. **Serretti A, Macciardi F, Catalano M, Bellodi L, Smeraldi E.** Genetic variants of dopamine receptor D4 and psychopathology. Schizophr Bull 1999;25:609-18.
31. **Serretti A, Cusin C, Lattuada E, Lilli R, Lorenzi C, Di Bella D, et al.** No interaction between serotonin transporter gene and dopamine receptor D4 gene in symptomatology of major psychoses. Am J Med Genet 1999;88:481-5.
32. **Chotai J, Serretti A, Lattuada E, Lorenzi C, Lilli R.** Gene-environment interaction in psychiatric disorders as indicated by season of birth variations in tryptophan hydroxylase (TPH), serotonin transporter (5-HTTLPR) and dopamine receptor (DRD4) gene polymorphisms. Psychiatry Res 2003; 119:99-111.
33. **DerSimonian R, Laird N.** Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.
34. **Villar J, Piaggio G, Carroli G, Donner A.** Factors affecting the comparability of meta-analyses and largest trials results in perinatology. J Clin Epidemiol 1997;50:997-1002.
35. **Egger M, Davey Smith G, Schneider M, Minder C.** Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
36. **American Psychiatric Association.** Diagnostic and Statistical Manual of Mental Disorders-IV. Washington DC, 1994.
37. **Endicott J, Spitzer RL.** A diagnostic interview: the schedule for affective disorders and schizophrenia. Arch Gen Psychiatry 1978;35:837-44.
38. **Spitzer RL, Endicott J, Robins E.** Research diagnostic criteria. Psychopharmacol Bull 1975;11:22-5.
39. **Risch NJ.** Searching for genetic determinants in the new millennium. Nature 2000;405: 847-56.
40. **Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG.** Replication validity of genetic association studies. Nat Genet 2001;29:306-9.
41. **Rothman J.** Modern Epidemiology. 2 ed. Philadelphia, PA: Lippincott, 1998.
42. **Oak JN, Oldenhof J, Van Tol HH.** The dopamine D(4) receptor: one decade of research. Eur J Pharmacol 2000;405:303-27.
43. **Wedzony K, Chocyk A, Mackowiak M, Fijal K, Czyrak A.** Cortical localization of dopamine D4 receptors in the rat brain--immunocytochemical study. J Physiol Pharmacol 2000;51:205-21.
44. **Tarazi FI, Campbell A, Yeghiayan SK, Baldessarini RJ.** Localization of dopamine receptor subtypes in corpus striatum and nucleus accumbens septi of rat brain: comparison of D1-, D2-, and D4-like receptors. Neuroscience 1998;83:169-76.
45. **D'Aquila PS, Collu M, Gessa GL, Serra G.** The role of dopamine in the mechanism of action of antidepressant drugs. Eur J Pharmacol 2000;405:365-73.
46. **Tremblay LK, Naranjo CA, Cardenas L, Herrmann N, Busto UE.** Probing brain reward system function in major depressive disorder: altered response to dextroamphetamine. Arch Gen Psychiatry 2002;59:409-16.
47. **Spyraki C, Fibiger HC.** Behavioural evidence for supersensitivity of postsynaptic dopamine receptors in the mesolimbic system after chronic administration of desipramine. Eur J Pharmacol 1981;74:195-206.
48. **Willner P.** The mesolimbic dopamine system as a target for rapid antideressant acction. Int Clin Psychopharmacol 1997;12:S7-S14.
49. **Post RM, Gerner RH, Carman JS, Gillin JC, Jimerson DC, Goodwin FK, et al.** Effects of a dopamine agonist piribedil in depressed patients: relationship of pretreatment homovanillic acid to antidepressant response. Arch Gen Psychiatry 1978;35:609-15.
50. **D'Aquila PS, Collu M, Gessa GL, Serra G.** Role of alpha 1 receptors in the behavioural supersensitivity to D2 agonists induced by chronic treatment with imipramine. Pharmacol Res 1992;25:95-101.

51. **Collu M, Poggiu AS, Devoto P, Serra G.** Behavioural sensitization of mesolimbic dopamine D2 receptors in chronic fluoxetine-treated rats. Eur J Pharmacol 1997;322:123-7.
52. **Nordin C, Siwers B, Bertilsson L.** Bromocriptine treatment of depressive disorders. Clinical and biochemical effects. Acta Psychiatr Scand 1981;64:25-33.
53. **Silverstone T.** Response to bromocriptine distinguishes bipolar from unipolar depression. Lancet 1984;1:903-4.
54. **Masand P, Pickett P, Murray GB.** Psychostimulants for secondary depression in medical illness. Psychosomatics 1991;32:203-8.
55. **Katon W, Raskind M.** Treatment of depression in the medically ill elderly with methylphenidate. Am J Psychiatry 1980;137:963-5.
56. **Kaufmann MW, Murray GB, Cassem NH.** Use of psychostimulants in medically ill depressed patients. Psychosomatics 1982;23:817-9.
57. **Kaplitz SE.** Withdrawn, apathetic geriatric patients responsive to methylphenidate. J Am Geriatr Soc 1975;23:271-6.
58. **Woods SW, Tesar GE, Murray GB, Cassem NH.** Psychostimulant treatment of depressive disorders secondary to medical illness. J Clin Psychiatry 1986;47:12-5.
59. **Wang E, Ding YC, Flodman P, Kidd JR, Kidd KK, Grady DL, et al.** The genetic architecture of selection at the human dopamine receptor D4 (DRD4) gene locus. Am J Hum Genet 2004;74:931-44.
60. **Schinka JA, Letsch EA, Crawford FC.** DRD4 and novelty seeking: results of meta-analyses. Am J Med Genet 2002;114:643-8.
61. **Faraone SV, Biederman J, Weiffenbach B, Keith T, Chu MP, Weaver A, et al.** Dopamine D4 gene 7-repeat allele and attention deficit hyperactivity disorder. Am J Psychiatry 1999;156:768-70.
62. **Glatt SJ, Faraone SV, Tsuang MT.** Schizophrenia is not associated with DRD4 48-base-pair-repeat length or individual alleles: results of a meta-analysis. Biol Psychiatry 2003;54:629-35.
63. **Yamaguchi I, Harmon SK, Todd RD, O'Malley KL.** The rat D4 dopamine receptor couples to cone transducin (Galphat2) to inhibit forskolin-stimulated cAMP accumulation. J Biol Chem 1997;272:16599-602.
64. **Asghari V, Sanyal S, Buchwaldt S, Paterson A, Jovanovic V, Van Tol HH.** Modulation of intracellular cyclic AMP levels by different human dopamine D4 receptor variants. J Neurochem 1995;65:1157-65.
65. **Watts VJ, Vu MN, Wiens BL, Jovanovic V, Van Tol HH, Neve KA.** Short- and long-term heterologous sensitization of adenylate cyclase by D4 dopamine receptors. Psychopharmacology (Berl) 1999;141:83-92.
66. **Obadiah J, Avidor-Reiss T, Fishburn CS, Carmon S, Bayewitch M, Vogel Z, et al.** Adenylyl cyclase interaction with the D2 dopamine receptor family; differential coupling to Gi, Gz, and Gs. Cell Mol Neurobiol 1999;19:653-64.
67. **Tang L, Todd RD, Heller A, O'Malley KL.** Pharmacological and functional characterization of D2, D3 and D4 dopamine receptors in fibroblast and dopaminergic cell lines. J Pharmacol Exp Ther 1994;268:495-502.
68. **Sanyal S, Van Tol HH.** Dopamine D4 receptor-mediated inhibition of cyclic adenosine 3',5'-monophosphate production does not affect prolactin regulation. Endocrinology 1997;138: 1871-8.
69. **Cohen AI, Todd RD, Harmon S, O'Malley KL.** Photoreceptors of mouse retinas possess D4 receptors coupled to adenylate cyclase. Proc Natl Acad Sci U S A 1992;89:12093-7.
70. **Petronis A.** The genes for major psychosis: aberrant sequence or regulation? Neuropsychopharmacology 2000;23:1-12.
71. **Reik W, Walter J.** Genomic imprinting: parental influence on the genome. Nat Rev Genet 2001;2: 21-32.

4

No association between the angiotensin-converting enzyme gene and major depression: a case-control study and meta-analysis

Abstract

The *ACE* I/D polymorphism has been studied as a candidate gene for major depression, but findings are inconsistent. To re-evaluate this association we conducted a case-control study and a meta-analysis. The case-control study was embedded within the Rotterdam Study, a population-based cohort of subjects aged 55 years and older. For the meta-analysis, we searched Medline for studies on the association between the *ACE* I/D polymorphism and major depression. We calculated odds ratios to compare genotype frequencies between cases and controls. The case-control study included 64 cases and 3,980 controls. The meta-analysis comprised six studies, encompassing 586 cases and 5,169 controls. No evidence was found for an association between the *ACE* I/D polymorphism and major depression in either study. Our results indicate that the *ACE* I/D polymorphism is not associated to major depression.

Introduction

Several lines of evidence in experimental, pharmacological and physiological research [1-3] suggest that the angiotensin converting enzyme (ACE) is involved in major depression. Furthermore, it is well known that the *ACE* gene is involved in the metabolism of the neuropeptide substance P, which in turn is involved in the regulation of affective behavior.[4] The gene that codes for ACE is located on chromosome 17q23. It contains a polymorphism that has either an insertion or a deletion (I/D) of a 287 base-pairs fragment in the 16th intron. The *ACE* gene has been studied as a candidate gene for major depression, but findings are inconsistent. Arinami et al. reported an increased risk of major depression for individuals carrying the *ACE* D homozygous genotype (*ACE* DD), and a higher level of substance P in subjects with this genotype.[4] Later studies were unable to replicate this finding,[5-8] although one observed a higher risk associated with the *ACE* heterozygote genotype (*ACE* ID).[8] Most studies were of limited size (n<200) raising the question whether lack of statistical power explains the negative findings.

We conducted a case-control study on the relationship between the *ACE* I/D polymorphism and major depression in a population-based cohort. Additionally we combined all available studies in a meta-analysis to investigate the overall evidence for a role of the *ACE* gene in major depression.

Methods

Case-Control Study

The cross sectional case-control study was embedded within the Rotterdam Study, a population based cohort of subjects aged 55 years and older (n=7,983).[9] All participants gave informed consent and the Medical Ethical Committee of the Erasmus University Medical Center approved the scientific protocols. Cases and controls were selected among subjects who participated in the third assessment of the cohort (n=4,797). A total of 4,652 subjects (97%) completed the Center of Epidemiological Studies Depression Scale (CES-D) and underwent extensive medical examinations. All participants with a CES-D score higher than 16 (n=336, 7.2%) were subsequently evaluated according to the DSM-IV using the Schedules for Clinical Assessment in Neuropsychiatry. Cases (n=64) were subjects who fulfilled the DSM-IV criteria for major depression. Controls (n=3,980) were subjects who scored lower than 16 on the CES-D and had no history of depression.

DNA was isolated from blood samples using salting out method standard procedures. The II, ID, and DD genotypes were detected by using the polymerase chain-reaction (PCR) technique. The relationship between the *ACE* I/D polymorphism and

major depression was assessed by odds ratios (ORs) using logistic regression analysis adjusting for age and sex. ORs with 95% confidence intervals (CI) were calculated for the ID and DD genotypes using the II genotype as the reference group. Analyses were performed on SPSS software version 11.5 (SPSS, Chicago, USA).

Meta-analysis

For the meta-analysis, we searched Medline for case-control and cohort studies on the association between the *ACE* I/D polymorphism and major depression published before October 2005. The search strategy was based on the key words *mood, affective, unipolar* or *depression* combined with *angiotensin* or *ACE*. We limited our search to human populations and articles written in English. References in retrieved articles were screened for additional studies.

Pooled ORs were calculated for the ID and DD genotype using random-effects models of DerSimonian and Laird taking the II genotype as the reference group. The degree of heterogeneity between the study results was tested with the chi-square test. Publication bias was evaluated by examination of funnel plots. The meta-analysis was performed with the Cochrane Review Manager Version 4.2. P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

Case-Control Study

The study population consisted of 64 unrelated cases of major depression (prevalence=1.4%; mean age=76.2 years, SD=7.8; 30% men) and 3,980 controls (mean age=72.7 years, SD=7.3; 43% men). There were no significant differences in the frequencies of the *ACE* alleles and the genotypes between cases (II=28%, ID=42%, DD=30%) and controls (II=22%, ID=51%, DD=27%). We found no statistically significant association to major depression for either the ID genotype (adjusted OR=0.61, 95% CI=0.33-1.12, p=0.11) or the DD genotype (adjusted OR=0.80, 95% CI=0.42-1.54, p=0.50).

Meta-analysis

Of the 362 articles retrieved, six investigated the association of the *ACE* I/D polymorphism and major depression. All were case-control studies. These and the case-control study from this paper were included in the meta-analysis. The genotype frequencies of cases and controls were in Hardy Weinberg Equilibrium. All studies used structured diagnostic interviews based on the DSM-III-R or DSM-IV criteria.

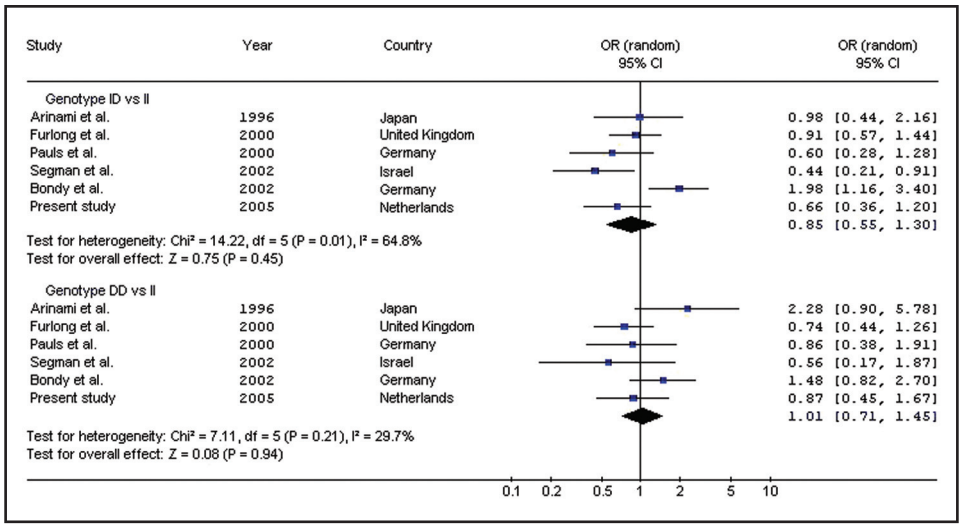

Figure 1 Meta-analysis on the association between the *ACE* I/D polymorphism and major depression

A total of 586 cases and 5,169 controls were included. The ORs (95% CI) of the individual studies and the pooled results are presented in Figure 1. The pooled OR was 0.85 (95% CI=0.55-1.30) for the ID genotype and 1.01 (95% CI=0.71-1.45) for the DD genotype. Both ORs were not statistically significant (p>0.05). There was significant heterogeneity between studies for the ID genotype (Chi^2=14.22, df=5, p=0.01), but not for the DD genotype (Chi^2=7.11, df=5, p=0.21), meaning that for the ID genotype the results of the individual studies differed more than what would be expected by chance. The positive association reported by Bondy et al [8] introduced heterogeneity by acting as an outlier in our analysis. The exclusion of the study did not materially change the results. The funnel plot showed a symmetrical distribution, indicating there is no evidence for publication bias.

Discussion

In this meta-analysis we pooled the data of 586 depressed cases and 5,169 controls from available published studies to re-evaluate the association between the *ACE* I/D polymorphism and major depression. We found no evidence of association. This meta-analysis demonstrates that the contradicting findings on the relation between *ACE* I/D polymorphism and major depression were not due to small sample sized populations.

ACE is a component of the renin angiotensin system (RAS), a system that is assumed to be involved in depression. Evidence for this relationship comes from research showing that ACE inhibitors have antidepressant effects,[10] antidepressants block ATII effects,[11] and the RAS modulates the hypothalamus-pituitary-adrenocortical axis by stimulating corticotrophin hormones.[12] Of all the genes in the RAS, the *ACE* is the only gene that has been investigated in relation to depression. The results of our study show conclusively that the *ACE* gene does not play a direct role in symptoms of depression. Given the role of the RAS in the etiology of depression, we recommend that future research should explore the contribution of other genes in this system.

References

1. **Zubenko GS, Nixon RA.** Mood-elevating effect of captopril in depressed patients. Am J Psychiatry 1984;141:110-1.
2. **Jezova D, Ochedalski T, Kiss A, Aguilera G.** Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. J Neuroendocrinol 1998;10: 67-72.
3. **Okuyama S, Sakagawa T, Sugiyama F, Fukamizu A, Murakami K.** Reduction of depressive-like behavior in mice lacking angiotensinogen. Neurosci Lett 1999;261:167-70.
4. **Arinami T, Li L, Mitsushio H, Itokawa M, Hamaguchi H, Toru M.** An insertion/deletion polymorphism in the angiotensin converting enzyme gene is associated with both brain substance P contents and affective disorders. Biol Psychiatry 1996;40:1122-7.
5. **Furlong RA, Keramatipour M, Ho LW, Rubinsztein JS, Michael A, Walsh C, et al.** No association of an insertion/deletion polymorphism in the angiotensin I converting enzyme gene with bipolar or unipolar affective disorders. Am J Med Genet 2000;96:733-5.
6. **Pauls J, Bandelow B, Ruther E, Kornhuber J.** Polymorphism of the gene of angiotensin converting enzyme: lack of association with mood disorder. J Neural Transm 2000;107:1361-6.
7. **Segman RH, Shapira Y, Modai I, Hamdan A, Zislin J, Heresco-Levy U, et al.** Angiotensin converting enzyme gene insertion/deletion polymorphism: case-control association studies in schizophrenia, major affective disorder, and tardive dyskinesia and a family-based association study in schizophrenia. Am J Med Genet 2002;114:310-4.
8. **Bondy B, Baghai TC, Zill P, Bottlender R, Jaeger M, Minov C, et al.** Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? Mol Psychiatry 2002;7:1120-6.
9. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
10. **Vuckovic A, Cohen BM, Zubenko GS.** The use of captopril in treatment-resistant depression: an open trial. J Clin Psychopharmacol 1991;11:395-6.
11. **Gard PR.** Angiotensin as a target for the treatment of Alzheimer's disease, anxiety and depression. Expert Opin Ther Targets 2004;8:7-14.
12. **Plotsky PM, Owens MJ, Nemeroff CB.** Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. Psychiatr Clin North Am 1998;21:293-307.

5

Angiotensinogen M235T polymorphism and symptoms of depression in a population-based study and a family-based study

Abstract

Background: Evidence suggests that the angiotensinogen (*AGT*) gene is involved in depression. The aim of this paper is to examine the association between the *AGT* M235T polymorphism and symptoms of depression in two independent populations, a population-based study, and a family-based study. **Method**: Symptoms of depression were scored using the Center of Epidemiological Studies Depression Scale (CES-D) and compared between the MM, MT, and TT genotype groups. The extent to which *AGT* M235T explains the heritability of the scores was examined using a variance components analysis. **Results**: A significant relation between the *AGT* M235T polymorphism and CES-D scores was found in men in both populations. The heritability estimate was 32%. The *AGT* genotype contributed to 1% of the total variance of the CES-D scores. **Conclusions**: Our findings suggest that the *AGT* gene is involved in the etiology of symptoms of depression in men.

