ARCHIVAL REPORT

A Genome-Wide Association Study of Depressive Symptoms

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Background: Depression is a heritable trait that exists on a continuum of varying severity and duration. Yet, the search for genetic variants associated with depression has had few successes. We exploit the entire continuum of depression to find common variants for depressive symptoms.

Methods: In this genome-wide association study, we combined the results of 17 population-based studies assessing depressive symptoms with the Center for Epidemiological Studies Depression Scale. Replication of the independent top hits ($p < 1 \times 10^{-5}$) was performed in five studies assessing depressive symptoms with other instruments. In addition, we performed a combined meta-analysis of all 22 discovery and replication studies.

Results: The discovery sample comprised 34,549 individuals (mean age of 66.5) and no loci reached genome-wide significance (lowest $p = 1.05 \times 10^{-7}$). Seven independent single nucleotide polymorphisms were considered for replication. In the replication set ($n =$ 16,709), we found suggestive association of one single nucleotide polymorphism with depressive symptoms (rs161645, 5q21, $p =$ 9.19×10^{-3}). This 5q21 region reached genome-wide significance ($p = 4.78 \times 10^{-8}$) in the overall meta-analysis combining discovery and replication studies ($n = 51{,}258$).

Conclusions: The results suggest that only a large sample comprising more than 50,000 subjects may be sufficiently powered to detect genes for depressive symptoms.

Key Words: Center for Epidemiologic Studies Depression Scale, CHARGE consortium, depression, depressive symptoms, genetics, genome-wide association study, meta-analysis

Major depressive disorder (MDD) is a complex disease with an underlying heritable component. Family and twin studies report a high familial tendency of the disorder and heritability estimates of 31% to 42% (1,2). However, the long

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search for genetic variants associated with depression has had few successes. Several linkage studies for major depressive disorder have been performed and these identified only one relevant locus (3,4). In addition, hundreds of candidate genes have been investigated in association studies, but only six variants have been confirmed in meta-analyses (5,6). Recent efforts to find new candidate genes via genome-wide association studies (GWAS) have also been largely unsuccessful (7–15). Genome-wide association studies identified interesting regions, but associations with MDD reached standard levels of genome-wide significance at only one locus (15). Furthermore, only few previously reported candidate genes were replicated in genome-wide association studies (7,13,16).

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

In the current study, we exploit the entire continuum of depression, defined as the number and severity of depressive symptoms a person experiences. We assessed depressive symptoms with one of the most widely used instruments in the general population, namely the Center for Epidemiological Studies

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Depression (CES-D) scale. This scale assesses the following major dimensions of depression: depressed mood, feelings of guilt and worthlessness, feelings of helplessness and hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance. The CES-D detects cases of MDD with high sensitivity and specificity (22) and has proven to be relatively stable over time (82% of older adults had stable CES-D scores over four measurement rounds in 10 years) (23,24). In addition, a high CES-D score, like a diagnosis of MDD, is associated with cardiovascular disease and mortality (25,26). Moreover, heritability estimates of depressive symptoms, as measured with the CES-D, range from 15% to 34% (27–29).

We present the results of a meta-analysis combining genome-wide association results of depressive symptoms from 17 population-based studies of European ancestry (n = 34,549). In addition, we sought to replicate our findings in five samples that used instruments other than the CES-D to quantify depressive symptoms (n = 16,709). Finally, we performed a combined meta-analysis of all discovery and replication studies that included 51,258 individuals.

Methods and Materials

Discovery Samples

This discovery set included results from 17 population-based studies comprising a total of 34,549 persons of European descent. The following studies collaborating in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (30) in the United States and Europe were included: the Atherosclerosis Risk In Communities 1 and 2 studies (ARIC1 and ARIC2) (31), the Cardiovascular Health Study (CHS) (32), the Framingham Heart Study (FHS) (33,34), and the Rotterdam Study I, II, and III (RS-I, RS-II and RS-III) (35). The following population-based studies joined the discovery analyses: the Baltimore Longitudinal Study of Aging (BLSA) (36); The Erasmus Rucphen Family (ERF) (37) study; the Health, Aging and Body Composition study (Health ABC); the Invecchiare in Chianti (Aging in the Chianti area; InCHIANTI) (38) study; Helsinki Birth Cohort Study (HBCS) (39); Multi-Ethnic Study of Atherosclerosis (MESA) (40); Nurses' Health Study (NHS) (41); Rush Memory and Aging Project (MAP) (42); Religious Orders Study (ROS) (43), and SardiNIA study (44). All studies were approved by their local institutional review boards and all participants provided written informed consent.