Introduction

While there is substantial evidence for the involvement of the renin angiotensin system (RAS) in depression,[1-4] little is known about whether specific RAS genes are involved. Investigations have focused mainly on the *ACE* and *AGT* genes. In a recent meta-analysis of 6 studies we demonstrated that the *ACE* gene is not associated with major depression.[5] Evidence for a role of the *AGT* gene is still minimal. A population-based study demonstrated that the M allele of the *AGT* M235T polymorphism was associated with bipolar depression,[6] knock-out mice lacking the *AGT* gene have a reduction of depressive-like behaviours,[7] and a significant linkage of mood disorders was found on chromosome 1q42 (LOD score=4.5), the area where *AGT* is localized.[8] The aim of our study was to further investigate the association of the *AGT* M235T polymorphism with symptoms of depression in two independent populations: a population-based study, and a genetically isolated family-based study.

Methods

Study populations and procedures

The population-based study was conducted within the Rotterdam Study, a cohort study in the Netherlands of 7,983 men and women aged 55 years and older. Baseline data were collected from 1990 to 1993. Analyses were performed on data from individuals who participated in the fourth assessment of the study (n=3,550), which took place from 2002 to 2004.

The family-based study was embedded in the Erasmus Rucphen Family Study, which is part of the Genetic Research in Isolated Populations programme. This study investigates the genetic origin of complex genetic disorders and traits in a genetically isolated community in the Southwest of the Netherlands. Genealogical data of this community date back to the middle of the sixteenth century. All descendants from 20 couples living in the isolate from 1850 to 1900 were ascertained and living descendants of 18 years and older were invited to participate. Data were collected from 2002 to 2005.

In both studies participants underwent extensive medical examinations and completed several questionnaires. The studies have been previously described.[9-10] The Medical Ethical Committee of the Erasmus University Medical Center approved both studies and all participants gave informed consent.

Measurements

Symptoms of depression were assessed using the Center of Epidemiological Studies Depression Scale (CES-D). The CES-D is a 20-item inventory that measures the

presence and severity of symptoms of depression. The psychometric properties of the CES-D score have been well established.[11] Total scores range from 0 to 60 with higher scores indicating more symptoms of depression. We used the CES-D score as a continuous scale.

The medical examination included assessments of height, weight, blood pressure and medication use. Blood pressure was taken twice in the sitting position from the right upper arm. The average of the two measurements was used as a continuous variable. Hypertension was defined as mean systolic blood pressure higher than 160 mmHg, mean diastolic blood pressure higher than 100 mmHg or the use of antihypertensive medication.

Genotyping

DNA was isolated from blood samples using standard procedures (salting out method).[12] The MM, MT, TT genotypes of the *AGT* M235T polymorphism were detected using a set of oligonucleotide primers flanking the polymorphic site in exon 2 (forward primer, 5'CTG GCT CCC ATC AGG3', reverse primer, 5'CTG GCT CCC GTC AGG3').

Statistical analysis

To examine the effect of the *AGT* genotype on symptoms of depression, mean CES-D scores were compared between *AGT* genotype groups by regression analysis adjusting for age, sex, and use of antidepressant medication. Since the CES-D score was not normally distributed, a square root transformation was applied in the analysis. As the results did not change with or without the transformation, we report in this paper the CES-D score without the transformation for ease of interpretation.

The heritability of the CES-D scores was estimated using a variance component analysis that was adjusted for age, sex, and antidepressant medication. To investigate the extent to which *AGT* M235T contributes to the heritability of the CES-D, we repeated the analysis with *AGT* genotype status as a covariate. Dummy variables were created for the MT and TT genotypes, thereby taking MM genotype as the reference group. In addition, to investigate whether symptoms of depression increased with the number of T alleles present (p for trend), we considered genotype status as a continuous variable by the number of risk alleles.

The contribution of the *AGT* genotype in the total variance of CES-D was quantified by estimating the difference in the proportion of variance explained by the covariates of the two analyses, and tested using the likelihood ratio test for the difference between the two analyses (χ^2 statistic at 2 degrees of freedom).

To investigate if hypertension is an intermediate factor in the relationship between *AGT* genotype and depression we repeated the analyses including hypertension. All analyses were performed using SPSS software, version 11.5 (SPSS, Chicago, USA) for the population-based analysis and SOLAR 2.1.2 (software package Southwest Foundation for Biomedical Research, San Antonio, Texas, USA) for the family-based analysis.[13] P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

Population-based study

Of 3,550 persons who participated in the fourth assessment of the Rotterdam Study, 3,134 persons (88%) completed the CES-D questionnaire and had genotype information available. The mean age of the participants was 75.9 years (SD ± 6.4) and 41% were men. General characteristics of the participants are presented in Table 1. The distribution of the *AGT* genotypes was in Hardy Weinberg Equilibrium (p=0.32). There were no statistically significant differences in allele or genotype frequencies between men and women (Table 2).

The mean CES-D scores are presented overall and stratified by men and women (Figure 1). Individuals with the MT and TT genotypes had significantly higher CES-D scores than individuals with the MM variant ($p<0.05$). The stratified analysis showed that mean CES-D scores differ between the genotype groups in men, but not in women. In men, the mean CES-D score for the MM carriers was 4.6 (SD±7.0), for the MT carriers 5.5 (SD±6.9), and for the TT carriers 6.7 (SD±6.9). Men with the MT and TT genotypes had significantly higher CES-D scores than individuals with the MM variant ($p<0.05$). CES-D scores in men increased with the number of T alleles present (p for trend <0.001).

Family-based study

Out of 825 relatives, 783 persons (95%) completed the CES-D questionnaire and had genotype information available. The mean age was 52.0 years (SD±14.5) and 41% of the participants were men. The age ranged from 18 to 89 years because the participants comprised three generations. General characteristics for men and women are presented in Table 1. The distribution of the *AGT* genotypes was in Hardy Weinberg Equilibrium (p=0.88). There were no statistical significant differences in allele or genotype frequencies between men and women (Table 2).

Table 1 General characteristics of the study populations

	Population-based study			Family-based study		
	Men (n = 1,279)	Women (n = 1,855)	p value	Men (n = 318)	Women (n = 465)	p value
Age, years	75.3 (6.0)	76.3 (6.6)	< 0.001	53.6 (14.0)	50.8 (14.7)	0.01
CES-D score	5.4 (6.7)	8.3 (8.6)	< 0.001	10.4 (10.1)	12.3 (10.4)	0.01
Use of antidepressants	3.4	6.5	< 0.001	5.0	10.1	0.01
Hypertension	49	50	0.18	47	43	0.13
Use of antihypertensives	29	26	0.06	37	35	0.49
Level of Education						
Elementary school	33	57		72	67	
Intermediate education	48	38	< 0.001	22	30	0.01
Higher education	19	5		6	3	
Body mass index, kg/m^2	27.0 (3.4)	27.7 (4.5)	< 0.001	27.4 (4.1)	26.5 (4.7)	0.01

Values are mean (standard deviations) for continuous variables and percentages for categorical variables.
p values obtained by ANOVA for continuous variables and χ^2 for categorical variables.
CES-D: Center of Epidemiological Studies Depression Scale.

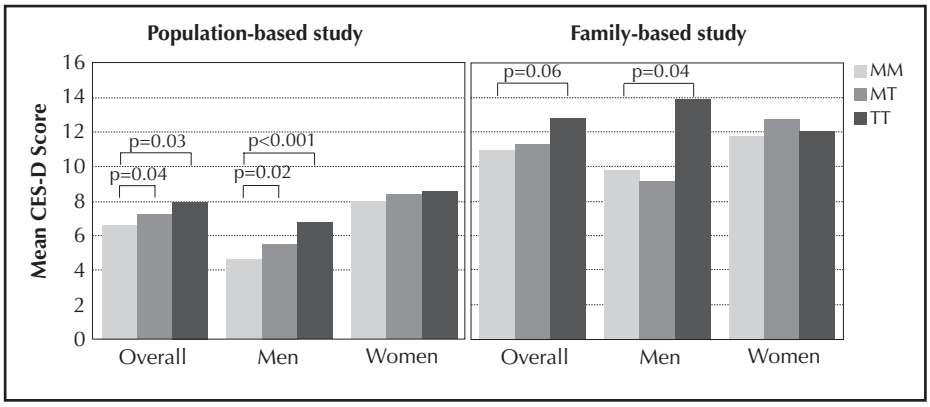

Figure 1 Mean CES-D scores adjusted for age and antihypertensive medication by *AGT* genotypes

Figure 1 shows mean adjusted CES-D scores stratified by *AGT* genotype for the overall group and for men and women separately. In this population we also found that among men, the CES-D scores increased with the number of T alleles present (p for trend=0.01). Men with the TT genotype had significantly higher CES-D scores

than individuals with the MM variant (p=0.01). For men the mean scores of the CES-D increased from 9.8 (SD±9.7) for the MM carriers to 13.9 (SD±9.7) for the TT carriers. No relationship between *AGT* genotype and CES-D scores was found among women.

The heritability of the CES-D scores was 32% (p<0.001) and the proportion of variance explained by all covariates was 0.09. When *AGT* genotype status and the genotype multiplied sex interaction were included in the analysis, the heritability estimates changed to 31% (p<0.001). The proportion of variance explained by all covariates changed to 0.10 indicating that the *AGT* genotype contributed to 1% of the total variance in the CES-D scores. Neither in the regression analyses nor in the heritability analysis did the effect of *AGT* change when hypertension was added to the model, indicating that hypertension is not an intermediate variable in the relationship between *AGT* and depression.

Table 2 Genotype and allele frequencies in the two study populations

	Population-based study			**Family-based study**		
	Men	**Women**		**Men**	**Women**	
	(n = 1,279)	**(n = 1,855)**	**p value**	**(n = 318)**	**(n = 465)**	**p value**
Genotype frequency, in % (n)						
MM	37 (473)	36 (676)	0.55	27 (87)	27 (126)	0.99
MT	48 (609)	47 (866)		50 (160)	50 (233)	
TT	15 (197)	17 (313)		22 (71)	23 (106)	
Allele frequency, in %						
M	61	60	0.39	53	52	0.50
T	39	40		47	48	

HWE = Hardy Weinberg Equilibrium

Adjusted for age, sex degree of consanguinity and use of antidepressant medication

Discussion

In two independent populations we found that the *AGT* M235T polymorphism was associated with symptoms of depression in men. In the population-based study men with the MT or the TT genotype reported significantly more symptoms of depression than those with the MM genotype, and in the family-based study men carrying the TT genotype had a higher level of symptoms of depression. Furthermore, we demonstrated that the *AGT* gene accounts for 1% of the overall variance in symptoms of depression.

Before discussing the implications of our findings, one methodological issue needs to be addressed. In the population-based study we found that carriers of the T allele (MT and TT genotype) on average had significantly higher CES-D scores than those with the MM genotype, while in the family-based study only individuals with the TT genotype had significantly higher CES-D scores. While the studies may suggest different modes of inheritance for the relationship between *AGT* and depression, these findings could be due to the age differences between the two cohorts. The weaker effect in heterozygous carriers could be attributable to the younger age of the family-based cohort, as symptoms of depression may appear at a later age. The stronger effect in homozygous carriers may become apparent earlier in life, and hence showed up in both of our populations. In both populations, we only found an association between the *AGT* M235T polymorphism and symptoms of depression in men.

Given that in both populations women had higher depression scores than men, it can be argued that other causal factors may outweigh the role of *AGT* in women and mask an eventual effect of the gene in this subgroup. Yet, there is some earlier evidence that the RAS may have different roles in the etiology of depression for men and women. For example, a study suggested gender differences in RAS regulation between men and women because in contrast with earlier studies that found an increase in aldosterone and renin plasma concentrations in depressed men,[14,15] they found no differences in these concentrations in women.[16] Another study found an association between panic disorders and men carrying the *AGT* T allele, this association was not found in women.[17]

The *AGT* gene has been widely studied for its relationship with hypertension.[18] We studied whether increased blood pressure was an intermediate factor in the relationship between *AGT* and symptoms of depression, but we found no evidence for this. An alternative mechanism that could explain the association of the *AGT* gene with depression is the relationship of RAS with the hypothalamic pituitary adrenal (HPA) axis. RAS directly modulates the HPA axis by stimulating corticotrophin hormones.[19,20] The presence of the T allele of the *AGT* gene increases the angiotensinogen level,[21] this increase could enhance the HPA feedback and lead to symptoms of depression. In depressed patients there has been a consistent finding suggesting hyperactivity of the HPA axis increases both corticotrophin releasing hormone (CHR) and adrenocotropic hormone (ACTH). This feedback leads to high amounts of cortisol and aldosterone,[22] which are typically seen in patients with depression.[23]

The only study that has evaluated the relationship between the *AGT* gene and affective disorders found that the M allele was associated with bipolar disorder and stated that the TT genotype could have a protective effect for bipolar disorder.[6] We particularly found that individuals with the TT genotype had higher levels of symptoms of depression than those with the M allele, which seems to contradict the study on bipolar depression. It can be argued that the presence of manic episodes suggest different pathophysiological mechanisms are involved in bipolar depression. For example, when the TT genotype upregulates the RAS and increases the risk for depression,[21] it can be speculated that the MM genotype downregulates the RAS and increases the risk for mania.

In conclusion we found that the *AGT* M235T polymorphism is associated with symptoms of depression in two independent populations, which supports the role of the *AGT* gene and the RAS in the etiology of mood disorders. In both the population-based and the family-based study, the association was only found in men, which suggests that the RAS may have a different causal role in depression among men and women. This finding contributes to the understanding of the pathogenesis of mood disorders, and may open new avenues to our understanding and treatment of mood disorders.

References

1. **Gard PR, Mandy A, Whiting JM, Nickels DP, Meakin AJ.** Reduction of responses to angiotensin II by antidepressant drugs. Eur J Pharmacol 1994;264:295-300.
2. **Gard PR.** Angiotensin as a target for the treatment of Alzheimer's disease, anxiety and depression. Expert Opin Ther Targets 2004;8:7-14.
3. **Murck H, Uhr M, Ziegenbein M, Kunzel H, Held K, Antonijevic IA, et al.** Renin-angiotensin-aldosterone system, HPA-axis and sleep-EEG changes in unmedicated patients with depression after total sleep deprivation. Pharmacopsychiatry 2006;39:23-9.
4. **Aguilera G, Kiss A, Luo X, Akbasak BS.** The renin angiotensin system and the stress response. Ann N Y Acad Sci 1995;771:173-86.
5. **López-León S, Janssens ACJW, Hofman A, Claes SJ, Breteler MB, Tiemeier H, et al.** No Association between the Angiotensin Converting Enzyme Gene and Major Depression: A Case-Control Study and Meta-analysis. Psychiatric Genetics 2006; 16:225-6.
6. **Meira-Lima IV, Pereira AC, Mota GF, Krieger JE, Vallada H.** Angiotensinogen and angiotensin converting enzyme gene polymorphisms and the risk of bipolar affective disorder in humans. Neurosci Lett 2000;293:103-6.
7. **Okuyama S, Sakagawa T, Sugiyama F, Fukamizu A, Murakami K.** Reduction of depressive-like behavior in mice lacking angiotensinogen. Neurosci Lett 1999;261:167-70.
8. **Blackwood DH, Fordyce A, Walker MT, St Clair DM, Porteous DJ, Muir WJ.** Schizophrenia and affective disorders--cosegregation with a translocation at chromosome 1q42 that directly disrupts brain-expressed genes: clinical and P300 findings in a family. Am J Hum Genet 2001;69: 428-33.
9. **Pardo LM, MacKay I, Oostra B, van Duijn CM, Aulchenko YS.** The effect of genetic drift in a young genetically isolated population. Ann Hum Genet 2005;69:288-95.

10. **Hofman A, Grobbee DE, de Jong PT, van den Ouweland FA.** Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. Eur J Epidemiol 1991;7:403-22.
11. **Radloff LS.** The CES-D scale: a self-report depression scale for research in the general population. Appl Psychol Measurement 1977;3:385-401.
12. **Miller SA, Dykes DD, Polesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
13. **Almasy L, Blangero J.** Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.
14. **Charloux A, Gronfier C, Lonsdorfer-Wolf E, Piquard F, Brandenberger G.** Aldosterone release during the sleep-wake cycle in humans. Am J Physiol 1999;276:E43-9.
15. **Vieweg WV, Veldhuis JD, Carey RM.** Temporal pattern of renin and aldosterone secretion in men: effects of sodium balance. Am J Physiol 1992;262:F871-7.
16. **Murck H, Held K, Ziegenbein M, Kunzel H, Koch K, Steiger A.** The renin-angiotensin-aldosterone system in patients with depression compared to controls--a sleep endocrine study. BMC Psychiatry 2003;3:15.
17. **Olsson M, Annerbrink K, Westberg L, Melke J, Baghaei F, Rosmond R, et al.** Angiotensin-related genes in patients with panic disorder. Am J Med Genet B Neuropsychiatr Genet 2004;127:81-4.
18. **Mondry A, Loh M, Liu P, Zhu AL, Nagel M.** Polymorphisms of the insertion / deletion ACE and M235T AGT genes and hypertension: surprising new findings and meta-analysis of data. BMC Nephrol 2005;6:1.
19. **Jezova D, Ochedalski T, Kiss A, Aguilera G.** Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. J Neuroendocrinol 1998;10: 67-72.
20. **Plotsky PM, Owens MJ, Nemeroff CB.** Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. Psychiatr Clin North Am 1998;21:293-307.
21. **Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al.** Molecular basis of human hypertension: role of angiotensinogen. Cell 1992;71:169-80.
22. **Claes SJ.** CRH, stress, and major depression: a psychobiological interplay. Vitam Horm 2004;69: 117-50.
23. **Emanuele E, Geroldi D, Minoretti P, Coen E, Politi P.** Increased plasma aldosterone in patients with clinical depression. Arch Med Res 2005;36:544-8.

Analyses of co-morbidity in families

6

Genetic factors influence the clustering of depression among individuals with lower socioeconomic status

Abstract

Background: Depression and socioeconomic status are both partly determined by genetic factors. We aimed to investigate the role of shared genetic factors in the co-occurrence of symptoms of depression and socioeconomic status, and to examine if neuroticism or intelligence are involved in these pathways. **Methods:** Subjects were 2,383 participants of the Erasmus Rucphen Family Study. Symptoms of depression were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D) and the Hospital Anxiety and Depression Scale (HADS-D), premorbid intelligence by the Dutch Adult Reading Test (DART) and neuroticism by the NEO-Five Factor Inventory. Socioeconomic status was obtained as the highest level of education. The role of shared genetic factors was quantified by estimating genetic correlations (ρG) between symptoms of depression and education level, with and without adjustment for DART premorbid intelligence and NEO-FFI neuroticism scores. **Results:** Higher level of education was associated with lower depression scores (partial correlation coefficient -0.09 for CES-D and -0.17 for HADS-D). Significant genetic correlations were found between education and both CES-D (ρG=-0.65) and HADS-D (ρG=-0.50). The genetic correlations remained statistically significant after adjusting for premorbid intelligence and neuroticism scores. **Conclusions:** Our study suggests that shared genetic factors play a role in the co-occurrence of lower socioeconomic status and symptoms of depression.

Introduction

Depression is the leading cause of disability among individuals between 15 to 44 years of age, and is expected to become the second leading source of disability across all ages by 2020.[1] While depression occurs in individuals from all layers of society, there is consistent evidence that the prevalence of depression is higher among individuals with lower socioeconomic status.[2]

The nature of the clustering between depression and socioeconomic status is multifactorial.[2,3] Lower socioeconomic status may increase the risk of depression, as individuals from lower socioeconomic levels tend to have more stressful life events, poorer coping styles, and weaker social support networks, which put them at increased risk of developing depression.[4] Alternatively, depression may lead to lower socioeconomic status, for example depression in young adulthood may increase the risk of job loss, and lead to lower socioeconomic status and depression later in life.[3] Another hypothesis is that the clustering of depression and lower socioeconomic status is explained by shared causal pathways that lead to depression and lower socioeconomic status. For example, serotonin and dopamine pathways have been found to be involved in both traits and may be involved in both traits independently.[5,6] Alternatively, low intelligence or neuroticism may share causal determinants as they are closely related to symptoms of depression and socioeconomic status. It has been known for long that neurotic persons are more vulnerable to depression,[7] and that persons with lower intelligence have a lower socioeconomic status.[7,8] Further there is some evidence that higher levels of neuroticism are associated with lower academic performance,[9] and that lower intelligence is associated with a slight increase in the risk of depression.[10]

When shared causal pathways are involved in the co-occurrence of depression and lower socioeconomic status, shared genes in these pathways may also be important. Both depression and socioeconomic status are partly determined by genetic predisposition. Heritability estimates range from 0.30 to 0.50 for socioeconomic status,[11-14] and from 0.17 to 0.78 for depression,[15] depending on the population investigated. The first aim of this study was to investigate the extent to which shared genetic factors can explain the clustering of depression among individuals with lower socioeconomic status. The second aim of the study was to examine if neuroticism or intelligence are involved in the clustering.

Subjects and methods

Subjects

Subjects were participants of the Erasmus Rucphen Family (ERF) Study, a family-based study that investigates the genetic origins of complex diseases in a genetically isolated community in the Southwest of the Netherlands. The study population essentially consists of one extended family, whose members are descendants from 20 related couples that lived in the isolate in the period between 1850 and 1900. All descendants were ascertained and descendants of 18 years and older were invited to participate. Spouses were invited only for family members that had children of 18 years and older.

Procedures

All participants filled out questionnaires and underwent extensive medical examinations at the research center. The examinations were done by physicians of the academic center according to a standardized research protocol. Level of education, symptoms of depression, personality and premorbid intelligence were ascertained by questionnaires. Participants were asked to bring all medication to the research center and the use of antidepressant medication was verified by the physician. Data were collected between 2002 and 2005. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam and all participants gave informed consent.

Measurements

Symptoms of depression were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D),[16] and the depression subscale of the Hospital Anxiety Depression Scale (HADS-D).[17] Both scales are valid and reliable self-report measures of symptoms of depression.[18] The CES-D consists of 20 items with total scores ranging from 0 to 60, and the HADS-D of 7 items with scores ranging from 0 to 21. Higher scores indicate more symptoms of depression.

Socioeconomic status was assessed as the highest level of education.[2] Seven education levels were distinguished and ranked from one (unfinished elementary, grade or primary school) to seven (college or university). Premorbid intelligence was assessed using the validated Dutch Adult Reading Test (DART).[19] DART premorbid intelligence scores range from zero to 100 with higher scores indicating higher levels of premorbid intelligence. Neuroticism was measured using the NEO five factor inventory (NEO-FFI), a validated self-report questionnaire addressing five core personality traits.[20] The NEO-FFI neuroticism scale consists of 12 statements with total scores ranging from 0 to 48. Higher scores indicate higher levels of neuroticism.

Statistical analysis

General characteristics were compared between men and women and tested using ANOVA for continuous variables and chi-squared test for dichotomous variables. To quantify the strength of the phenotypic association between symptoms of depression and education, partial correlations (ρ) were calculated adjusting for age, sex, degree of consanguinity and the use of antidepressant medication. Multiple linear regression models were fitted to examine the association of covariates with symptoms of depression and to assess the distributional assumption of normality. The normality of residuals was tested using a one-sample Kolmogorov-Smirnov test. These analyses were performed using SPSS 11.0 for Windows.

Heritability estimates (h^2) were calculated as the ratio of the variance of the trait explained by additive polygenic effects to the total phenotypic variance of the trait. Bivariate analyses were performed to estimate the genetic and environmental correlations between the symptoms of depression and education.[21,22] The genetic and environmental correlations can be calculated from the phenotypic correlations (ρ_P) by the following formula ρ_P = [square root]h_1^2[square root]$h_2^2\rho_G$ + [square root](1-h_1^2)[square root](1-h_2^2)ρ_E,[23,24] where h_1^2 and h_2^2 are the heritability estimates of the traits for which the phenotypic correlation is calculated, and ρ_G and ρ_E are the genetic and environmental correlations between these two traits. Significance of the additive genetic and environmental correlations was determined using a likelihood ratio test. To test whether a given correlation between two traits was significantly different from zero, the likelihood of a model in which this correlation was constrained to zero was compared with a model in which the same correlation was estimated. Twice the difference in ln-likelihoods of these models yields a test statistic that is asymptotically distributed as a chi-squared statistic with degrees of freedom equal to the difference in number of parameters estimated in the two models.

Analyses were adjusted for age, sex, use of medication, degree of consanguinity and sibship effects. The degree of consanguinity, indicating the degree to which parents of each participant are related to each other through their ancestors, was estimated using the PEDIG software,[23] based on the pedigree of the total population. PEDIG yields a coefficient for each participant, which was then entered as a covariate in the calculation of the heritability and genetic correlations. Sibship effects denote the exposure to early environmental factors that are shared by children of the same household. In this study, sibship effect estimates were phenotypic similarities induced in the progeny of the same mother because of the small number of half-sibs in our sample. This effect is a combination of effects induced by shared early life environment and dominance genetic effects.

Finally, to investigate the extent to which neuroticism and intelligence were intermediate factors in the causal pathway between the shared genetic factors and the co-occurrence of symptoms of depression and lower socioeconomic status, the analyses were additionally adjusted for NEO-FFI neuroticism scores and DART premorbid intelligence scores. In this analysis we assume that if neuroticism and intelligence are intermediate factors in the pathway, (genetic) correlations will disappear when adjusting for these factors. SOLAR 2.1.2 software package (Southwest Foundation for Biomedical Research, San Antonio, Texas, USA) was used for the calculation of heritability estimates and for the genetic and environmental correlations. P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

Of the 3,092 participants of the ERF Study, data on 2,383 were available for our analyses. Mean age of the participants was 48.7 years (SD 15.1) and 56.9% were women. Women reported more symptoms of depression on the CES-D scale and had higher NEO-FFI neuroticism scores (Table 1). Nine percent of the women reported the use of antidepressants compared to 4.3% of the men ($p < 0.001$). Higher levels of education were associated with lower scores on the CES-D (ρ=-0.09, $p<0.001$) and HADS-D (ρ=-0.17, $p<0.001$; Table 2) scales. Higher DART premorbid intelligence scores were significantly correlated with higher levels of education (ρ=-0.48, $p<0.001$), and higher NEO-FFI neuroticism scores were significantly associated with higher scores on both scales of symptoms of depression scales (CES-D ρ=-0.51, $p<0.001$; HADS-D ρ=-0.50, $p<0.001$).

Heritability estimates were 0.24 and 0.22 for the CES-D and HADS-D scores, 0.36 for education level, 0.54 for DART premorbid intelligence scores and 0.28 for NEO-FFI neuroticism scores. The heritability estimate of education decreased to 0.15 ($p<0.001$) after adjusting for DART premorbid intelligence scores, and the heritability estimates of CES-D and HADS-D depression scores decreased after adjusting for neuroticism (CES-D to 0.11 ($p<0.001$) and HADS-D to 0.12 ($p<0.001$).

Significant negative genetic correlations were found between education and both symptoms of depression scales (CES-D: ρG=-0.65, $p<0.001$; HADS-D: ρG=-0.50; $p<0.001$; Table 3). Genetic correlations between symptoms of depression and both DART premorbid intelligence scores and NEO-FFI neuroticism scores were statistically significant. The genetic correlations between education and symptoms of depression scores remained unchanged and statistically significant after additional adjustment for DART premorbid intelligence and NEO-FFI neuroticism scores (data

not shown). The environmental correlation was statistically significant for the association of education with CES-D scores (ρE=0.10, p=0.05) but not with HADS-D scores (ρE=0.01, p=0.89), and for the association of NEO-FFI neuroticism scores with CES-D and HADS-D scores (ρE=0.51, p<0.001 and ρE=0.42, p=0.001).

Table 1 General characteristics of the study population

	Men (n=1,028)	**Women (n=1,355)**	**p-Value**
Age, years	48.8 (14.7)	48.6 (15.3)	0.79
Education			
Lower	34.1	30.5	<0.001
Intermediate	58.1	65.8	
Higher	7.8	3.7	
Symptoms of depression			
CES-D score	9.1 (8.6)	11.9 (10.2)	<0.001
HADS-D score	6.0 (4.1)	6.1 (4.5)	0.62
Use of antidepressant medication	4.3	9.0	<0.001
DART score	61.5 (19.4)	58.6 (19.2)	0.002
NEO-FFI neuroticism score	29.7 (7.6)	32.6 (8.0)	<0.001

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale - Depression subscale, DART = Dutch Adult Reading Test (premorbid intelligence), NEO-FFI = NEO Five Factor Inventory.

Values are means (standard deviations) for continuous variables and percentages for categorical variables. p-values were obtained using χ^2-statistics for categorical variables and univariate analysis of variance for continuous variables.

Table 2 Phenotypic correlations between the study variables

	CES-D	HADS-D	Education	DART score
Education	-0.09***	-0.17***		
Education, adjusted for DART score	-0.06**	-0.10**		
Education, adjusted for neuroticism	-0.02	-0.12***		
DART premorbid intelligence	-0.07**	-0.16***	0.49***	
NEO-FFI neuroticism	0.51***	0.51***	-0.15***	-0.15***

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale - Depression subscale, DART = Dutch Adult Reading Test (premorbid intelligence), NEO-FFI = NEO Five Factor Inventory. Adjusted for age, sex, degree of consanguinity and use of antidepressant medication. *p<0.05, **p<0.01, ***p<0.001

Table 3 Genetic and environmental correlations between symptoms of depression and education, intelligence and neuroticism

	CES-D		HADS-D	
	ρG	ρE	ρG	ρE
Education	-0.65 (0.14)***	0.10 (0.05)*	-0.50 (0.13)***	0.01 (0.05)
DART premorbid intelligence	-0.31 (0.11)***	0.04 (0.06)	-0.45 (0.11)***	0.04 (0.06)
NEO-FFI neuroticism	0.88 (0.06)***	0.51 (0.03)***	0.77 (0.10)***	0.42 (0.04)***

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale - Depression subscale, DART = Dutch Adult Reading Test, NEO-FFI = NEO Five Factor Inventory. ρG = genetic correlation, ρE = environmental correlation.

Analyses are adjusted for age, sex, use of antidepressant medication, degree of consanguinity and sibship effects. Values are genetic correlations with standard errors.

*p<0.05, **p<0.01, ***p<0.001

Discussion

Our study demonstrates significant genetic correlations between symptoms of depression and level of education, suggesting that shared genetic factors play a role in the clustering of depression among individuals with lower socioeconomic status. This genetic correlation most likely reflects shared causal pathways with genetic factors that play a role in both depression and socioeconomic status. We further showed that these shared genetic pathways did not involve neuroticism or intelligence, as the genetic correlations remained unchanged after adjusting for these personality traits.

Before interpreting the findings, two issues should be addressed. First, we used education as a proxy of socioeconomic status. Education is the most frequently used proxy, but this may be less appropriate in a three-generation study. Older participants with lower education levels may have acquired higher socioeconomic status by their work history, whereas younger participants did not have this opportunity yet. Hence, education level may be a less suitable proxy for socioeconomic status in older participants. Second, we observed slight differences in the results obtained for the CES-D and the HADS-D scores. While both scales are validated for the assessment of symptoms of depression,[18] the scales differ in the items included and may therefore lead to different results. Note that while the estimates of the genetic correlations differed in magnitude, the overall pattern of association was the same for the CES-D and the HADS-D scales.

The heritability estimates for both symptoms of depression and socioeconomic status in our population were in line with previously published studies.[11-15] However, the variation in heritability estimates is large due to the differences between study populations, study design and methodology.[24] The heritability estimates in our large extended family population were lower than in twin studies, and studies in smaller nuclear families generally give higher estimates. This is expected as in the latter studies the environmental and genetic background is more homogeneous, while in our study the environment and genetic background is more heterogeneous because the study spans multiple generations.

The negative genetic correlations between education level and CES-D and HADS-D suggest that the same underlying genetic factors lead to more symptoms of depression and lower socioeconomic status. We investigated whether intelligence or neuroticism were intermediate factors in this genetic pathway, but found no evidence for this hypothesis. Genetic factors that predispose to intelligence do contribute to the heritability of education, and predisposing genes for neuroticism contribute to the heritability of depression, but they do not explain the co-occurrence of symptoms of depression and lower socioeconomic status. To date, only a few studies have assessed the association between socioeconomic status and genetic variants. Candidate genes which have been studied for both depression and socioeconomic status are the *DRD4* gene and the *APOE* gene.[25-28] However, for socioeconomic status no clear evidence has been established as the findings of genetic association studies have not yet been reproduced.

Improving health through the reduction of socioeconomic inequalities has been a public health goal for decades.[2] Depression is among other disorders such as cardiovascular disease, an outcome consistently associated to lower socioeconomic

status.[2] Our results show that the co-occurrence may be partly explained by shared genetic factors. However, this does not mean that we cannot prevent depression among individuals with lower socioeconomic status by improving the environmental context. Most likely the depression is the consequence of a complex interaction between genes and environment. Developing programs to promote educational achievement and coping with life stresses in genetically vulnerable people will be crucial.

References

1. **Murray CJ, López AD.** Evidence-based health policy-lessons from the Global Burden of Disease Study. Science 1996; 274: 740–743.
2. **Lorant V, Deliege D, Eaton W, Robert A, Philippot P, Ansseau M.** Socioeconomic inequalities in depression: a meta-analysis. Am J Epidemiol 2003;157:98-112.
3. **Mackenbach JP.** Genetics and health inequalities: hypotheses and controversies. J Epidemiol Community Health 2005;59:268-73.
4. **Turner RJ, Lloyd DA.** The stress process and the social distribution of depression. J Health Soc Behav 1999;40:374-404.
5. **Manuck SB, Bleil ME, Petersen KL, Flory JD, Mann JJ, Ferrell RE, et al.** The socio-economic status of communities predicts variation in brain serotonergic responsivity. Psychol Med 2005;35:519-28.
6. **Levinson DF.** The genetics of depression: a review. Biol Psychiatry 2006;60:84-92.
7. **Fanous AH, Kendler KS.** The genetic relationship of personality to major depression and schizophrenia. Neurotox Res 2004;6:43-50.
8. **Turkheimer E, Haley A, Waldron M, D'Onofrio B, Gottesman, II.** Socioeconomic status modifies heritability of IQ in young children. Psychol Sci 2003;14:623-8.
9. **Chamorro-Premuzic T, Furnham A.** Personality predicts academic performance: Evidence from two longitudinal university samples. J Res Pers 2003;37:319-338.
10. **Ravnkilde B, Videbech P, Clemmensen K, Egander A, Rasmussen NA, Rosenberg R.** Cognitive deficits in major depression. Scand J Psychol 2002;43:239-51.
11. **Baker LA, Treloar SA, Reynolds CA, Heath AC, Martin NG.** Genetics of educational attainment in Australian twins: sex differences and secular changes. Behav Genet 1996;26:89-102.
12. **Teasdale TW, Owen DR.** Heredity and familial environment in intelligence and educational level--a sibling study. Nature 1984;309:620-2.
13. **Vogler GP, Fulker DW.** Familial resemblance for educational attainment. Behav Genet 1983;13:341-54.
14. **Tambs K, Sundet JM, Magnus P, Berg K.** Genetic and environmental contributions to the covariance between occupational status, educational attainment, and IQ: a study of twins. Behav Genet 1989;19:209-22.
15. **Sullivan PF, Neale MC, Kendler KS.** Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157:1552-62.
16. **Radloff.** The CES-D scale: a self report depression scale for research in the genreal population. Appl Psychol Measurement 1977;3:385-401.
17. **Zigmond A.** The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.
18. **Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ.** Assessing depressive symptoms in five psychiatric populations: a validation study. Am J Epidemiol 1977;106:203-14.
19. **Schmand B, Bakker D, Saan R, Louman J.** [The Dutch Reading Test for Adults: a measure of premorbid intelligence level]. Tijdschr Gerontol Geriatr 1991;22:15-9.

20. **Hoekstra HAO, F.** NEO-personality index (NEO-PI and NEO-FFI). Lisse: Sweets Test Services (STS) The Netherlands; 1996.
21. **Almasy L, Dyer TD, Blangero J.** Bivariate quantitative trait linkage analysis: pleiotropy versus coincident linkages. Genet Epidemiol 1997;14:953-8.
22. **Williams JT, Van Eerdewegh P, Almasy L, Blangero J.** Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. Am J Hum Genet 1999;65:1134-47.
23. **Falconer DS, MacKay TFC.** Introduction to Quantitative Genetics. London: Longman, 1989.
24. **Luynch M, Walsh B.** Genetics and data analysis of quantitative traits. Sunderland, MA: Sinauer, 1998.
25. **Boichard D.** PEDIG: A FORTRAN package for pedigree analysis studied for large populations. In 7th World Congr. Genet. Appl. Livest. Prod.; Montpelier, FR: 2002.
26. **López-León S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM.** The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005;57:999-1003.
27. **Keltikangas-Jarvinen L, Elovainio M, Kivimaki M, Ekelund J, Peltonen L.** Novelty seeking as a mediator in relationships between type 4 dopamine receptor gene polymorphism and predisposition to higher education. Learn and Indiv Dif 2002;14:23-30.
28. **Hubacek JA, Pitha J, Skodova Z, Adamkova V, Lanska V, Poledne R.** A possible role of apolipoprotein E polymorphism in predisposition to higher education. Neuropsychobiology 2001;43:200-3.
29. **López-León S, Janssens ACJW, González-Zuloeta Ladd AM, Del-Favero J, Claes S, Oostra BA, et al.** Meta-analyses of Genetic Studies on Major Depressive Disorder. Mol Psychiatry 2008.

7

Shared genetic factors in the co-occurrence of symptoms of depression and cardiovascular risk factors

Abstract

Objectives: We aim to investigate the extent to which shared genetic factors play a role in the co-occurrence of symptoms of depression and cardiovascular risk factors. **Methods:** The analyses included 2,383 individuals from a genetically isolated population in the Netherlands. Symptoms of depression were assessed using the Center for Epidemiology Studies Depression Scale (CES-D) and the Depression subscale of the Hospital Anxiety and Depression Scale (HADS-D). Assessment of cardiovascular risk factors included systolic and diastolic blood pressure, plasma, glucose levels, high and low density lipoprotein (HDL, LDL) and total cholesterol levels. The degree of shared genetic and shared environmental factors in the co-occurrence of traits was quantified by estimating genetic and environmental correlations. **Results:** Overall, we found that HADS-D scores were significantly associated to total cholesterol levels (ρ=0.05; p=0.05), and inversely associated to HDL (ρ=-0.06; p=0.03). Significant genetic correlations were found between CES-D scores and total plasma cholesterol (ρG=0.30, p=0.02), LDL (ρG=0.31, p=0.02) and total cholesterol/HDL ratios (ρG=0.25, p=0.04). For HADS-D scores, a significant genetic correlation was found with total cholesterol/HDL ratios (ρG=0.27, p=0.02). Environmental correlations with an opposite direction were found between CES-D and both total cholesterol (ρE=-0.16, p<0.001) and LDL (ρE=-0.15, p<0.001). **Conclusions:** Our study shows that from the cardiovascular risk factors studied, there is evidence for shared genetic factors contributing to the co-occurrence of symptoms of depression and lipid levels. This finding suggests a joint genetic pathogenesis.