Phenotype Definition

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

Adjustment for Use of Antidepressants

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

Genotyping and Imputation

Genome-wide genotyping was performed by the individual studies on Illumina (Illumina, Inc., San Diego, California) or Affymetrix (Affymetrix, Santa Clara, California) platforms. All studies imputed their genotype data to $\sim$2.5 million single nucleotide polymorphisms (SNPs) to account for the different genotyping platforms. HapMap release 22 CEU (HapMap sample comprised of Utah residents with Northern and Western European ancestry) build 36 was generally used as reference for imputation (two studies used build 35). Genotype and imputation quality control were performed in each study separately. Genotype and quality control procedures for each study can be found in Table S1 in Supplement 1.

Data Analysis

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

Meta-Analysis

We performed a p value based meta-analysis weighted by sample size. This is a valid approach to account for the different CES-D versions to measure depressive symptoms and for the

different distributions of depressive symptoms. The meta-analysis test statistic was computed as follows:

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

The meta-analysis was performed with METAL (http://www.sph.umich.edu/csg/abecasis/metal/) (47). The beta (β) of each individual study *i* was matched to a common coded allele (the minor allele) for each SNP across all studies. Single nucleotide polymorphisms with a minor allele frequency less than 2.5% or an observed to expected variance ratio (imputation quality) less than .30 were excluded on a per study basis. Single nucleotide polymorphisms for which the total sample size was lower than 5000 were removed from the results. Genomic control correction was applied to each study's results.

Replication

Independent top SNPs with a p value $< 1 \times 10^{-5}$ in the discovery meta-analysis were selected with the clumping function in PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) (48) ($R^2 < .05$, 500 kilobase [kb]) for replication in five studies that measured depressive symptoms with other instruments (total $n = 16{,}709$). Persons included in the replication studies were independent from those in the discovery studies. Although replication with other instruments than the CES-D might introduce some heterogeneity, all instruments measure depressive symptoms. Further, a positive replication would ensure that our top hits are not instrument-dependent.

Age, Gene, Environment Susceptibility–Reykjavik Study (AGES) (49), the ARIC 3 study (31), Monitoring of Trends and Determinants of Cardiovascular Disease/Cooperative Health Research in the Region of Augsburg F3 and F4 (MONICA/KORA F3 and F4) (50), and the Study of Health in Pomerania (SHIP) (51,52) measured depressive symptoms with the Geriatric Depression Scale (GDS), Maastricht Questionnaire, Patient Health Questionnaire (PHQ-9), and the Beck Depression Inventory-II (BDI-II), respectively. The BDI-II, GDS, and PHQ-9 aim to screen for depression and are highly correlated (53,54). The BDI-II is based on the DSM-IV criteria for MDD and comprises 21 items on a scale of 0 to 3 with higher scores indicating more severe depressive symptoms over the past 2 weeks. The PHQ-9 is, like the BDI-II, based on the DSM-IV criteria for MDD, but it consists of nine items on a scale of 0 to 3 to assess depressive symptoms over the past 2 weeks. The GDS was specifically designed to screen for depression in older adults and comprised 15 items answered with "yes" or "no." The Maastricht Questionnaire (21 items), although designed to measure vital exhaustion, correlates with measures of depressive symptoms (55) and was previously used to assess depressive symptoms (56,57).

Replication was considered significant if the Bonferroni-corrected p value for testing seven SNPs was ≤.050 (uncorrected p value ≤ 7.1×10^{-3}).

Pathway Analysis

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

Candidate Gene Search

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

Results

Meta-Analysis of Depressive Symptoms

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

The genomic control inflation factor lambda (λ_{gc}) for each study ranged between .997 and 1.024. A meta-analysis of 17 studies ($n = 34{,}549$) with depressive symptoms measured by CES-D was performed (Q-Q and Manhattan plots in Figure S1 in Supplement 1). The total number of SNPs analyzed was 2,391,896. No association reached the prespecified genome-wide significance level of 5×10^{-8} for the association with the depressive symptom score. However, we identified 117 SNPs with a p value $< 1 \times 10^{-5}$, which included seven independent top SNPs ($R^2 < .05$ in 500 kb, Table 2). The SNP with the lowest p value was rs8020095 ($p = 1.05 \times 10^{-7}$) and maps to an intronic region of *GPHN* on chromosome 14. Of the seven top SNPs, none had a heterogeneity p value (tested by Cochran's Q) below .05 in the discovery meta-analysis.