Introduction

Depression and cardiovascular disease (CVD) are two of the most common disorders in developed countries.[1] According to the World Health Organization estimates, 29% percent of all deaths globally are attributed to CVD each year, and depressive disorders are the most frequently occurring psychiatric disease with a life-time incidence of around 25%.[2] The prevalence of depression in patients with CVD is estimated at 20% to 35%.[2,3] The causal mechanism behind this association is multidirectional, in that depression may lead to CVD, it may worsen CVD symptoms or it may result from CVD.[4]

In recent years, there has been an increasing interest in the hypothesis that depression and CVD risk factors may share common genetic pathways. Both depression and CVD are partly determined by genetic predisposition. For both traits there is substantial evidence for a strong genetic component.[5-9] The hypothesis of a joint genetic etiology is supported by genetic association studies that show association of the angiotensin I converting enzyme (*ACE*) gene, methylenetetrahydrofolate reductase (*MTHFR)* gene, tyrosine hydroxylase (*TH*) gene, dopamine receptor D4 (*DRD4*) gene, G-protein B3 subunit (*GNB3*) gene, and the serotonin transporter (*SLC6A4*) gene to depression and to CVD and its major risk factors such as hypertension and lipid levels.[10-14]

Apart from these candidate gene studies, there has been limited research on the role of shared genetic pathways in the association between depression and CVD. Only one study has investigated, the genetic correlation between depression and CVD risk factors.[15] This study, among male twins from the Vietnam Era Twin Registry, showed a significant genetic correlation between symptoms of depression and hypertension and heart disease.[15] The aim of our study was to investigate the extent to which shared genetic factors explain the association between depression and CVD risk factors in a large family-based study.

Subjects and methods

Subjects

Subjects were participants of the Erasmus Rucphen Family (ERF) Study, a family-based study that investigates the genetic origin of complex diseases in a genetically isolated community in the Southwest of the Netherlands. The study population essentially consists of one extended family of descendants from 20 related couples that lived in the isolate between 1850 and 1900. All descendants were ascertained and descendants of 18 years and older were invited to participate. Spouses were invited only for family members that had children of 18 years and older.

Procedures

All participants filled out questionnaires and underwent extensive medical examinations. These examinations were conducted by physicians of the academic centers according to a standardized research protocol. The questionnaires addressed symptoms of depression, among other variables. The medical exam included the assessment of medical history, medication use and blood pressure measurements. Fasting blood samples were drawn in order to measure lipid and glucose levels according to standardized procedures.[16,17] Data were collected between 2002 and 2005. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam and all participants gave informed consent.

Measurements

Symptoms of depression were assessed using the Center for Epidemiology Studies Depression Scale (CES-D),[18] and the Depression subscale of the Hospital Anxiety Depression Scale (HADS-D).[19] Both scales are validated and reliable self-report measures of symptoms of depression.[18,19] The CES-D consists of 20 items with total scores ranging from 0 to 60 and the HADS-D consists of 7 items with scores ranging from 0 to 21. Higher scores indicate more symptoms of depression.

CVD risk factors included blood pressure, glucose level and lipid levels. Blood pressure was measured twice on the right upper arm with the subject in a sitting position. Glucose and lipid levels (total cholesterol, high and low density lipoprotein levels (HDL, LDL)) blood chemistry were analysed on a spectrophotometric chemistry analyser (Synchron LX20; Beckman, Fullerton, California, USA).

Statistical analysis

General characteristics were compared between men and women and tested using ANOVA for continuous variables and chi-squared test for dichotomous variables. To quantify the strength of the phenotypic association between symptoms of depression and cardiovascular risk factors, partial correlations (ρ) were calculated adjusting for age, sex, degree of consanguinity and the use of antidepressant medication. Multiple linear regression models were fitted to assess the distributional assumption of normality. Systolic and diastolic blood pressure, plasma glucose levels, HDL, LDL and total cholesterol levels were natural log-transformed to ensure normally distributed residuals. The normality of residuals was tested using a one-sample Kolmogorov-Smirnov test. These analyses were performed using SPSS 11.0 for Windows.

Heritability estimates (h^2) were calculated as the ratio of the variance of the trait explained by additive polygenic effects to the total phenotypic variance of the trait. Bivariate analyses were performed to estimate the genetic and environmental correlations between the depression scores and CVD risk factors. The genetic and environmental correlations can be calculated from the phenotypic correlations (ρ_P) by the following formula: ρ_P = [square root]h_1^2[square root]$h_2^2\rho_G$ + [square root](1-h_1^2)[square root](1-h_2^2)ρ_E,[22, 23] where h_1^2 and h_2^2 are the heritability estimates of the traits, for which the phenotypic correlation is calculated, and ρ_G and ρ_E are the genetic and environmental correlations between these two traits. Significance of the additive genetic and environmental correlations was determined using a likelihood ratio test. To test whether a given correlation between two traits was significantly different from zero, the likelihood of a model in which this correlation was constrained to zero was compared with a model in which the same correlation was estimated. Twice the difference in ln-likelihoods of these models yields a test statistic that is asymptotically distributed as a chi-squared statistic with degrees of freedom equal to the difference in number of parameters estimated in the two models.

Analyses were adjusted for age, sex, use of medication, degree of consanguinity and sibship effects. The degree of consanguinity, indicating the degree to which parents of each participant are related to each other through their ancestors, was estimated using the PEDIG software,[24] based on the pedigree of the total population. PEDIG yields a coefficient for each participant, which was then entered as a covariate in the calculation of the heritability and genetic correlations. Sibship effects denote the exposure to early environmental factors that are shared by children of the same household. In this study, sibship effect estimates were phenotypic similarities induced in the progeny of the same mother because of the small number of half-sibs in our sample. This effect is a combination of effects induced by shared early life environment and dominance genetic effects. SOLAR 2.1.2 software package (Southwest Foundation for Biomedical Research, San Antonio, Texas, USA) was used for the calculation of heritability estimates and for the genetic and environmental correlations. P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

The study included 2,383 participants, 1,355 women and 1,028 men. Women reported more symptoms of depression and more frequently used antidepressive medication (Table 1). Men had worse cardiovascular profiles with significantly higher

systolic and diastolic blood pressure, higher glucose levels, higher total cholesterol levels and lower HDL levels. Furthermore, men more frequently used antihypertensive medication and lipid modifying agents. Table 2 shows that there was a moderate correlation between the CES-D and HADS-D depression scores and between the cardiovascular risk factors. HADS-D was significantly associated to total cholesterol/HDL ratio levels (ρ=0.05; p=0.05) and inversely associated to HDL (ρ=-0.06; p=0.03).

Table 1 General characteristics of the study population

	Men (n=1,028)	**Women (n=1,355)**	**p-Value**
Age, years	48.8 (14.7)	48.6 (15.3)	0.79
Symptoms of depression			
CES-D score	9.1 (8.6)	11.9 (10.2)	<0.001
HAD-D score	6.0 (4.1)	6.1 (4.5)	0.62
Cardiovascular risk factors			
Systolic blood pressure (mm Hg)	142.7 (17.6)	136.4 (21.0)	<0.001
Diastolic blood pressure (mm Hg)	81.6 (9.6)	78.7 (9.8)	<0.001
Glucose (mmol/l)	4.7 (1.1)	4.4 (0.8)	<0.001
Total cholesterol (mmol/l)	5.5 (1.1)	5.6 (1.1)	0.01
High density lipoprotein (mmol/l)	1.1 (0.3)	1.4 (0.4)	<0.001
Total cholesterol/HDL ratio	5.1 (1.5)	4.2 (1.2)	<0.001
Low density lipoprotein (mmol/l)	3.8 (1.0)	3.7 (1.0)	0.54
Medication use			
Antidepressants	4.3	9.0	<0.001
Antihypertensives	37.3	28.7	0.001
Insulin	4.3	2.6	0.06
Lipid modifying agents	26.2	16.3	<0.001

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale. Values are means (standard deviations) for continuous variables and percentages for categorical variables. p-values were obtained using univariate analysis of variance for continuous variables and χ^2-statistics for categorical variables.

T

Table 2 Phenotypic correlations between study variables

	CES-D	HADS-D	SBP	DBP	Glucose	Cholesterol	HDL	Total cholesterol/HDL ratio
Systolic blood pressure (mmHg)	-0.01	-0.01						
Diastolic blood pressure (mmHg)	0.02	-0.01	0.60***					
Glucose (mmol/l)	-0.03	-0.01	0.23***	0.15***				
Cholesterol (mmol/l)	0.02	0.01	0.17***	0.10***	0.05			
High density lipoprotein (mmol/l)	-0.02	-0.06**	0.04	-0.01	-0.11***	0.26***		
Total cholesterol/HDL ratio	0.03	0.05*	0.07*	0.09**	0.12***	0.40***	-0.73***	
Low density lipoprotein (mmol/l)	0.02	0.03	0.13***	0.10**	0.03	0.93***	-0.01	0.56***

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale, SBP = systolic blood pressure, DBP = diastolic blood pressure. Adjusted for age, sex, degree of consanguinity and medication (antidepressants, antihypertensives, insulin and lipid modifying agents)

*p<0.05, **p<0.01, ***p<0.001

Table 3 Heritability estimates of depression and cardiovascular risk factors

	H^2 (se)
HADS-D	0.24 (0.04)
CES-D	0.22 (0.04)
Systolic blood pressure (mmHg)	0.18 (0.05)
Diastolic blood pressure (mmHg)	0.29 (0.04)
Glucose (mmol/l)	0.23 (0.05)
Cholesterol (mmol/l)	0.26 (0.05)
High density lipoprotein (mmol/l)	0.46 (0.05)
Total cholesterol/HDL ratio	0.36 (0.05)
Low density lipoprotein (mmol/l)	0.28 (0.05)

All p-values <0.001, se = standard error.

Adjusted for age, sex, degree of consanguinity and use of antidepressant medication

Table 4 Genetic and environmental correlation between symptoms of depression and cardiovascular risk factors

	CES-D		HADS-D	
	ρ**G**	ρ**E**	ρ**G**	ρ**E**
Systolic blood pressure (mmHg)	-0.15 (0.16)	-0.03 (0.05)	0.11 (0.18)	-0.07 (0.04)
Diastolic blood pressure (mmHg)	0.008 (0.12)	-0.04 (0.04)	0.15 (0.13)	-0.08 (0.04)
Glucose (mmol/l)	0.12 (0.14)	-0.05 (0.05)	0.24 (0.17)	-0.03 (0.04)
Cholesterol (mmol/l)	0.30 (0.13)*	-0.16 (0.05)***	0.21 (0.15)	-0.07 (0.05)
High density lipoprotein (mmol/l)	-0.10 (0.11)	-0.03 (0.05)	-0.20 (0.11)	-0.003 (0.05)
Total cholesterol/HDL ratio	0.25 (0.12)*	-0.06 (0.05)	0.27 (0.12)*	-0.02 (0.05)
Low density lipoprotein (mmol/l)	0.31 (0.13)*	-0.15 (0.05)***	0.20 (0.14)	-0.05 (0.05)

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale, HDL = high-density lipoprotein. ρG = genetic correlation, ρE = environmental correlation.

Values between brackets are standard errors. Analyses are adjusted for age, sex, use of medication (antidepressants, antihypertensives, insulin and lipid modifying agents), degree of consanguinity and sibship effects.

*p<0.05, **p<0.01, ***p<0.001

Heritability estimates were 0.24 (p<0.001) for CES-D and 0.22 (p<0.001) for HADS-D scores (Table 3). Heritability estimates for CVD risk factors ranged from 0.18 (p<0.001) for systolic blood pressure to 0.46 (p<0.001) for HDL levels. Table 4 presents the genetic and environmental correlations between symptoms of depression

and CVD risk factors. Significant genetic correlations were found for CES-D scores with total cholesterol (ρG=0.30; p=0.02) and LDL levels (ρG=0.31; p=0.02) and for both CESD-D and HADS-D scores with total cholesterol/HDL ratios (ρG=0.25; p=0.04 and ρG=0.27; p=0.02) respectively. No evidence for a genetic correlation was found for glucose or blood pressure to either HADS-D or CES-D scores. Statistically significant environmental correlations in the opposite direction were found between CES-D scores and both total cholesterol (ρE=-0.16) and LDL levels (ρE=-0.15).

Discussion

We investigated genetic correlations between symptoms of depression and CVD risk factors and found significant genetic correlations of CES-D scores with total cholesterol, LDL and total cholesterol/HDL ratios and of HADS-D scores with total cholesterol/HDL ratios. Furthermore, we observed statistically significant negative environmental correlations of CES-D scores with total cholesterol and LDL.

The only study to date that has assessed the genetic relationship between vascular risk factors and depression found an association between hypertension and symptoms of depression.[15] We did not find any correlation between blood pressure and symptoms of depression in the overall group, or when analyzing men only.

There is strong support for the hypothesis that abnormal lipid and related fatty acid metabolism contributes to depression.[25] The enzymes, and other proteins that regulate the phospholipid metabolism, are for a large part genetically determined and clearly of interest in relation to depression and symptoms of depression. A review listed more than 100 candidate genes encoding for proteins involved in the metabolism of phospholipids and fatty acids which are localized in regions previously associated to psychiatric disorders.[26] To date the apolipoprotein E gene has been associated to depression,[14] and the prostaglandin and lipoprotein lipase genes have been related to bipolar disorder.[26] To what extend other lipids are involved remains to be determined.

Of note is that the environmental correlations between the CES-D scores and CVD risk factors showed an inverse relation. While shared genetic factors contribute to the co-occurrence of symptoms of depression and higher plasma total cholesterol and higher plasma LDL levels, shared environmental factors contributed to the co-occurrence of depression and lower plasma total cholesterol, and lower plasma LDL levels. When comparing the genetic and environmental correlations, genetic correlations were higher resulting in an overall positive correlation when ignoring the genetic or environmental origin of the correlation. The significant inverse

environmental correlation may be related to the fact that (extremely) low cholesterol levels have been related to a higher prevalence of symptoms of depression and suicide.[25] Although there may be biological explanations for this inverse relationship, alternatively one may hypothesize that the low lipid levels result from dietary habits or medication that are a consequence of symptoms of depression.

In conclusion, we found evidence for a shared genetic origin of the co-occurrence of symptoms of depression and blood lipid levels as well as for a shared environmental factor explaining the opposite direction. These findings are compatible with the view of a shared genetic origin of symptoms of depression and blood lipid levels, or, alternatively of symptoms of depression and brain lipid levels.

References

1. **World Health Organization.** ICD-10: International statistical classification of disease and related health problems. Geneva, Switzerland, 1992.
2. **Rozanski A, Blumenthal JA, Kaplan J.** Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 1999;99:2192-217.
3. **Lett HS, Blumenthal JA, Babyak MA, Sherwood A, Strauman T, Robins C, et al.** Depression as a risk factor for coronary artery disease: evidence, mechanisms, and treatment. Psychosom Med 2004;66:305-15.
4. **Plante GE.** Depression and cardiovascular disease: a reciprocal relationship. Metabolism 2005;54:45-8.
5. **Sullivan PF, Neale MC, Kendler KS.** Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157:1552-62.
6. **Knuiman MW, Divitini ML, Welborn TA, Bartholomew HC.** Familial correlations, cohabitation effects, and heritability for cardiovascular risk factors. Ann Epidemiol 1996;6:188-94.
7. **Watanabe RM, Valle T, Hauser ER, Ghosh S, Eriksson J, Kohtamaki K, et al.** Familiality of quantitative metabolic traits in Finnish families with non-insulin-dependent diabetes mellitus. Finland-United States Investigation of NIDDM Genetics (FUSION) Study investigators. Hum Hered 1999;49:159-68.
8. **Chien KL, Hsu HC, Su TC, Yang CY, Lee YT.** Consistency of genetic inheritance mode and heritability patterns of triglyceride vs. high density lipoprotein cholesterol ratio in two Taiwanese family samples. BMC Genet 2003;4:7.
9. **Ober C, Abney M, McPeek MS.** The genetic dissection of complex traits in a founder population. Am J Hum Genet 2001;69:1068-79.
10. **Bondy B, Baghai TC, Zill P, Bottlender R, Jaeger M, Minov C, et al.** Combined action of the ACE D- and the G-protein beta3 T-allele in major depression: a possible link to cardiovascular disease? Mol Psychiatry 2002;7:1120-6.
11. **Elovainio M, Puttonen S, Heponiemi T, Reuter M, Kivimaki M, Viikari J, et al.** Relationship between DRD4 polymorphism and lipid metabolism: what is the role of novelty seeking? Neuropsychobiology 2005;51:53-8.
12. **Camus V, Kraehenbuhl H, Preisig M, Bula CJ, Waeber G.** Geriatric depression and vascular diseases: what are the links? J Affect Disord 2004;81:1-16.
13. **Otte C, McCaffery J, Ali S, Whooley MA.** Association of a serotonin transporter polymorphism (5-HTTLPR) with depression, perceived stress, and norepinephrine in patients with coronary disease: the Heart and Soul Study. Am J Psychiatry 2007;164:1379-84.

14. **López-León S, Janssens AC, Gonzalez-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, et al.** Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2008.
15. **Scherrer JF, Xian H, Bucholz KK, Eisen SA, Lyons MJ, Goldberg J, et al.** A twin study of depression symptoms, hypertension, and heart disease in middle-aged men. Psychosom Med 2003;65:548-57.
16. **Neeley WE.** Simple automated determination of serum or plasma glucose by a hexokinase-glucose-6 -phosphate dehydrogenase method. Clin Chem 1972;18:509-15.
17. **van Gent CM, van der Voort HA, de Bruyn AM, Klein F.** Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. Clin Chim Acta 1977;75:243-51.
18. **Radloff LS.** The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Measurement 1977;1:385-401.
19. **Zigmond AS, Snaith RP.** The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.
20. **Almasy L, Dyer TD, Blangero J.** Bivariate quantitative trait linkage analysis: pleiotropy versus coincident linkages. Genet Epidemiol 1997;14:953-8.
21. **Williams JT, Van Eerdewegh P, Almasy L, Blangero J.** Joint multipoint linkage analysis of multivariate qualitative and quantitative traits. I. Likelihood formulation and simulation results. Am J Hum Genet 1999;65:1134-47.
22. **Falconer D, MacKay TItQG, 1996. LAWPC.** Introduction to Quantitative Genetics London: Addison Wesley Publishing Company, 1996.
23. **Luynch M, Walsh B.** Genetics and data analysis of quantitative traits. Sunderland, MA: Sinauer, 1998.
24. **Boichard D.** PEDIG: A FORTRAN package for pedigree analysis studied for large populations. In 7th World Congr. Genet. Appl. Livest. Prod.; Montpelier, FR: 2002.
25. **Horrobin DF, Bennett CN.** New gene targets related to schizophrenia and other psychiatric disorders: enzymes, binding proteins and transport proteins involved in phospholipid and fatty acid metabolism. Prostaglandins Leukot Essent Fatty Acids 1999;60:141-67.
26. **Bennett CN, Horrobin DF.** Gene targets related to phospholipid and fatty acid metabolism in schizophrenia and other psychiatric disorders: an update. Prostaglandins Leukot Essent Fatty Acids 2000;63:47-59.

8

EVALUATING THE EVIDENCE OF SHARED ETIOLOGY OF SYMPTOMS OF DEPRESSION WITH FAT AND MUSCLE MASS

Abstract

Objective: Both obesity and lean mass have been correlated with symptoms of depression. We aimed to investigate the contribution of genetic and environmental factors in the co-occurrence of these traits, and examined the role of personality and education in the shared etiological pathways. **Methods:** Subjects were 2,383 participants of the Erasmus Rucphen Family (ERF) Study**.** Symptoms of depression were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D) and the Hospital Anxiety and Depression Scale (HADS-D). The NEO-Five Factor Inventory was used to assess personality traits. Anthropometric and Dual Energy X-ray Absorptiometry (DEXA) total body scans were obtained for the assessment of body composition. The role of shared genetic and shared environmental factors was quantified by estimating genetic (ρG) and environmental (ρE) correlations between symptoms of depression and measures of body composition, with and without adjustment for personality traits, education and sibship effects. **Results:** Phenotypic correlations between body composition and symptoms of depression ranged from -0.08 to 0.08. Heritability estimates for body composition ranged from 0.40 to 0.46 ($p<0.001$) in women and from 0.35 to 0.51 ($p<0.001$) in men, and heritability estimates for depression scores were higher in women (0.34 and 0.37) than in men (0.13 and 0.21). No consistent genetic correlations between measures of body composition and symptoms of depression were found. We did find a significant consistent environmental correlation between depression scores and lean mass index (ρE = -0.23 for CES-D and -0.31 for HADS-D). These correlations did not change after additional adjustment for education and personality. **Conclusions:** In our study, there is no evidence that the co-occurrence of symptoms of depression and body composition result from a common genetic pathway.

Introduction

Both obesity and lean mass have been correlated with symptoms of depression. Symptoms of depression and obesity frequently co-occur,[1-3] while an inverse relationship between lean mass and depression is seen.[4] There are multiple explanations for the clustering of obesity and depression traits, in either direction.[5,6] First, obese persons may be more likely to become depressed due to psychosocial and behavioral implications of obesity, such as lack of self-confidence, isolation and inactivity.[1] Second, depressed persons may be more likely to develop obesity due to physiologic mechanisms such as overeating and physical inactivity.[1] And third, obesity and depression may result from the same underlying causes, such as education, personality, disturbances of the hypothalamic-pituitary-adrenal axis and serotonin/dopamine pathways, which are known to play major roles in both traits.[7-13] Evidence for common etiological pathways is supported by the results of genetic association studies, which have shown that several genes such as the glucocorticoid receptor gene,[14,15] the corticotrophin releasing hormone receptor gene,[16,17] the serotonin 2A and 2C receptor gene,[18-21] and the dopamine receptor D4 gene,[22,23] have been associated to both obesity and depression. Also the causal mechanism for the relationship between lean mass and depression may be multidirectional. Indeed, determinants of lean mass such as physical activity may be also associated to depression, but it can also be argued that depression leads to inactivity and a reduction in lean mass. Regarding the latter hypothesis, there is increasing interest in the role of physical activity in the treatment of depression.[24]

Obesity, lean mass and depression are both substantially caused by genetic determinants.[25-27] Heritability estimates, indicating the proportion of variance of a trait that is explained by additive genetic factors, vary widely for obesity related factors, ranging from 50% to 70% for body mass index (BMI),[25,26] 36% to 61% for waist-to-hip ratio (WHR), 72% to 82% for waist circumference,[28] and 60% to 79% for lean mass.[29,30] Heritability estimates for depression range from 17% to 78%.[27] Given the high heritability, and their co-occurrence, one may hypothesize that genetic factors underlie the clustering of these traits. The aim of this study was to investigate the extent to which shared genetic and shared environmental factors contribute to the co-occurrence of symptoms of depression with fat and muscle mass.

Subjects and methods

Subjects

Subjects were participants of the Erasmus Rucphen Family (ERF) Study, a family-based study that investigates the genetic origins of complex diseases in a genetically isolated community in the Southwest of the Netherlands. The study population essentially consists of one extended family of descendants from 20 related couples that lived in the isolate between 1850 and 1900. All descendants were ascertained and descendants of 18 years and older were invited to participate. Spouses were invited only for family members that had children of 18 years and older.

Procedures

All participants filled out questionnaires and underwent extensive medical examinations. These examinations were done by research physicians of the academic center according to a standardized protocol. The questionnaires addressed education level, symptoms of depression, and personality, and the medical exam included assessment of medical history, antidepressant use, anthropometrics and body composition. Data were collected between 2002 and 2005. The study was approved by the Medical Ethical Committee of the Erasmus University Medical Center Rotterdam and all participants gave informed consent.

Measurements

Measures of body composition were obtained from anthropometric assessments, which included height, weight, waist and hip circumference, and a Dual Energy X-ray Absorptiometry (DEXA) total body scan. Waist circumference was measured midway between the lower costal margin and the iliac crest, and hip circumference at the widest diameter of the hip. Waist to hip ratio (WHR) was computed as the waist circumference divided by the hip circumference. Body Mass Index (BMI) was computed as weight in kilograms divided by the squared height in meters. DEXA scans were performed in a calibrated Prodigy total body fanbeam densitometer and analyzed with enCORE 2002 software version 6.70.021 (GE Lunar, Madison, WI, USA). DEXA measurements were used to calculate the fat mass and lean mass indices, which respectively are the total fat mass and lean mass in kilograms divided by the squared height in meters, similar to how we calculated BMI.

Symptoms of depression were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D),[31] and the depression subscale of the Hospital Anxiety Depression Scale (HADS-D).[32] Both the CES-D and HADS-D are validated and reliable self-report measures of symptoms of depression.[33] The CES-D consists

of 20 items with total scores ranging from 0 to 60, and the HADS-D consists of 7 items with scores ranging from 0 to 21. Higher scores indicate more symptoms of depression.

Personality was assessed using the NEO five factor inventory (NEO-FFI), measuring five major domains of personality, including neuroticism, extraversion, openness to experience, agreeableness and conscientiousness.[34] The NEO-FFI is a self-report questionnaire consisting of 60 statements answered on a five-point Likert scale with domain scores ranging from 0 to 48 each.

Statistical analysis

General characteristics were compared between men and women and tested using ANOVA for continuous variables and chi-squared test for dichotomous variables. To quantify the strength of the phenotypic association between symptoms of depression and measures of body composition, partial correlations (ρ) were calculated adjusting for age, sex, degree of consanguinity and the use of antidepressant medication. Individuals with a BMI lower than 18.5 kg/m^2 were excluded from the main analyses. Multiple linear regression models were fitted to assess the distributional assumption of normality. The normality of residuals was tested using a one-sample Kolmogorov-Smirnov test. These analyses were performed using SPSS 11.0 for Windows.

Heritability estimates (h^2) were calculated as the ratio of the variance of the trait explained by additive polygenic effects to the total phenotypic variance of the trait. Bivariate analyses were performed to estimate the genetic and environmental correlations between the depression scores and measures of body composition. The genetic and environmental correlations were calculated from the phenotypic correlations (ρP) by the following formula: $\rho P = [\text{square root}]h_1^2\ [\text{square root}]h_2^2\rho G + [\text{square root}](1-h_1^2)[\text{square root}](1-h_2^2)\rho E$, [35, 36] where h_1^2 and h_2^2 are the heritability estimates of the traits, for which the phenotypic correlation is calculated, and ρG and ρE are the genetic and environmental correlations between these two traits. Significance of the additive genetic and environmental correlations was determined using a likelihood ratio test. To test whether a given correlation between two traits was significantly different from zero, the likelihood of a model in which this correlation was constrained to zero was compared with a model in which the same correlation was estimated. Twice the difference in ln-likelihoods of these models yields a test statistic that is asymptotically distributed as a chi-squared statistic with degrees of freedom equal to the difference in the number of parameters estimated in the two models.

Analyses were adjusted for age, sex, use of medication, degree of consanguinity and sibship effects. The degree of consanguinity, indicating the degree to which parents of each participant are related to each other through their ancestors, was estimated using the PEDIG software,[37] based on the pedigree of the total population. PEDIG yields a coefficient for each participant, which was then entered as a covariate in the calculation of the heritability and genetic correlations. Sibship effects denote the exposure to early environmental factors that are shared by children of the same household. In this study, sibship effect estimates were phenotypic similarities induced in the progeny of the same mother because of the small number of half-sibs in our sample. This effect is a combination of effects induced by shared early life environment and dominance genetic effects.

Finally, to investigate the extent to which education and personality were intermediate factors in the causal pathway between the shared genetic factors and the co-occurrence of body composition and symptoms of depression, analyses were additionally adjusted for education and personality in separate models. SOLAR 2.1.2 software package (Southwest Foundation for Biomedical Research, San Antonio, Texas, USA) was used for the calculation of heritability estimates and for the genetic and environmental correlations. P values lower than 0.05 (two-tailed) were considered statistically significant.

Results

Of the 3,092 participants of the study, data on 2,383 were available for the outcomes studied in the present analyses. Mean age of the participants was 48.7 years (SD 15.1) and 56.9% were women. A total of 55.6% of the women and 70.5% of the men were overweight or obese (Table 1; $p<0.001$). Women had significantly higher fat mass index, higher CES-D depression scores and more frequently used antidepressants. Men had higher lean mass index and more abdominal obesity.

In women and men, a higher waist to hip ratio was correlated to a significantly higher level of symptoms of depression as measured with the HADS-D but not with the CESD-D scores (Table 2). In men, a consistently significant inverse correlation was seen between the lean mass index and the CES-D and HADS-D scores and also a higher BMI was correlated to a significantly lower level of symptoms of depression as measured with the CESD-D but not with the HADS-D scores. When studying the correlation between body composition and depression with the putative confounders education and personality (Table 3), we found that education was inversely association to CES-D ($p<0.001$), HADS-D scores ($p<0.001$) and to waist to hip ratio

(p<0.001). Symptoms of depression were associated with all NEO-FFI personality types (Table 3), and there were only scattered correlations with body composition measures. Extraversion was significantly correlated to lean mass index, conscientiousness was significantly inversely correlated to fat mass index and neuroticism and openness were correlated to the waist to hip ratio in opposite directions.

Table 1 General characteristics of the study population

	Women (n=1,355)	**Men (n=1,028)**	**p-Value**
Age, years	48.6 (15.3)	48.8 (14.7)	0.79
Body mass index, kg/m²	26.4 (4.9)	27.2 (4.3)	<0.001
Underweight (<18.5)	1.2	0.7	
Normal weight (18.5 – 24.9)	43.2	28.8	
Overweight (25.0 – 29.9)	35.7	49.3	
Obese (> 30.0)	19.9	21.2	
Lean mass index, kg/m²	15.2 (1.6)	19.0 (2.0)	<0.001
Fat mass index, kg/m²	10.1 (3.7)	7.3 (2.9)	<0.001
Waist to hip ratio	0.80 (0.08)	0.94 (0.08)	<0.001
Abdominal obesity†	12.0	22.1	<0.001
Symptoms of depression			
CES-D	11.9 (10.2)	9.1 (8.6)	<0.001
HADS-D	6.1 (4.5)	6.0 (4.1)	0.62
Use of antidepressant medication	9.0	4.3	<0.001
Education level			
Lower	30.5	34.1	<0.001
Intermediate	65.8	58.1	
Higher	3.7	7.8	
Personality type (NEO-FFI)			
Neuroticism	32.6 (8.0)	29.7 (7.6)	<0.001
Extraversion	39.7 (6.5)	40.3 (6.5)	0.05
Openness	33.5 (5.9)	33.1 (5.5)	0.21
Agreeableness	45.0 (5.4)	42.0 (5.4)	<0.001
Conscientiousness	46.3 (5.9)	46.5 (5.8)	0.62

CES-D = Center for Epidemiologic Studies Depression Scale, HADS-D = Hospital Anxiety and Depression Scale-Depression subscale, NEO-FFI = NEO five factor inventory.

Values are means (standard deviations) for continuous variables and percentages for categorical variables.

† Abdominal obesity = Percentage of individuals with waist to hip ratio ≥0.9 in men and ≥1.0 in women.

Table 2 Correlations of body composition with symptoms of depression by sex

	CES-D		HAD-D	
	Women	**Men**	**Women**	**Men**
Body mass index	-0.01	0.02	-0.07*	-0.03
Lean mass index	-0.01	0.02	-0.08*	-0.08*
Fat mass index	-0.02	0.02	-0.05	0.01
Waist to hip ratio	0.05	0.08*	-0.01	0.08*

CES-D = Center for Epidemiologic Studies Depression Scale
HAD-D = Hospital Anxiety and Depression Scale –Depression subscale
Adjusted for antidepressant use
*p<0.05

Table 4 shows that for both depression scales, heritability estimates were higher in women (0.34 and 0.37) than in men (0.13 and 0.21). Heritability estimates for body composition ranged from 0.40 to 0.46 ($p<0.001$) in women and from 0.35 to 0.51 ($p<0.001$) in men (Table 3). There were statistically significant sibship effects for all measures of body composition in women ($c^2 = 0.11$ to 0.20, $0.05<p<0.001$), but not in men (Table 4).

When decomposing the phenotypic correlations into genetic and environment correlations, the positive correlation between waist-to-hip ratio and symptoms of depression did not remain statistically significant. There was no evidence for a role of shared genetic factors in the co-occurrence of body composition and symptoms of depression in men or women (Table 5). Genetic correlations were highest between the HADS-D depression scores and waist-to-hip ration in men, but these were not statistically significant. We found an effect of shared environmental factors in the relation of lean mass index with CES-D and HADS-D depression scores in men ($\rho E = -0.23$ and -0.31, $p<0.05$), but not in women. Shared environmental factors also related higher body mass index to lower depression scores as measured with the HADS-D ($\rho E = -0.22$, $p<0.05$). These correlations did not change after additional adjustment for education and personality in separate analyses (data not shown).

Table 3 Correlations between the study variables body composition and depression with putative confounders education and personality

	Education	Personality type (NEO-FFI)				
		Neuroticism	Extraversion	Openness	Agreeableness	Conscientiousness
Symptoms of depression						
CES-D	-0.09***	0.51***	-0.39***	-0.03*	-0.21***	-0.24***
HADS-D	-0.17***	0.51***	-0.45***	-0.14***	-0.19***	-0.23***
Body Composition						
Body mass index	0.01	0.01	0.01	-0.02	-0.03	-0.03
Lean mass index	-0.02	-0.01	0.04*	-0.02	0.00	0.02
Fat mass index	0.02	0.03	-0.01	-0.01	-0.03	-0.05*
Waist to hip ratio	-0.05***	0.03*	-0.01	-0.04*	-0.01	-0.03

CES-D = Center for Epidemiologic Studies Depression scale

HADS-D = Depression subscale of the Hospital Anxiety and Depression scale

Adjusted for age, sex, and degree of consanguinity. Analyses for CES-D and HADS-D were additionally adjusted for use of antidepressant medication.

* $p < 0.05$, ** $p < 0.01$, *** $p<0.001$

Table 4. Heritability estimates and sibship effects in body composition and symptoms of depression

	Women		Men	
	Heritability (h^2)	**Sibship effect (c^2)**	**Heritability (h^2)**	**Sibship effect (c^2)**
Symptoms of depression				
CES-D	0.34***	0.00	0.13	0.00
HADS-D	0.37***	0.00	0.21**	0.00
Body composition				
Body mass index	0.40***	0.20**	0.44***	0.00
Lean mass index	0.44***	0.20***	0.51***	0.00
Fat mass index	0.46***	0.13**	0.46***	0.00
Waist to hip ratio	0.46***	0.11*	0.35***	0.06

CES-D = Center for Epidemiologic Studies Depression scale, HADS-D = Depression subscale of the Hospital Anxiety and Depression Scale.

Analyses were adjusted for age, degree of consanguinity and sibship effects. Analyses for CES-D and HADS-D were additionally adjusted for use of antidepressant medication.

* $p < 0.05$, ** $p < 0.01$, *** $p<0.001$

Discussion

We found significant evidence for a relationship between body composition and symptoms of depression. In women and men, higher waist to hip ratio was correlated to a significantly higher level of symptoms of depression as measured with the HADS-D. In men, a consistent significant inverse correlation was seen between the lean mass index and both the CES-D and HADS-D scores. In men, also a higher BMI was correlated to a significantly lower level of symptoms of depression as measured with the CESD-D scores. We found no evidence that the co-occurrence of the fat or lean mass related outcomes and symptoms of depression are explained by the same genetic factors. We did find a consistent and significant positive environmental correlation between lean mass index and symptoms of depression in men, suggesting that both lean mass index and symptoms of depression are affected through the same environmental factors. An evident candidate for this shared pathway is physical activity, which increases lean mass index by acquiring more muscles, and decreases symptoms of depression by maintaining low base endorphin levels.[38] Also body mass index showed a statistically significant inverse environmental correlation to HADS-D scores, but this may be a false positive finding as the association to the CES-D scores was not significant. An explanation for this finding may be that BMI measures both fat and lean mass and that the inverse association between BMI and HADS-D is explained by the inverse association of lean mass index to HADS-D.

Before interpreting the results, two methodological issues need to be discussed. First, overall phenotypic correlations between body composition and symptoms of depression were low in our study. However, findings of other studies show similar correlations.[39] When decomposing the correlation into genetic and environment correlations, the overall phenotypic positive correlation between waist to hip ratio and symptoms of depression did not remain statistically significant. Although we have studied a large data set, this finding could be explained by sheer lack of power. Second, we used two depression scales and found slight differences in the results obtained for the CES-D and the HADS-D scores. While both scales are validated for the assessment of symptoms of depression,[33] the correlation between the depression scales was high (Pearson correlation coefficient=0.75, $p<0.001$) but not perfect. The scales may assess slightly different symptoms and may lead to differences in results. It is of note that while the estimates of the genetic correlations differed in magnitude, the overall pattern of association was the same for the CES-D and the HADS-D, except for the instances when the correlation for one scale or both scales was virtually zero.

Our study showed that the heritability estimates of body composition and depression were high, in particular in women. Of further interest is the consistent finding of significant sibship effects for body composition in women and not in men. This effect could be explained by dietary behaviors. Like in most traditional societies where women are responsible for the cooking, we may see a sibship effect in women when they have adopted the cooking and dietary habits from their mothers. An alternative explanation is that sibship effects reflect dominance variance, which cannot be excluded from the present analyses. An interesting finding is that symptoms of depression were significantly associated with education, which is a proxy measure for socioeconomic status.[40]

Our study did not show evidence for shared genetic factors influencing the co-occurrence between obesity and depression nor the inverse relationship between lean mass and depression. This implies that in our study there is no evidence that symptoms of depression, fat mass, or lean mass are caused by the same underlying genetic pathways. Our findings did not change when we additionally adjusted for education and personality. Overall, we observed evidence for a phenotypic relation between symptoms of depression and waist to hip ratio, which is an important determinant of metabolic syndrome. Our study does not answer the question whether this co-occurrence is explained by shared genetic or environmental pathways, as neither of these was significant. Although genetic factors may play a role in the co-occurrence of a high waist to hip ratio with symptoms of depression, they do not seem to dominate over the role of environmental ones. These findings do not exclude that genetic factors underlie obesity and that obesity consequently leads to depression, or alternatively that genetic factors underlie depression and that the depression consequently leads to obesity.