We reran the analysis for the independent top SNPs excluding people on antidepressants; p values of the top SNPs shifted toward one (e.g., rs8020095 p value 1.56×10^{-6}, rs161645 p value 1.71×10^{-3}). Adding five points to the total score for people using antidepressants in a subsample (RS-I, RS-II, RS-III, $n = 7925$) resulted in the same top SNPs and similar p values for the top SNPs tested here.

Replication

Table 2 presents the results of the replication analysis and the overall meta-analysis across discovery sample and replication sample. The mean observed to expected variance ratio for the seven top SNPs across all cohorts ranged between .91 and .98 (Table S2 in Supplement 1). In the replication sample, an SNP on chromosome 5 showed an association with depressive symptoms (5q21, rs161645, $p = 9.19 \times 10^{-3}$, Table 2), but this association

Table 1. Study Sample Characteristics of Discovery and Replication Samples

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

ARIC1, ARIC2, ARIC3, RSI, RSII, RSIII, MK F3, and MK F4 included unique individuals.

AGES-RS, Age, Gene, Environment Susceptibility–Reykjavik Study; ARIC, Atherosclerosis Risk in Communities study; BDI-II, Beck Depression Inventory-II; BLSA, Baltimore Longitudinal Study of Aging; CES-D, Center for Epidemiologic Studies Depression scale; CHS, Cardiovascular Health Study; ERF, Erasmus Rucphen Family study; FHS, Framingham Heart Study; GDS, Geriatric Depression Scale; HABC, Health, Aging and Body Composition study; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti; MESA, Multi-Ethnic Study of Atherosclerosis; MK, Monitoring of trends and determinants of cardiovascular disease/cooperative health research in the region of Augsburg (MONICA/KORA); MQ, Maastricht Questionnaire; NA, not applicable; NHS, Nurses Health Study; PHQ-9, Patient Health Questionnaire-9 items; RS, Rotterdam Study; Rush MAP, Rush Memory and Aging Project; Rush ROS, Rush Religious Orders Study; SardiNIA, SardiNIA study; SHIP, Study of Health In Pomerania; SD, standard deviation.

[a]Cutoff for screen positives was 9 for ARIC1, 8 for CHS, 9 for NHS, 3 for Rush MAP and Rush ROS, 6 for AGES-RS, 24 for ARIC3, and 17 for SHIP.

[b]Level 0: preprimary education; level 1: primary education or first stage of basic education; level 2: lower secondary education or second stage of basic education; level 3: (upper) secondary education; level 4: postsecondary nontertiary education; level 5: first stage of tertiary education; level 6: second stage of tertiary education.

Table 2. Meta-Analysis Results of CES-D Depressive Symptom Score in Discovery Studies, Replication of Results in Studies that Measured Depressive Symptoms with Other Instruments, and Overall Meta-Analysis of All Studies

								Discovery Meta-Analysis CES-D $n = 34,549$		Replication Other Instruments $n = 16,709$		Overall Meta-Analysis $n = 51,258$	
SNP[a]	Chr	Position	SNPs (n)[b]	Closest Gene	Distance (Base Pair)	Allele	MAF	Overall Direction (Per Study)	p Value	Overall Direction (Per Study)	p Value	Overall Direction	p Value
rs8020095	14	66,523,611	2	*GPHN*	intron	A/G	.17	+ (+++−+++++++−+++−+?)	1.05e-07	− (−?−−+)	.79	+	3.04e-06
rs8038316	15	52,560,732	3	*UNC13C*	intron	A/G	.05	− (−?−−−−−−−−+−−−−+−)	1.24e-06	− (−−−−+)	.42	−	9.64e-06
rs161645	5	104,097,816	3	*NUDT12*	1,171,427	A/G	.34	+ (++++++++++++−++−+?)	2.32e-06	+ (+++−+)	9.19e-03	+	8.39e-08[c]
rs357282	5	38,904,792	0	*OSMR*	intron	T/G	.13	+ (++++++++−−++++−++)	7.56e-06	+ (−+−−+)	.87	+	1.60e-04
rs4653635	1	223,662,313	3	*LBR*	intron	A/G	.16	− (−−−+−−−−+−−−−−−−−)	8.14e-06	+ (−++−−)	.55[d]	−	8.89e-04
rs4594522	20	30,718,645	5	*COMMD7*	35,508	C/T	.36	− (−−−−+−−−+−−−−−+−−)	9.29e-06	− (−++−−)	.80	−	1.56e-04
rs13137117	4	94,673,387	9	*GRID2*	intron	T/A	.25	+ (+++−+++−++++++++−−)	9.77e-06	+ (−+−+−)	.97	+	2.63e-04