The most pronounced finding was the consistent and significant evidence for a role of shared environmental factors in the association between lean and fat mass index and symptoms of depression. It has been recognized for long that there is a relationship between lean mass and symptoms of depression. However, few studies addressed the relation of fat mass index and symptoms of depression. Lean mass has been more difficult to address, but the DEXA allowed us to disentangle to assess this outcome directly.

Elucidating the common etiology of obesity and depression is relevant for public health. If genetic factors underlie the clustering, different approaches are needed than is the case when environmental factors underlie the clustering. From a public health perspective the most important finding of our study is that co-occurrence of higher lean mass and less symptoms of depression is primarily of environmental

origin.[38] There is increasing interest in the role of physical activity in the treatment of depression and our findings suggest that further research is justified.[24]

References

1. **Stunkard AJ, Faith MS, Allison KC.** Depression and obesity. Biol Psychiatry 2003;54:330-7.
2. **Faith MS, Matz PE, Jorge MA.** Obesity-depression associations in the population. J Psychosom Res 2002;53:935-42.
3. **Simon GE, Von Korff M, Saunders K, Miglioretti DL, Crane PK, van Belle G, et al.** Association between obesity and psychiatric disorders in the US adult population. Arch Gen Psychiatry 2006;63:824-30.
4. **Cugini P, Cilli M, Salandri A, Ceccotti P, Di Marzo A, Rodio A, et al.** Anxiety, depression, hunger and body composition: III. Their relationships in obese patients. Eat Weight Disord 1999;4:115-20.
5. **Goodman E, Whitaker RC.** A prospective study of the role of depression in the development and persistence of adolescent obesity. Pediatrics 2002;110:497-504.
6. **Herva A, Laitinen J, Miettunen J, Veijola J, Karvonen JT, Laksy K, et al.** Obesity and depression: results from the longitudinal Northern Finland 1966 Birth Cohort Study. Int J Obes (Lond) 2006;30:520-7.
7. **Everson SA, Maty SC, Lynch JW, Kaplan GA.** Epidemiologic evidence for the relation between socioeconomic status and depression, obesity, and diabetes. J Psychosom Res 2002;53:891-5.
8. **Kendler KS, Gatz M, Gardner CO, Pedersen NL.** Personality and major depression: a Swedish longitudinal, population-based twin study. Arch Gen Psychiatry 2006;63:1113-20.
9. **Ryden A, Sullivan M, Torgerson JS, Karlsson J, Lindroos AK, Taft C.** Severe obesity and personality: a comparative controlled study of personality traits. Int J Obes Relat Metab Disord 2003;27:1534-40.
10. **Ryden A, Sullivan M, Torgerson JS, Karlsson J, Lindroos AK, Taft C.** A comparative controlled study of personality in severe obesity: a 2-y follow-up after intervention. Int J Obes Relat Metab Disord 2004;28:1485-93.
11. **Bornstein SR, Schuppenies A, Wong ML, Licinio J.** Approaching the shared biology of obesity and depression: the stress axis as the locus of gene-environment interactions. Mol Psychiatry 2006;11:892-902.
12. **Lucki I.** The spectrum of behaviors influenced by serotonin. Biol Psychiatry 1998;44:151-62.
13. **Noble EP.** D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. Am J Med Genet B Neuropsychiatr Genet 2003;116:103-25.
14. **Lin RC, Wang XL, Dalziel B, Caterson ID, Morris BJ.** Association of obesity, but not diabetes or hypertension, with glucocorticoid receptor N363S variant. Obes Res 2003;11:802-8.
15. **van West D, Van Den Eede F, Del-Favero J, Souery D, Norrback KF, Van Duijn C, et al.** Glucocorticoid receptor gene-based SNP analysis in patients with recurrent major depression. Neuropsychopharmacology 2006;31:620-7.
16. **Challis BG, Luan J, Keogh J, Wareham NJ, Farooqi IS, O'Rahilly S.** Genetic variation in the corticotrophin-releasing factor receptors: identification of single-nucleotide polymorphisms and association studies with obesity in UK Caucasians. Int J Obes Relat Metab Disord 2004;28:442-6.
17. **Liu Z, Zhu F, Wang G, Xiao Z, Tang J, Liu W, et al.** Association study of corticotropin-releasing hormone receptor1 gene polymorphisms and antidepressant response in major depressive disorders. Neurosci Lett 2007;414:155-8.
18. **Christiansen L, Tan Q, Iachina M, Bathum L, Kruse TA, McGue M, et al.** Candidate gene polymorphisms in the serotonergic pathway: influence on depression symptomatology in an elderly population. Biol Psychiatry 2007;61:223-30.
19. **Rosmond R, Bouchard C, Bjorntorp P.** 5-HT2A receptor gene promoter polymorphism in relation to abdominal obesity and cortisol. Obes Res 2002;10:585-9.

20. **Lerer B, Macciardi F, Segman RH, Adolfsson R, Blackwood D, Blairy S, et al.** Variability of 5-HT2C receptor cys23ser polymorphism among European populations and vulnerability to affective disorder. Mol Psychiatry 2001;6:579-85.
21. **Pooley EC, Fairburn CG, Cooper Z, Sodhi MS, Cowen PJ, Harrison PJ.** A 5-HT2C receptor promoter polymorphism (HTR2C - 759C/T) is associated with obesity in women, and with resistance to weight loss in heterozygotes. Am J Med Genet B Neuropsychiatr Genet 2004;126:124-7.
22. **López-León S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM.** The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005;57:999-1003.
23. **Poston WS, Ericsson M, Linder J, Haddock CK, Hanis CL, Nilsson T, et al.** D4 dopamine receptor gene exon III polymorphism and obesity risk. Eat Weight Disord 1998;3:71-7.
24. **Ernst E, Rand JI, Stevinson C.** Complementary therapies for depression: an overview. Arch Gen Psychiatry 1998;55:1026-32.
25. **Allison DB, Kaprio J, Korkeila M, Koskenvuo M, Neale MC, Hayakawa K.** The heritability of body mass index among an international sample of monozygotic twins reared apart. Int J Obes Relat Metab Disord 1996;20:501-6.
26. **Stunkard AJ, Harris JR, Pedersen NL, McClearn GE.** The body-mass index of twins who have been reared apart. N Engl J Med 1990;322:1483-7.
27. **Sullivan PF, Neale MC, Kendler KS.** Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000;157:1552-62.
28. **Rose KM, Newman B, Mayer-Davis EJ, Selby JV.** Genetic and behavioral determinants of waist-hip ratio and waist circumference in women twins. Obes Res 1998;6:383-92.
29. **Hsu FC, Lenchik L, Nicklas BJ, Lohman K, Register TC, Mychaleckyj J, et al.** Heritability of body composition measured by DXA in the diabetes heart study. Obes Res 2005;13:312-9.
30. **Livshits G, Kato BS, Wilson SG, Spector TD.** Linkage of genes to total lean body mass in normal women. J Clin Endocrinol Metab 2007;92:3171-6.
31. **Radloff LS.** The CES-D scale: A self-report depression scale for research in the general population. Appl Psychol Measurement 1977;1:385-401.
32. **Zigmond A.** The hospital anxiety and depression scale. Acta Psychiatr Scand 1983;67:361-70.
33. **Weissman MM, Sholomskas D, Pottenger M, Prusoff BA, Locke BZ.** Assessing depressive symptoms in five psychiatric populations: a validation study. Am J Epidemiol 1977;106:203-14.
34. **Hoekstra HA, Ormel J, de Fruyt F.** NEO Persoonlijkheidsvragenlijsten NEO-PI-R en NEO-FFI: Handleiding [NEO Personality Inventories: NEO-PI-R and NEO-FFI. Manual]. 1996.
35. **Almasy L, Blangero J.** Multipoint quantitative-trait linkage analysis in general pedigrees. Am J Hum Genet 1998;62:1198-211.
36. **Falconer DS MTF.** Introduction to quantitative genetics. fourth ed. Essex: Pearson Prentice Hall, 1996:312-313.
37. **Boichard D.** PEDIG: A FORTRAN package for pedigree analysis studied for large populations. In 7th World Congr. Genet. Appl. Livest. Prod.; Montpelier, FR: 2002.
38. **Lobstein DD, Ismail AH, Rasmussen CL.** Beta-endorphin and components of emotionality discriminate between physically active and sedentary men. Biol Psychiatry 1989;26:3-14.
39. **Friedman MA, Brownell KD.** Psychological correlates of obesity: moving to the next research generation. Psychol Bull 1995;117:3-20.
40. **Lorant V, Deliege D, Eaton W, Robert A, Philippot P, Ansseau M.** Socioeconomic inequalities in depression: a meta-analysis. Am J Epidemiol 2003;157:98-112.

General discussion

9

General discussion

The aim of this genetic epidemiological thesis was to study candidate genes that play a role in the etiology of depression and to obtain new insights about biological pathways that may be involved in this disorder. In this chapter, the findings of the study are reviewed and discussed, and topics for future research are addressed. Before discussing and interpreting the main findings, methodological issues with regard to the design of the study and the instruments used are reviewed. Advantages and disadvantages of the various approaches are discussed.

Methodological issues

Definition and measurement of depression

As discussed in the introduction, depression can refer to the presence of symptoms of depression or to the clinical diagnosis of MDD. The presence of symptoms of depression is generally assessed using a self-report questionnaire designed for use in research or for primary care.[1] MDD is diagnosed by a psychiatrist after an in-depth interview following the criteria of the DSM-IV. [1]

We chose to measure symptoms of depression instead of diagnosing MDD, and to analyze the sum scores of the self-report questionnaires as continuous variables for several reasons. The first reason is a practical reason, self-report questionnaires are easy to administer in large epidemiological studies. Second, including the depression scores as a continuous variable, prevents misclassification of individuals with sub-clinical depression. When formal diagnostic criteria are applied, individuals with substantial symptoms of depression who do not fulfil the formal diagnostic criteria are commonly erroneously assigned to the control group.[1] And third, analyzing the depression scores as a continuous variable increases the statistical power considerably.[2] There are also disadvantages of using self-report questionnaires for the assessment of symptoms of depression compared to using in-depth psychiatric interviews for MDD diagnosis. The subgroup that has high depression scores does not only include MDD patients, but a more heterogeneous group such as individuals with normal grief, bipolar disorder, anxiety, substance abuse, and dysphoria.[3] This means that when localizing genes, we cannot explicitly assume that these are specifically involved in MDD as the clinical entity of interest but rather are predisposing to a more heterogeneous phenotype. This makes it difficult to target genes predisposing for a specific disorder such as MDD. Despite the obvious advantages of studying endophenotypes from a genetic perspective, from a clinical perspective MDD as an outcome may be more relevant for diagnostic, prognostic and therapeutic applications. However, in most cases, the two different methods of assessing depression can be complimentary to each other. When studying symptoms of depression we can maximize the statistical power, and increase our probability of finding susceptibility genes for depression. Once genes are localized, these can be subsequently studied in MDD patients to validate their role.

Family-based versus population-based studies

This thesis describes results from a family-based and a population-based association study. The two approaches are complementary to each other and both have advantages and disadvantages. The family-based Erasmus Rucphen Family Study (ERF) was embedded in the Genetic Research in Isolated Populations (GRIP) programme,

which investigates the genetic origin of complex genetic disorders and traits. The advantage of the GRIP population is that the relationships between inhabitants are well documented. This allows research on the co-segregation of diseases over multiple generations to study the question whether the co-occurrence of depression with other disorders can be explained by a common genetic origin. Another opportunity is to conduct linkage studies of complex genetic traits, which aim at identifying rare genetic variants that have large effects on the risk of disease. Linkage studies have been conducted successfully in GRIP and other populations such as Iceland.[4-6]

Most of the genetic epidemiological studies performed to date are being conducted in population-based studies. Population-based studies provide a powerful setting for candidate gene studies and for the recently introduced genome wide association studies. This new approach targets the discovery of common variants with small effects. Although the successes for genome wide association studies have been limited for depression, it has been extremely successful for a variety of complex traits such as diabetes, multiple sclerosis and breast cancer. By enlarging the study size of studies of MDD and other depressive disorders, the genome wide association approach may lead to success for these disorders as well. Despite the fact that genome wide association studies are most powerful in population-based studies, they can and have been conducted successful in family-based studies such as the GRIP programme.

Meta-analysis

We reviewed all MDD case-control genetic association studies published before June 2007 and performed meta-analyses for polymorphisms that had been investigated in at least three studies. With this method we were able to combine the results of many studies and increase statistical power. This is particularly important in studies of depression, as most studies have been performed in small samples.[7] Two known methodological issues in meta-analyses are between-study heterogeneity and bias. Between-study heterogeneity may reflect real differences between study populations, but it may also be due to methodological differences between studies. True differences between study populations, for instance, can occur if study populations differ in age, sex or ethnicity of the participants. Because of the limited number of genetic association studies that have been performed on depression, we were not able to perform stratified analyses to study possible sources of heterogeneity in the several meta-analyses that showed substantial between-study heterogeneity. This implies that more replication studies are needed. Because several meta-analyses showed evidence for between-study heterogeneity, more studies are needed to investigate the causes of heterogeneity in more detail.

The second methodological issue in meta-analyses is bias. There are three common biases in meta-analyses that should be considered when interpreting the results: (1) the winner's curse or first study bias, (2) the large number of false positive findings in small studies, and (3) publication bias, which is related to the fact that journals are more likely to publish papers with positive findings.[8,9] First, the winner's curse predicts that often the first study published demonstrates a strong significant association that is very extreme compared to the true effect and is therefore not replicated by subsequent studies. It is possible to identify this type of bias by removing the first study from the analysis. If the overall effect estimate (relative risk or odds ratio) shifts towards the null-hypothesis or becomes non-significant, it is likely that the initial positive finding is explained by this type of bias. Second, small studies tend to give not only false negative findings due to a lack of power, but also often give false positive findings, which are not replicated in larger studies. This makes it not only crucial to weigh for the study size in the meta-analysis, but also to examine the excess of positive findings, if sufficient studies are available. Finally, due to publication bias, studies with statistically significant findings are more likely overrepresented in the literature and therefore also in meta-analyses, biasing the findings to statistically significant overall effects, which then more likely are false positive effects. Publication bias is generally examined using funnel plots,[10] but this is one of the most difficult biases to exclude. Due to the limited number of genetic association studies that have been performed in depression, we were not always able to perform appropriate bias checks.

Studies on the co-occurrence of disease

Our study focused for a large part on the co-occurrence of depression with other traits. This is a unique analysis that can be performed in family- based studies. Yet these analyses also have their limitations. First, we distinguished genetic effects from shared early environment effects by adjusting the bivariate analyses for sibship effects. The advantage is that the heritability estimates then indicates a true genetic effect, but this may be an underestimation because sibship effects not only denote shared early environment, but also genetic dominance. A first limitation is whether we can distinguish sibship effects explaining early shared environment from dominance variance. Sophisticated programmes such as SOLAR cannot make that distinction. This imposes serious limitations for the interpretations of the findings on body composition, which can for a large part be determined by early childhood nutrition and exercise. Although the sibship effect seen may most likely denote environmental effects, we cannot exclude dominance variance.

Another issue is the question of adjustment of the variables under study. We chose to adjust only for shared determinants with a strong impact on the outcome. We did not adjust for factors such as smoking, which may have a strong environmental background but may be partly under genetic control. Theoretically, the genetic correlation between depression and lipids and depression and socioeconomic status may be explained by a genetic correlation of smoking. We did not explore this further in the present study. In our analysis we adjusted for the fact that there is an increased level of distant consanguinity in an isolated population, which may inflate heritability. We did not adjust for other population genetic features such as assortative mating.

Main Findings

Candidate Genes

Association Studies

We investigated two polymorphisms in two genes involved in the renin angiotensin system (RAS). We studied the *ACE* I/D polymorphism in relation to MDD in the Rotterdam Study, and the *AGT* M235T polymorphism in relation to symptoms of depression in the Rotterdam Study and the ERF Study. We found no association between the *ACE* I/D polymorphism and MDD, but did find an association between the *AGT* M235T polymorphism and symptoms of depression in both the Rotterdam and ERF Studies.

The association of the *AGT* gene with symptoms of depression may be explained by the relationship between the RAS and the hypothalamic pituitary adrenal (HPA) axis. RAS directly modulates the HPA axis by stimulating corticotrophin hormones.[11,12] The presence of the T allele of the *AGT* gene increases the angiotensinogen level,[13] which in turn could enhance the HPA feedback and lead to symptoms of depression. In depressed patients there has been a consistent finding suggesting that hyperactivity of the HPA axis increases both corticotrophin releasing hormone (CHR) and adrenocotropic hormone (ACTH). This feedback leads to higher levels of cortisol and aldosterone,[14] which are typically seen in patients with depression.[15]

We found the strongest association between *AGT* M235T and symptoms of depression in men. This may raise questions about a different role of the *AGT* gene in men and women. Given that in both populations women had higher depression scores than men, it can be argued that other causal factors outweigh the role of *AGT* in women and mask an eventual effect of the gene in this subgroup. Alternatively, there is some earlier evidence that the RAS may have different roles in the etiology of depression for men and women. For example, aldosterone and renin plasma concen-

trations, which are hormones that are part of the renin-angiotensin-aldosterone system, were increased in depressed men,[16,17] but not in depressed women.[18] Another study found an increased frequency of panic disorders among men carrying the *AGT* T allele compared to non-carriers, but this association was not found in women.[19] However, it is important to note that we have to be careful with the interpretation of subgroup analysis. A recent review of Patsopoulos et al. showed that differences in effects across gender often did not replicate, nor was there an a priori reason to study differential effects.[20] Our finding therefore awaits replication.

Contrary to our expectations, we found no association between the *ACE* gene and MDD, but we cannot completely rule out that the gene is not involved in depression since we have studied only one polymorphism. Recently, three studies reported statistically significant associations for two other polymorphisms in the *ACE* gene (rs4291, rs4295) with MDD.[21,22] One of these studies included more than 1200 individuals in their study.[22] Because we only investigated one polymorphism, we cannot exclude that other polymorphisms in the ACE gene are associated.

Meta-analysis

Our meta-analyses clearly showed that the number of genetic association studies in MDD is still very limited. Only 22 polymorphisms (6%) out of the 393 polymorphisms studied were investigated in at least three studies, but even in these, the total number of cases and controls was often not large enough to detect even a moderate association (OR ~1.2-1.5). Furthermore, most genes were investigated for only one polymorphism in the gene. Even if this single polymorphism appears not to be associated, there may still be other variants in the gene that are associated to MDD. It is evident that more research is needed.

By reviewing and performing meta-analyses for all MDD case-control genetic association studies (Chapter 2), we found statistically significant associations for six polymorphisms in the following genes: apolipoprotein E (*APOE)*, guanine nucleotide-binding protein (*GNB3*), methylene-tetrahydrofolate reductase (*MTHFR*), serotonin transporter (*SLC6A4*), dopamine transporter (*SLC6A3*) and the dopamine receptor D4 (*DRD4*).

The *SLC6A4* gene plays a role in the serotoninergic system. Serotonin has been the main focus in the research of depression.[23] The hypothesis that this neurotransmitter is involved in the etiology of depression derives retrospectively from pharmacological experience. Serotonin has been the target of treatment of depression for the last fifty years.[23] The effect of the *SLC6A4* gene was however very small. In fact, it was the gene with the lowest odds ratio (OR in alleles = 1.11 compared to 0.51,

1.20, 1.38, 1.73, 2.06 for the *APOE* ρ2, *MTHFR* 677T, *GNB3* 825T *DRD4* 48bp and *SLC6A3*).

The effect of the *SLC6A3* is also of interest, as it is involved in dopamine metabolism. Depression is seen in Parkinson's disease, one of the most common dopamine related disorders. Of interest is also the association we found in our meta-analyses that *APOE, GNB3* and *MTHFR* are associated to MDD. Each of these genes has also been implicated in vascular disease. These associations are of interest in light of the co-occurrence of depression and symptoms of depression with vascular pathology (see next paragraph).

Shared etiological pathways between depression and other traits

To guide and support genetic association studies, we investigated the role of genetic predisposition in the co-occurrence of symptoms of depression with socioeconomic status, cardiovascular risk factors and body composition. The rationale behind this research is to identify overlapping etiological pathways. Genes involved in these pathways can be studied as candidate genes in relation to depression. The following vascular risk factors were investigated: blood pressure, glucose levels, lipid levels and body composition. In our research, we found a genetic association between symptoms of depression and lipid parameters, and between symptoms of depression and socioeconomic status.

The finding that there is a genetic association between lipids and symptoms of depression in our population is in line with our finding that the *APOE* gene is associated with MDD in the meta-analysis. It is interesting to see that the *APOE* was found to be the most strongly associated gene to depression in terms of the OR in our meta-analysis. The OR of 0.51 implies an almost 2-fold reduction in MDD risk for carriers of the ε2 allele compared to the ε3 allele. Our finding is in line with earlier reviews concluding that genes in the phospholipids metabolism are of interest in relation to depression.[24,25] A recent paper listed more than 100 candidate genes for proteins involved in the metabolism of phospholipids and fatty acids that could be associated to psychiatric disease.[24]

In recent years, there is increasing interest in the hypothesis that depression and cardiovascular risk factors may share common genetic pathways.[26-28] The *GNB3* gene, which codes for an important signal traducing component of the cell membrane, has been found to be associated to hypertension,[29] and to depression in our meta-analysis (Chapter 2). The *MTHFR* gene, which is implicated in folate metabolism, was found to yield a threefold increase in risk for premature cardiovascular disease,[30] and a meta-analysis showed that individuals with the TT genotype have

a significantly higher risk of coronary heart disease.[31] We found a significant association of the *MTHFR* gene to MDD in our meta-analysis. In our analyses on the co-occurrence of symptoms of depression and cardiovascular risk factors, we only found a genetic association between symptoms of depression and lipid parameters, and could not confirm the results of the only previous study on this, which found a genetic association of depression with hypertension and cardiovascular disease.[32]

In addition to the genetic correlation between symptoms of depression and lipid variables, we also found an association between symptoms of depression and socioeconomic status. Socioeconomic status is often viewed of as a purely environmental factor. However, our study showed that the heritability of socioeconomic status was even higher than that of symptoms of depression. The heritability estimate in our study was in line with previous studies that reported heritability estimates for socioeconomic status ranging from 0.30 to 0.50.[33-36] Several studies have studied genetic polymorphisms in relation to socioeconomic status.[37,38] In line with this finding of genetic correlation and with the results of our meta-analysis, which found an association between MDD and *APOE*, an association between the *APOE* gene and socioeconomic status has been reported.[38] It remains to be determined whether this finding is replicated by other studies.

Suggestions for future research

Even though association studies in relation to depression have been performed since thirty years ago,[39] our meta-analyses clearly showed that the number of genetic associations studies of MDD is still limited, and that only very few genetic variants have been studied. Clearly many more studies are needed. To date, very few candidate gene studies on MDD have targeted lipids. Candidate gene studies seem an outdated approach with the availability of new methods such as genome wide association studies, but these still need replication studies in candidate regions.

The recent successes of the genome wide association studies have overshadowed the candidate gene studies that have been less successful. However, the key feature of the success of genome wide association studies has been that findings have been replicated by 3 or more other studies before publication. Such rigid, self-imposed criteria have never been used for candidate gene studies.

Genetic association studies now enter a new era of genome wide association studies. After 30 years of research using traditional association studies that investigated a few polymorphisms in a single study, this approach enables the screening of hundreds of thousands for association simultaneously. Genome wide association

will be used more often, especially as ongoing technical developments allow for easy and relatively low-cost genotyping, which can now rapidly test 500,000 to 1,000,000 single nucleotide polymorphisms (SNPs) across the human genome.[40] This method enables us to localize common genetic variants with small effects in depression as well.

As with single gene association studies, it is expected that a lack of power will be a drawback for finding genes associated to depression. One of the solutions is collaboration between centers. Collaborative efforts in genome-wide association studies are performed by the Genetic Association Information Network (GAIN) consortium.[41] A key issue will be to standardize studies a priori, to enable stratified analyses and interaction analyses in future meta-analyses.

Studies have shown that environmental factors as well as genetic factors play an important role in the etiology of depression. Caspi et al have stressed the role of gene-environment interaction,[40] and showed that depression less likely occurs in the absence of specific environment or life events. Furthermore, there is interest in the role of dietary factors. Deficiencies of folate, vitamin B12, iron, zinc, omega-3 fatty acid, and selenium tend to be more common among depressed than non-depressed persons.[42] Additionally, folate deficiency may reduce the response to antidepressants.[43] It would be interesting to study, in relation to depression, the effect of iron-containing food on the *MTHFR* gene, and the effects of diet in relation to the *APOE* gene.

Finally, there is increasing interest in pharmacogenetics. Several studies have addressed the question of how antidepressants react differently to people with different genetic variants. For example there is considerable progress showing a role of P450 genes in the response to antidepressants.[44] Pharmacogenetic epidemiology of depression will be an important area of research.

In conclusion, candidate gene and genome wide association approaches are useful in the identification of depression susceptibility genes. The most important avenue to follow will be to enlarge the ongoing studies in terms of number of patients included, and to include sufficiently sized replication studies. Also linkage studies may still yield valuable findings on major genes, but these studies will also require large series of relatives and sufficiently sized replication sets.

References

1. **Kaplan H SB.** Sypnosis of Psychiatry 8th Edition. Baltimore, USA. Williams & Wilkins, 2005.
2. **Almasy L, Blangero J.** Endophenotypes as quantitative risk factors for psychiatric disease: rationale and study design. Am J Med Genet 2001;105:42-4.
3. **American Psychiatric Association.** Diagnostic and Statistical Manual of Mental Disorders-IV. Washington DC, 1994.
4. **Bonifati V, Rizzu P, van Baren MJ, Schaap O, Breedveld GJ, Krieger E, et al.** Mutations in the DJ-1 gene associated with autosomal recessive early-onset parkinsonism. Science 2003;299:256-9.
5. **Njajou OT, Vaessen N, Joosse M, Berghuis B, van Dongen JW, Breuning MH, et al.** A mutation in SLC11A3 is associated with autosomal dominant hemochromatosis. Nat Genet 2001;28:213-4.
6. **Thorleifsson G, Magnusson KP, Sulem P, Walters GB, Gudbjartsson DF, Stefansson H, et al.** Common sequence variants in the LOXL1 gene confer susceptibility to exfoliation glaucoma. Science 2007;317:1397-400.
7. **Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN.** Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat Genet 2003;33:177-82.
8. **Egger M, Smith GD.** Bias in location and selection of studies. BMJ 1998;316:61-6.
9. **Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG.** Replication validity of genetic association studies. Nat Genet 2001;29:306-9.
10. **Egger M, Davey Smith G, Schneider M, Minder C.** Bias in meta-analysis detected by a simple, graphical test. BMJ 1997;315:629-34.
11. **Jezova D, Ochedalski T, Kiss A, Aguilera G.** Brain angiotensin II modulates sympathoadrenal and hypothalamic pituitary adrenocortical activation during stress. J Neuroendocrinol 1998;10:67-72.
12. **Plotsky PM, Owens MJ, Nemeroff CB.** Psychoneuroendocrinology of depression. Hypothalamic-pituitary-adrenal axis. Psychiatr Clin North Am 1998;21:293-307.
13. **Jeunemaitre X, Soubrier F, Kotelevtsev YV, Lifton RP, Williams CS, Charru A, et al.** Molecular basis of human hypertension: role of angiotensinogen. Cell 1992;71:169-80.
14. **Claes SJ.** CRH, stress, and major depression: a psychobiological interplay. Vitam Horm 2004;69:117-50.
15. **Emanuele E, Geroldi D, Minoretti P, Coen E, Politi P.** Increased plasma aldosterone in patients with clinical depression. Arch Med Res 2005;36:544-8.
16. **Charloux A, Gronfier C, Lonsdorfer-Wolf E, Piquard F, Brandenberger G.** Aldosterone release during the sleep-wake cycle in humans. Am J Physiol 1999;276:E43-9.
17. **Vieweg WV, Veldhuis JD, Carey RM.** Temporal pattern of renin and aldosterone secretion in men: effects of sodium balance. Am J Physiol 1992;262:F871-7.
18. **Murck H, Held K, Ziegenbein M, Kunzel H, Koch K, Steiger A.** The renin-angiotensin-aldosterone system in patients with depression compared to controls--a sleep endocrine study. BMC Psychiatry 2003;3:15.
19. **Olsson M, Annerbrink K, Westberg L, Melke J, Baghaei F, Rosmond R, et al.** Angiotensin-related genes in patients with panic disorder. Am J Med Genet B Neuropsychiatr Genet 2004;127:81-4.
20. **Patsopoulos NA, Tatsioni A, Ioannidis JP.** Claims of sex differences: an empirical assessment in genetic associations. JAMA 2007;298:880-93.
21. **Mendlewicz J, Oswald P, Claes S, Massat I, Souery D, Van Broeckhoven C, et al.** Patient-control association study of substance P-related genes in unipolar and bipolar affective disorders. Int J Neuropsychopharmacol 2005;8:505-13.
22. **Baghai TC, Binder EB, Schule C, Salyakina D, Eser D, Lucae S, et al.** Polymorphisms in the angiotensin-converting enzyme gene are associated with unipolar depression, ACE activity and hypercortisolism. Mol Psychiatry 2006.
23. **Hardman JG, Limbird LE, Molinoff PB, Ruddon RW. Goodman & Gilman,** The Pharmacological Basis of Therapeutics. New York: A. McGraw-Hill, 1996.
24. **Bennett CN, Horrobin DF.** Gene targets related to phospholipid and fatty acid metabolism in schizophrenia and other psychiatric disorders: an update. Prostaglandins Leukot Essent Fatty Acids 2000;63:47-59.

25. **Meira-Lima IV, Vallada H.** [Genes related to phospholipid metabolism as risk factors related to bipolar affective disorder]. Rev Bras Psiquiatr 2003;25:51-5.
26. **Carney RM, Freedland KE.** Depression and coronary heart disease: more pieces of the puzzle. Am J Psychiatry 2007;164:1307-9.
27. **Bondy B.** Common genetic factors for depression and cardiovascular disease. Dialogues Clin Neurosci 2007;9:19-28.
28. **Mahley RW, Rall SC, Jr.** Apolipoprotein E: far more than a lipid transport protein. Annu Rev Genomics Hum Genet 2000;1:507-37.
29. **Siffert W, Rosskopf D, Siffert G, Busch S, Moritz A, Erbel R, et al.** Association of a human G-protein beta3 subunit variant with hypertension. Nat Genet 1998;18:45-8.
30. **Kluijtmans LA, van den Heuvel LP, Boers GH, Frosst P, Stevens EM, van Oost BA, et al.** Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. Am J Hum Genet 1996;58:35-41.
31. **Klerk M, Verhoef P, Clarke R, Blom HJ, Kok FJ, Schouten EG.** MTHFR 677C-->T polymorphism and risk of coronary heart disease: a meta-analysis. JAMA 2002;288:2023-31.
32. **Scherrer JF, Xian H, Bucholz KK, Eisen SA, Lyons MJ, Goldberg J, et al.** A twin study of depression symptoms, hypertension, and heart disease in middle-aged men. Psychosom Med 2003;65:548-57.
33. **Baker LA, Treloar SA, Reynolds CA, Heath AC, Martin NG.** Genetics of educational attainment in Australian twins: sex differences and secular changes. Behav Genet 1996;26:89-102.
34. **Teasdale TW, Owen DR.** Heredity and familial environment in intelligence and educational level - a sibling study. Nature 1984;309:620-2.
35. **Vogler GP, Fulker DW.** Familial resemblance for educational attainment. Behav Genet 1983;13:341-54.
36. **Tambs K, Sundet JM, Magnus P, Berg K.** Genetic and environmental contributions to the covariance between occupational status, educational attainment, and IQ: a study of twins. Behav Genet 1989;19:209-22.
37. **Keltikangas-Jarvinen L, Elovainio M, Kivimaki M, Ekelund J, Peltonen L.** Novelty seeking as a mediator in relationships between type 4 dopamine receptor gene polymorphism and predisposition to higher education. L Indiv Diff 2002;14:23-30.
38. **Hubacek JA, Pitha J, Skodova Z, Adamkova V, Lanska V, Poledne R.** A possible role of apolipoprotein E polymorphism in predisposition to higher education. Neuropsychobiology 2001;43:200-3.
39. **Beckman G, Beckman L, Cedergren B, Perris C, Strandman E.** Serum protein and red cell enzyme polymorphisms in affective disorders. Hum Hered 1978;28:41-7.
40. **Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al.** Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 2003;301:386-9.
41. **Boomsma DI, Willemsen G, Sullivan PF, Heutink P, Meijer P, Sondervan D, et al.** Genome-wide association of major depression: description of samples for the GAIN Major Depressive Disorder Study: NTR and NESDA biobank projects. Eur J Hum Genet 2008;16:335-42.
42. **Bodnar LM, Wisner KL.** Nutrition and depression: implications for improving mental health among childbearing-aged women. Biol Psychiatry 2005;58:679-85.
43. **Mischoulon D, Raab MF.** The role of folate in depression and dementia. J Clin Psychiatry 2007;68 Suppl 10:28-33.
44. **Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C.** Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 2007;116:496-526.

Summary

The aim of this genetic epidemiological thesis was to study candidate genes that play a role in the etiology of depression, and to obtain new insights about biological pathways that may be involved in the etiology of depression.

The identification of novel susceptibility genes and of shared etiological pathways was conducted in two large-scale studies: the Rotterdam Study and the Erasmus Rucphen Family (ERF) Study. The Rotterdam Study is a population-based cohort study in the Netherlands of 7,983 men and women aged 55 years and older. Baseline measurements were obtained between 1990 and 1993. All participants were subsequently examined in follow-up examination rounds every 2-3 years. Analyses were performed on data from individuals who participated in the fourth assessment of the study (n=3,550), which took place from 2002 to 2004. The ERF Study is a large-scale family-based study, which is part of the Genetic Research in Isolated Populations programmes. This programme investigates the genetic origin of complex genetic disorders and traits in a genetically isolated community in the Southwest of the Netherlands. Genealogical data of this community date back to the middle of the sixteenth century. All descendants from 20 couples living in the isolate from 1850 to 1900 were ascertained and living descendants of 18 years and older were invited to participate. Data were collected from 2002 to 2005.

The work presented in this thesis focuses for the largest part on symptoms of depression. These were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D), and the depression subscale of the Hospital Anxiety Depression Scale (HADS-D). Both scales are valid and reliable self-report measures of symptoms of depression. The CES-D consists of 20 items with total scores ranging from 0 to 60, and the HADS-D of 7 items with scores ranging from 0 to 21. Higher scores indicate more symptoms of depression. In addition, we investigated major depressive disorder (MDD), which was defined following standard diagnostic criteria according to the manual of mental disorders (DSM-IV) or the International Classification of Diseases (ICD-10).

The thesis starts with a review of all genetic case-control association studies on MDD published before June 2007 (**Chapter 2**). This review included all case-control studies that compared adults diagnosed with standard diagnostic criteria for MDD to controls from the general population. Studies were excluded if (1) cases were selected by questionnaires assessing symptoms of depression, (2) the study

population included bipolar patients, (3) the distribution of genotypes in the control population was not in Hardy-Weinberg Equilibrium, or (4) if the data were re-used in a larger study on the same polymorphism. We performed meta-analyses for genetic polymorphisms that had been investigated in at least three studies. The 183 papers that met our criteria addressed 393 polymorphisms in 102 genes. Twenty-two polymorphisms (6%) in 20 genes were investigated in at least three studies. Our meta-analyses in Chapter 2 showed statistically significant evidence for the following MDD susceptibility polymorphisms: *APOE* ε2 (OR, 0.51), *GNB3* 825T (OR, 1.38), *MTHFR* 677T (OR, 1.20), *SLC6A4* 44bp Ins/Del S (OR, 1.11) and the *SLC6A3* 40bpVNTR 9/10 genotype (OR, 2.06). In **Chapter 3** we report an association between the *DRD4* 2 allele 48bp (OR, 1.73) and MDD. To date, there is evidence for six MDD susceptibility genes: *APOE*, *DRD4*, *GNB3*, *MTHFR*, *SLC6A3* and *SLC6A4*.

We further investigated two polymorphisms in the renin angiotensin system (RAS), the *ACE* I/D polymorphism in relation to MDD in the Rotterdam Study, and the *AGT* M235T polymorphism in relation to symptoms of depression in both the Rotterdam Study as well as in the ERF Study. We found no association between the *ACE* I/D polymorphism and MDD in our study nor in a meta-analysis (**Chapter 4**), but an association between the *AGT* M235T polymorphism and symptoms of depression was found in both the Rotterdam and ERF Studies, with the strongest association in men (**Chapter 5**).

Chapters 6 to 8 focus on co-morbidity and aim at the identification of biological pathways that may be involved in the etiology of depression. For this purpose, the roles of shared genetic and shared environmental factors in the co-occurrence of symptoms of depression and other variables were quantified by genetic (ρG) and environmental (ρE) correlations. Genetic and environmental correlations range from -1 to +1 and express to what extent two traits are influenced by the same genes (ρG), and the same environmental factors (ρE), respectively. Analyses were adjusted for age, sex, use of antidepressant medication, degree of consanguinity and sibship effects. In **Chapter 6** the role of shared genetic factors in the co-occurrence of symptoms of depression and socioeconomic status was investigated. Significant genetic correlations were found between education and symptoms of depression (CES-D: $\rho G=-0.65$, $p<0.001$; HADS-D: $\rho G=-0.50$; $p<0.001$), suggesting that shared genetic factors play a role in the clustering of depression among individuals with lower socioeconomic status. **Chapter 7** describes the role of shared genetic factors in the co-occurrence of symptoms of depression and cardiovascular risk factors such as blood pressure, glucose levels and lipid parameters. Our study showed evidence for shared genetic factors contributing to the co-occurrence of symptoms of depression

and lipid parameters. Significant genetic correlations were found between CES-D scores and total plasma cholesterol (ρG=0.30), LDL (ρG=0.31) and total cholesterol/HDL ratios (ρG=0.25). Significant genetic correlations were also found for HADS-D scores with total cholesterol/HDL ratios (ρG=0.27). In **Chapter 8** we report that there is no evidence that the co-occurrence of symptoms of depression and body composition result from the same genetic factors.