Direction of effect discovery: Framingham Heart Study, Cardiovascular Health Study, Rotterdam Study-I/Rotterdam Study-II/ Rotterdam Study-III, Atherosclerosis Risk in Communities1, Atherosclerosis Risk in Communities2, Erasmus Rucphen Family study, Invecchiare in Chianti, Health, Aging and Body Composition, Baltimore Longitudinal Study of Aging, Helsinki Birth Cohort Study, Multi-Ethnic Study of Atherosclerosis, Nurses' Health Study (NHS)-breast cancer substudy, NHS-cardiovascular health disease substudy, NHS-kidney stones substudy, NHS-type 2 diabetes substudy, Rush-Memory and Aging Project, Rush-Religious Orders Study, and SardiNIA study. Direction of effect replication: Age, Gene, Environment Susceptibility–Reykjavik Study, Atherosclerosis Risk in Communities3, Monitoring of trends and determinants of cardiovascular disease/cooperative health research in the region of Augsburg (MONICA/KORA) F3, MONICA/KORA F4, and Study of Health In Pomerania. Allele = minor/major on the + strand, the minor allele is the coded allele.

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

[a]Independent SNPs with a p value $< 1 \times 10^{-5}$ in the discovery meta-analysis. The total n for SNP rs8020095 was 40,902, for rs8038316 was 48,103, for rs161645 was 49,820, and for the other SNPs was 51,258. The mean observed versus expected variance ratio (measure of imputation quality) for imputed SNPs ranged between .91 and .99. Table S2 in Supplement 1 includes this information detailed per SNP.

[b]Supporting SNPs: number of SNPs in linkage disequilibrium with the top SNP ($R^2 > .8$), with a p value $< 10^{-4}$.

[c]Lowest p value of the overall meta-analysis $p = 4.78 \times 10^{-8}$ for SNP rs40465 (G/T) that is in linkage disequilibrium ($R^2 = .80$) with rs161645, discovery $p = 2.58 \times 10^{-6}$ (++++++++++++++++−+?), replication $p = 5.00 \times 10^{-3}$ (+++−+).

[d]Heterogeneity p value $< .05$.

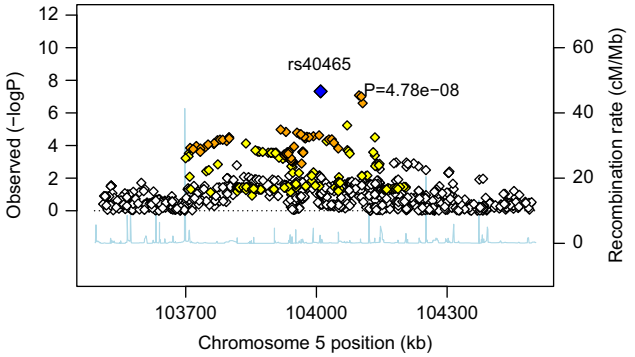

Figure 1. Association results in the 5q21 region. Summary of the association of single nucleotide polymorphisms (SNPs) on chromosome 5 (base 103,500,000 to 104,500,000) with depressive symptoms from the overall meta-analysis ($n = 51,258$). The SNP with the strongest association (rs40465) is highlighted in blue and its corresponding p value is given. Other SNPs are colored according to their degree of linkage disequilibrium (LD) with rs40465, ranging from high LD (orange, R^2 .5–1.0) to low LD (white, $R^2 < .2$). cM, centimorgan; kb, kilobase; Mb, megabase.

did not reach the predefined threshold for multiple testing (corrected for multiple testing $p = .064$). This SNP resides in a gene desert, with the closest gene *NUDT12* more than 1000 kb away.