In **Chapter 9** the results are interpreted from a genetic epidemiological perspective and suggestions for future research are discussed. The major conclusions of the thesis are that the number of genetic association studies of MDD is still limited, as only 393 genetic variants have been studied and that more genetic association studies are needed. Our data suggests that there is a genetic predisposition in the co-occurrence of symptoms of depression with both socioeconomic status and lipid parameters. This last finding may guide and support future genetic association studies.

Samenvatting

Het doel van dit genetisch-epidemiologisch proefschrift is het bestuderen van kandidaatgenen die een rol spelen in de etiologie van depressie, alsmede het verkrijgen van nieuwe inzichten in de biologische trajecten die betrokken kunnen zijn bij de etiologie van depressie.

De identificatie van nieuwe gevoeligheidsgenen en van gedeelde etiologische trajecten werd uitgevoerd in twee grootschalige studies: de Rotterdam Studie en de Erasmus Rucphen Familie (ERF) Studie. De Rotterdam Studie is een population-based cohort studie in Nederland van 7.983 mannen en vrouwen van 55 jaar en ouder. De eerste gegevens werden verzameld tussen 1990 en 1993. Alle deelnemers werden vervolgens elke 2 tot 3 jaar opnieuw onderzocht. Analyses werden uitgevoerd op de gegevens van personen die deelnamen aan de vierde evaluatieronde van het onderzoek (n=3.550), dat plaatsvond van 2002 tot 2004. De ERF Studie is een grootschalige familiegebaseerde studie, die deel uitmaakt van het Genetic Research in Isolated Populations programma. Dit programma onderzoekt de genetische oorsprong van complexe genetische aandoeningen en eigenschappen in een genetisch geïsoleerde gemeenschap in het zuidwesten van Nederland. Genealogische gegevens van deze gemeenschap dateren uit het midden van de zestiende eeuw. Alle nakomelingen van 20 echtparen die in het isolaat leefden van 1850 tot 1900 werden opgespoord en levende nakomelingen van 18 jaar en ouder werden uitgenodigd om deel te nemen aan het onderzoek. Gegevens zijn verzameld van 2002 tot 2005.

Het werk beschreven in dit proefschrift richt zich voor het grootste deel op symptomen van depressie. Deze werden bepaald aan de hand van de Center for Epidemiologic Studies Depression schaal (CES-D), en de depressie subschaal van de Hospital Anxiety Depression Scale (HADS-D). Beide schalen zijn valide en betrouwbare zelfgerapporteerde metingen van symptomen van depressie. De CES-D bestaat uit 20 items met totale scores variërend van 0 tot 60 en de HADS-D uit 7 items met scores variërend van 0 tot 21. Hogere scores duiden op meer symptomen van depressie. Daarnaast onderzochten we depressieve stoornis (MDD), die werd gedefiniëerd door middel van standaard diagnostische criteria volgens de handleiding psychische stoornissen (DSM-IV) of de International Classification of Diseases (ICD-10).

Het proefschrift begint met een overzicht van alle genetische patiënt-controle associatie studies naar MDD gepubliceerd vóór juni 2007 (**Hoofdstuk 2**). Dit overzicht omvatte alle patiënt-controle studies die volwassenen gediagnosticeerd met

standaard diagnostische criteria voor MDD (de cases) vergeleek met controles uit de algemene bevolking. Studies werden uitgesloten als (1) cases werden geselecteerd door vragenlijsten die symptomen van depressie beoordeelden, (2) de studie bipolaire patiënten omvatte, (3) de genotypenverdeling van de controle groep niet in Hardy-Weinberg evenwicht was, of (4) als de gegevens opnieuw werden gebruikt in een grotere studie van hetzelfde polymorfisme. We voerden meta-analyses uit voor genetische polymorfismen die waren onderzocht in ten minste drie studies. De 183 artikelen die aan onze criteria voldeden onderzochten in totaal 393 polymorfismen in 102 genen. Tweeëntwintig polymorfismen (6%) in 20 genen werden onderzocht in ten minste drie studies. Onze meta-analyses in Hoofdstuk 2 lieten statistisch significant bewijs zien voor de volgende polymorfismen: *APOE* ε2 (OR, 0.51), *GNB3* 825T (OR, 1.38), *MTHFR* 677T (OR, 1.20), *SLC6A4* 44bp Ins/Del S (OR, 1,11) en het *SLC6A3* 40bpVNTR 9/10 genotype (OR, 2.06). In **Hoofdstuk 3** rapporteren we over een associatie tussen de *DRD4* 2-allel 48bp (1.73) en MDD. Tot op heden is er bewijs voor zes MDD gevoeligheidsgenen: *APOE*, *DRD4*, *GNB3*, *MTHFR*, *SLC6A3* en *SLC6A4*.

We onderzochten verder twee polymorfismen in het renine-angiotensine systeem (RAS): het *ACE* I/D polymorfisme in relatie tot MDD in de Rotterdam Studie en het *AGT* M235T polymorfisme in verband met de symptomen van depressie in zowel de Rotterdam Studie en de ERF Studie. We vonden geen associatie tussen het ACE I/D polymorfisme en MDD in onze studie noch in een meta-analyse (**Hoofdstuk 4**), maar een associatie tussen het AGT M235T polymorfisme en symptomen van depressie werd gevonden in zowel de Rotterdam Studie en de ERF Studie, met de sterkste associatie bij mannen (**Hoofdstuk 5**).

Hoofdstukken 6 tot en met 8 richten zich op co-morbiditeit en trachten biologische trajecten die betrokken kunnen zijn bij de etiologie van depressie te identificeren. Voor dit doel werden de rollen van gedeelde genetische en omgevingsfactoren in het gezamenlijk optreden van de symptomen van depressie en andere variabelen gekwantificeerd door genetische (ρG) en omgevingscorrelaties (ρE). Genetische en omgevingscorrelaties variëren van -1 tot +1 en drukken uit in welke mate twee eigenschappen worden beïnvloed door respectievelijk dezelfde genen (ρG), en dezelfde omgevingsfactoren (ρE). Analyses werden gecorrigeerd voor leeftijd, geslacht, het gebruik van antidepressiva, de mate van bloedverwantschap en sibship effecten. In **Hoofdstuk 6** wordt de rol van gedeelde genetische factoren in het gezamenlijk optreden van de symptomen van depressie en de sociaal-economische status onderzocht. Aanzienlijke genetische correlaties zijn gevonden tussen onderwijs en symptomen van depressie (CES-D: ρG=-0,65, p<0,001; HADS-D: ρG=-0,50; p<0,001),

wat erop wijst dat gedeelde genetische factoren een rol spelen bij de clustering van depressie bij mensen met een lagere sociaal-economische status. **Hoofdstuk 7** beschrijft de rol van gedeelde genetische factoren in het gezamenlijk optreden van symptomen van depressie en cardiovasculaire risicofactoren zoals bloeddruk, glucose niveaus en lipidenconcentratie. Onze studie draagt bewijs aan voor het bestaan van gedeelde genetische factoren die bijdragen aan het gezamenlijk optreden van depressieve symptomen en lipidenconcentratie. Aanzienlijke genetische correlaties werden gevonden tussen de CES-D scores en het totale plasma cholesterol ($\rho G=0,30$), LDL ($\rho G=0,31$) en totaal cholesterol / HDL ratios ($\rho G=0,25$). Aanzienlijke genetische correlaties werden ook gevonden voor HADS-D scores met een totaal cholesterol / HDL ratio ($\rho G=0,27$). In **Hoofdstuk 8** melden wij dat er geen aanwijzingen zijn dat gezamenlijk optreden van de symptomen van depressie en lichaamssamenstelling het gevolg zijn van dezelfde genetische factoren.

In **Hoofdstuk 9** worden de resultaten geïnterpreteerd vanuit een genetisch-epidemiologisch perspectief en worden suggesties voor toekomstig onderzoek besproken. De belangrijkste conclusies van het proefschrift zijn dat het aantal genetische associatie studies van MDD nog steeds beperkt is, aangezien slechts 393 genetische varianten zijn onderzocht en dat er meer genetische associatie studies nodig zijn. Onze resultaten suggereren dat er sprake is van een genetische aanleg in het gezamenlijk optreden van symptomen van depressie met zowel sociaal-economische status en lipidenconcentratie. Deze laatste conclusie kan richting en steun bieden bij toekomstige genetische associatie studies.

Resumen

El objetivo de esta tesis es identificar genes que desempeñan un papel en la etiología de la depresión, y obtener nuevos conocimientos acerca de las vías biológicas que pudieran estar implicadas en dicha etiología.

La identificación de nuevos genes de susceptibilidad a la depresión y de vías genéticamente compartidas entre depresión y otras variables, fueron realizadas dentro del *Rotterdam Study* y el *Erasmus Rucphen Family Study* (ERF); dos estudios de gran escala. El *Rotterdam Study* es un estudio de cohorte poblacional llevado a cabo en los Países Bajos. En él participaron 7.983 hombres y mujeres mayores de 55 años. Los primeros datos de referencia se recolectaron entre 1990 y 1993. Cada dos a tres años todos los participantes fueron reexaminados. Los análisis incluidos en esta tesis engloban información de la cuarta evaluación del estudio (n = 3.550), que tuvo lugar entre 2002 y 2004. El *ERF Study* investiga el origen de enfermedades complejamente genéticas en una comunidad genéticamente aislada en el suroeste de los Países Bajos. Datos genealógicos de esta comunidad se remontan a mediados del siglo XVI. Todos los descendientes mayores de 18 años procedentes de 20 parejas que vivieron en la población fueron invitados a participar. Los datos se recolectaron entre los años 2002 a 2005.

Para evaluar síntomas de depresión se utilizaron dos escalas: la Escala de Depresión del Centro de Estudios Epidemiológicos (CES-D) y la Sub-escala de Depresión de la Escala Hospitalaria de Ansiedad y Depresión (HADS-D). La escala CES-D consta de 20 preguntas que derivan en una puntuación del 0 a 60. La escala HADS-D incluye 7 preguntas que derivan un puntaje del 0 a 21. Los puntajes más altos indican un mayor grado de síntomas de depresión. El diagnóstico de depresión mayor se definió siguiendo los criterios del Manual Diagnóstico y Estadístico de los Trastornos Mentales de la Asociación Psiquiátrica Americana (DSM-IV por sus siglas en inglés) o la Clasificación Estadística Internacional de Enfermedades y Otros Problemas de Salud (CIE-10).

La tesis comienza con una revisión de todos los estudios de asociación genética sobre depresión mayor publicados antes de junio del 2007 (**Capítulo 2**). Incluimos estudios en los que los casos fueron de adultos diagnosticados con depresión mayor, y en los que los controles fueron escogidos en la población general. Los criterios de exclusión de publicaciones fueron los siguientes: (1) si los casos fueron seleccionados por cuestionarios de evaluación de síntomas de la depresión, (2) si la población de estudio incluyó pacientes bipolares, (3) si la distribución de genotipos en los

controles no estaba en equilibrio Hardy-Weinberg, o (4) si los datos se repetían en otro estudio sobre el mismo polimorfismo. Se realizó un metaanálisis si al menos tres estudios investigaban el mismo polimorfismo genético. En total, 183 publicaciones cumplieron con los criterios de inclusión. En éstos, 393 polimorfismos en 102 genes fueron estudiados. Se valoraron con metaanálisis 22 polimorfismos (6%) en 20 genes. Se encontraron 5 polimorfismos genéticos de susceptibilidad a depresión mayor: *APOE* ε2 (OR 0,51), *GNB3* 825T (OR 1,38), *MTHFR* 677T (OR 1,20), *SLC6A4* 44bp Ins/Del S (OR 1,11) y el *SLC6A3* 40bpVNTR genotipo 9/10 (OR 2,06). En el **Capítulo 3** fue encontrada una asociación entre el alelo 2 del gen *DRD4* 48bp (OR 1,73) y depresión mayor. Nuestros metaanálisis reportan que hasta la fecha hay evidencia de que seis genes están asociados a depresión mayor: *APOE, DRD4, GNB3, MTHFR, SLC6A3* y *SLC6A4*.

En los siguientes capítulos se investigaron dos polimorfismos en el sistema de renina angiotensina (RAS), el polimorfismo *ACE* I/D en relación a depresión mayor en el *Rotterdam Study* y el polimorfismo *AGT* M235T en relación a síntomas de depresión tanto en el *Rotterdam Study* así como en el *ERF Study*. No se encontró asociación entre el polimorfismo *ACE* I/D y depresión mayor ni en nuestra población ni en nuestro metaanálisis (**Capítulo 4**). Así mismo, sí se encontró una asociación entre el polimorfismo *AGT* M235T y síntomas de depresión en ambas poblaciones que estudiamos. Esta asociación se encontró presente en hombres (**Capítulo 5**).

Los capítulos 6 a 8 se centraron en obtener nuevos conocimientos acerca de las vías biológicas que pudieran estar implicadas en la etiología de la depresión. Con este fin hemos determinado si variables correlacionadas con depresión comparten una base genética con síntomas de esta enfermedad. Las correlaciones genéticas (ρG) y las ambientales (ρE) se reportaron. Éstas van desde -1 a +1 y expresan en qué medida dos rasgos están influenciados por los mismos genes (ρG) o por los mismos factores ambientales (ρE). Los análisis fueron ajustados por edad, sexo, medicamentos para depresión, grado de consanguinidad y efecto de hermandad. En el **Capítulo 6** hemos investigado la presencia de factores genéticos compartidos en la concurrencia de síntomas de depresión y educación. Se encontraron correlaciones genéticas significativas entre educación y síntomas de depresión (CES-D: ρG=-0,65, p<0,001; HADS-D: ρG=-0,50, p<0,001). Estos resultados sugieren que existen factores genéticos compartidos entre síntomas de depresión y estatus socioeconómico. En el **Capítulo 7** hemos estudiado la existencia de factores genéticos compartidos entre síntomas de depresión y factores de riesgo cardiovascular. Las variables de riesgo cardiovascular incluidas fueron presión arterial, niveles de glucosa y parámetros de lípidos. Se encontraron correlaciones genéticas significativas entre CES-D

y colesterol total en plasma (ρG=0,30), LDL (ρG=0.31) y la proporción colesterol total/HDL (ρG=0.25). Respecto a HADS-D se encontraron correlaciones genéticas significativas con la proporción colesterol total /HDL (ρG=0,27). En el **Capítulo 8** hemos reportado que no hay pruebas de que la concurrencia de los síntomas de la depresión y la obesidad tengan una base genética común.

En el **Capítulo 9** se han interpretado estos resultados desde una perspectiva de epidemiología genética; además, se ofrecen sugerencias para investigación futura. Las principales conclusiones de la tesis son que el número de estudios de asociación genética de depresión mayor son limitados, ya que sólo 393 variantes genéticas han sido estudiadas. Nuestros metaanálisis muestran evidencia de que hasta la fecha hay seis genes que están asociados a depresión mayor: *APOE, DRD4, GNB3, MTHFR, SLC6A3* y *SLC6A4*. Las correlaciones genéticas mostradas sugieren que existe una predisposición genética en la concurrencia de síntomas de depresión tanto con el nivel socioeconómico, como con los parámetros lipídicos. Este último hallazgo pudiera guiar y respaldar estudios de asociación genética en el futuro.

List of publications

López-León S, Croes EA, Sayed-Tabatabaei FA, Claes S, Van Broeckhoven C, van Duijn CM. The dopamine D4 receptor gene 48-base-pair-repeat polymorphism and mood disorders: a meta-analysis. Biol Psychiatry 2005;57:999-1003.

Croes EA, El Galta R, Houwing-Duistermaat JJ, López-León S, Rademaker TA, Dekker MC, Oostra BA, Verhulst F, van Duijn CM. Phenotypic subtypes in attention deficit hyperactivity disorder in an isolated population. Eur J of Epidemiol 2005;20:789-794.

López MI, Tarasco M, Cuevas C, León NA, López-León S. Investigación de actitudes de los profesionales de la psicoterapia en relación a la actuación erótica dentro de la relación psicoterapéutica. Psiquiatría 2005;21:1-11.

López-León S, Janssens ACJW, Hofman A, Claes S, Breteler MMB, Tiemeier H, van Duijn CM. No association between the angiotensin-converting enzyme gene and major depression: a case-control study and meta-analysis. Psychiatr Genet 2006;16:225-6.

López-León S, Janssens ACJW, González-Zuloeta Ladd AM, Del-Favero J, Claes S, Oostra BA, van Duijn CM. Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2088.

López-León S, Janssens ACJW, Tiemeier H, Hofman A, Aulchenko YS, Snijders PJLM, Claes S, Oostra BA, van Duijn CM. Angiotensinogen M235T polymorphism and symptoms of depression in a population-based study and a family-based study. Psychiatr Genet 2008.

López-León S, Choy WC, Aulchenko YS, Claes S, Oostra BA, Mackenbach JP, van Duijn CM, Janssens ACJW. Genetic factors influence the clustering of depression among individuals with lower socioeconomic status. *Submitted.*

López-León S, Choy WC, Aulchenko YS, Claes S, Oostra BA, Mackenbach JP, van Duijn CM, Janssens ACJW. Shared genetic factors in the co-occurrence of symptoms of depression and cardiovascular risk factors. *Submitted.*

Choy WC, López-León S, Zillikens C, Aulchenko YS, Mackenbach JP, van Duijn CM, Janssens ACJW. Evaluating the evidence of shared etiology of symptoms of depression with fat and muscle mass. *Submitted.*

Janssens ACJW, González-Zuloeta Ladd AM, López-León S, Ioannidis JPA, Khoury MJ, Oostra BA, van Duijn CM. Value of meta-analyses on gene-disease association in the era of large-scale consortia and biobank studies. *Submitted.*

Schol-Gelok S, Janssens ACJW, Tiemeier H, Liu F, López-León S, van Swieten JC, de Koning I, Aulchenko YS, Oostra BA, van Duijn CM. A genome-wide screen for depression in a genetically isolated Dutch population. *Submitted.*

About the author

Dr. Sandra López León was born in Mexico City in 1976. She grew up in a family dedicated for several generations to mental health and education. She graduated from the American School Foundation and then from the Medical School of the Anahuac University in Mexico City. After completing her medical internship at the Jerusalem Hadassah Medical Center in Israel, she obtained her Doctor of Medicine (MD) degree in 2001. That same year she moved to the Netherlands where she obtained the certificate of Dutch as a Second Language. In 2002, she started the work described in this thesis at the Genetic Epidemiology Unit of the Department of Epidemiology & Biostatistics, in close collaboration with the Department of Clinical Genetics of the Erasmus University Medical Center, in Rotterdam, The Netherlands. In 2003, she obtained a Master of Science degree (MSc) and, in 2004, a Doctor of Science degree (DSc), both from the Netherlands Institute of Health Sciences. Subsequently she continued working towards a Doctor of Philosophy (PhD), from the Erasmus University. She has presented her work in various international forums and scientific meetings, and has published in several scientific journals. She is member of the International Society of Psychiatric Genetics, and of the Spanish Human Genetics Association (AEGH). Dr. Lopez's hobbies include writing, painting, playing piano, cooking and travelling. She is married to Albertus Ruiter and has two children, Leon and Maximiliano. Dr. López currently lives in Barcelona, Spain.

SANDRALOPEZLEON@GMAIL.COM