In the overall meta-analysis including discovery and replication samples ($n = 51,258$), SNP rs40465 reached genome-wide significance ($p = 4.78 \times 10^{-8}$). This SNP is in high linkage disequilibrium with SNP rs161645 ($R^2 = .80$). Rs40465 had a p value of 2.58×10^{-6} in the discovery meta-analysis and a p value of 5.00×10^{-3} in the meta-analysis of replication studies. An association plot of the 5q21 region is presented in Figure 1.

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

Pathway Analysis

One hundred four functional genes of the 170 genes that were annotated were mapped to biological processes. Relevant processes that were overrepresented among top SNPs (p value $< 10^{-4}$) of the overall meta-analysis were neurotransmitter secretion (Bonferroni-corrected p value $= 9.84 \times 10^{-3}$), vitamin transport (Bonferroni-corrected p value $= .014$), and synaptic transmission (Bonferroni-corrected p value $= .037$). A complete list of biological processes that were significantly overrepresented is presented in Table 3.

Candidate Gene Search

None of the 17 tested candidate genes were replicated in the current study (Table S4 in Supplement 1). Nine out of 17 associations had the same direction in our overall meta-analysis as in the published study, and none of the nine was significant (uncorrected for multiple testing).

Discussion

In this GWAS of depressive symptoms, we combined the results of 17 population-based studies with 34,549 individuals to find common variants for depressive symptoms. Including the five replication studies, this effort comprised data from 51,258 independent individuals. Of the seven SNPs we attempted to replicate, we found suggestive evidence for the observed association of one SNP in the 5q21 region with depressive symptoms. This region reached genome-wide significance when tested over all studies ($n = 51,258$).

Although evidence shows that depression can be well represented by a continuum of depressive symptoms, we observed a genome-wide significant hit in this large GWAS only when pooling all studies with depressive symptoms. This difficulty of finding signals is in line with GWAS of major depression. Nine GWAS of depression, of which the largest comprised ~6000 MDD cases and ~7000 control subjects, yielded only one genome-wide significant finding (15).

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

Table 3. Pathway Analysis

Biological Process	NCBI	Observed	Expected	Over/ Under	Adjusted p Value[a]
Neurotransmitter Secretion	346	6	1.81	+	9.84e-03
Vitamin Transport	95	3	.50	+	.014
Protein Metabolic Process	3240	26	16.92	+	.015
Synaptic Transmission	594	7	3.10	+	.037
Transport	2857	22	14.92	+	.038
Vesicle-Mediated Transport	1160	11	6.06	+	.040
Cation Transport	621	7	3.24	+	.045
Cell-Cell Signaling	1331	12	6.95	+	.045
Protein Transport	1646	14	8.60	+	.048
Intracellular Protein Transport	1646	14	8.60	+	.048

Enrichment of biological processes among the top results (overall meta-analysis p value $< 10^{-4}$) was statistically tested with a binomial test.

NCBI: number of genes in a biological process (reference). Observed: number of genes that belong to a biological process among the GWAS results. Expected: expected number of genes that belong to a biological process in the GWAS results. Over/under: overrepresentation or underrepresentation of the genes in the results.

GWAS, genome-wide association studies; NCBI, National Center for Biotechnology Information.

[a]A Bonferroni-correction was applied to correct for multiple testing.

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

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

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

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

We combined results from studies that measured depressive symptoms with instruments other than the CES-D to replicate the association between depressive symptoms and seven independent top SNPs. In an overall meta-analysis, we tested whether any variation introduced by different instruments was offset by the increased power. In the replication effort, one SNP (5q21 region) reached a *p* value below .05 but did not pass this threshold when controlling for multiple testing. Another SNP in the 5q21 region, however, reached genome-wide significance when the association across discovery and replication studies was tested (n = 51,258). The 5q21 region resides in a gene desert with the closest gene, *NUDT12*, lying more than 1000 kb away. NUDT12 has not been previously implicated in psychiatric disorders.

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

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

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

Third, lack of replication might be related to heterogeneity of the replication phenotype. In the replication approach, we combined the results of studies that measured depressive symptoms with different instruments. Instruments were also administered at different time points across studies. However, the instruments have been reported to be highly correlated (correlations between .77 and .86) and relatively stable genetic determinants over the life span were observed in an Australian Twin study (53,54,65,67,68).

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

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

depressive symptoms to provide more power to our search for potential candidate genes.

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