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Comprehensive Gene- and Pathway-Based Analysis of Depressive Symptoms in Older Adults

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Abstract. Depressive symptoms are common in older adults and are particularly prevalent in those with or at elevated risk for dementia. Although the heritability of depression is estimated to be substantial, single nucleotide polymorphism-based genome-wide association studies of depressive symptoms have had limited success. In this study, we PERFORMED genome-wide gene- and pathway-based analyses of depressive symptom burden. Study participants included non-Hispanic Caucasian subjects ($n = 6{,}884$) from three independent cohorts, the Alzheimer's Disease Neuroimaging Initiative (ADNI), the Health and Retirement Study (HRS), and the Indiana Memory and Aging Study (IMAS). Gene-based meta-analysis identified genome-wide significant associations (*ANGPT4* and *FAM110A*, q-value = 0.026; *GRM7-AS3* and *LRFN5*, q-value = 0.042). Pathway analysis revealed enrichment of association in 105 pathways, including multiple pathways related to ERK/MAPK signaling,GSK3

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signaling in bipolar disorder, cell development, and immune activation and inflammation. *GRM7*, *ANGPT4*, and *LRFN5* have been previously implicated in psychiatric disorders, including the *GRM7* region displaying association with major depressive disorder. The ERK/MAPK signaling pathway is a known target of antidepressant drugs and has important roles in neuronal plasticity, and GSK3 signaling has been previously implicated in Alzheimer's disease and as a promising therapeutic target for depression. Our results warrant further investigation in independent and larger cohorts and add to the growing understanding of the genetics and pathobiology of depressive symptoms in aging and neurodegenerative disorders. In particular, the genes and pathways demonstrating association with depressive symptoms may be potential therapeutic targets for these symptoms in older adults.

Keywords: ANGPT4, depressive symptoms, genome-wide association study, GRM7, GSK3, MAPK-ERK

INTRODUCTION

Neuropsychiatric symptoms such as depression are common in older adults, with clinically significant levels in up to 50%, with particular prevalence in those with or at elevated risk for dementia [1–3]. Furthermore, 25% of older adults with minor depression progress to major depression within two years, highlighting the importance of appropriate early diagnosis and therapy [1]. Chronic neurodegenerative disorders such as schizophrenia and Alzheimer's disease (AD) are well-known risk factors for depression and other neuropsychiatric symptoms [4, 5]. With the heritability of major depressive disorder estimated to be as high as 42% from family and twin studies, a better understanding of the genetic susceptibility for depressive symptoms is important for improved risk assessment and ultimately for the development of preventative and therapeutic strategies [6]. Furthermore, the heritability of depressive symptoms ranges from 15% to 34% [2, 7–9].

Genome-wide association studies (GWAS) testing millions of single nucleotide polymorphisms (SNPs) for association with depressive symptoms have had limited success [9, 10] and linkage and candidate gene studies have only identified a small number of variants [11–15], leaving a high ceiling for exploring the role of genetic variation in the pathogenesis of depressive symptoms [9, 16]. Recently, the largest GWAS study of depressive symptoms to date comprising more than 50,000 subjects identified one suggestive SNP in the 5q21 region, which reached genome-wide significance in meta-analysis with additional replication cohorts [9].

Gene- and pathway-based association analyses are effective complements to SNP-based GWAS, as they have increased power to identify true associations [17]. Both of these alternative approaches can aggregate potentially meaningful information from multiple susceptibility loci to identify new associations which otherwise might be concealed due to stringent correction for multiple testing at the individual SNP level in a GWAS [18].

Here we performed comprehensive gene- and pathway-based association analyses using three independent cohorts to identify new genetic associations to depressive symptoms in older adults.

MATERIALS AND METHODS

Subjects

All individuals used in this report were participants in the ADNI (Alzheimer's Disease Neuroimaging Initiative), the HRS (Health and Retirement Study), or the IMAS (Indiana Memory and Aging Study) cohorts. The ADNI initial phase (ADNI-1) was launched in 2003 to test whether serial magnetic resonance imaging (MRI), position emission tomography (PET), other biological markers, and clinical and neuropsychological assessment could be combined to measure the progression of mild cognitive impairment (MCI) and early AD. The ADNI-1 participants were recruited from 59 sites across the U.S. and Canada and include approximately 200 cognitively normal older individuals (healthy controls), 400 patients diagnosed with MCI, and 200 patients diagnosed with early probable AD aged 55–90 years. ADNI-1 has been extended in subsequent phases (ADNI-GO and ADNI-2) for follow-up of existing participants and additional new enrollments. Inclusion and exclusion criteria, clinical and neuroimaging protocols, and other information about ADNI have been published previously and can be found at http://www.adni-info.org/. Demographic information, raw scan data, *APOE* and whole-genome genotyping data, neuropsychological test scores, and diagnostic information are publicly available from the ADNI data repository (http://adni.loni.usc.edu/).

The HRS, a nationally representative longitudinal study launched in 1992, recruited more than 26,000 Americans over 50 years old, and used biennial

interviews to collect detailed information on the health, social, and economic status of participants. We analyzed cross-sectional data from HRS wave 8 because genomic DNA was obtained during HRS waves 8–9. A complete description of the HRS longitudinal panel survey design and methods is available elsewhere [19, 20].

The IMAS is an ongoing neuroimaging and biomarker study of memory circuitry in AD and MCI at the Indiana University School of Medicine. The sample included individuals with significant cognitive complaints without performance deficits, amnestic MCI, and mild clinical AD, as well as healthy controls. Participant recruitment, selection criteria, and characterization are described in detail elsewhere [21–24].

Written informed consent was obtained at the time of enrollment and/or genetic sample collection and protocols were approved by each participating study and sites' Institutional Review Board.

Genotyping and imputation

Genotyping was performed using the Illumina Human610-Quad BeadChip for the ADNI-1 participants and the Illumina HumanOmni Express BeadChip for participants initially enrolled in ADNI-GO or ADNI-2. For the IMAS, genotyping was performed using the HumanOmni Express BeadChip. For the ADNI and the IMAS, *APOE* genotyping was separately obtained using standard methods to yield the *APOE* ε4 allele defining SNPs (rs429358, rs7412) [25]. For the HRS, genotyping was performed at the Center for Inherited Disease Research using the HumanOmni2.5–4v1 array [26].

As the three cohorts used different genotyping platforms, we imputed un-genotyped SNPs separately in each cohort using MACH and the 1000 Genomes Project data as a reference panel. Before the imputation, we performed standard sample and SNP quality control procedures as described previously [27]: 1) for SNP, SNP call rate <95%, Hardy-Weinberg test $p < 1 \times 10^{-6}$, and minor allele frequency (<1%; 2) for sample, sample gender and identify check, and sample call rate <95%. Furthermore, in order to prevent spurious association due to population stratification, we selected only non-Hispanic Caucasian participants that clustered with HapMap CEU (Utah residents with Northern and Western European ancestry from the CEPH collection) or TSI (Toscani in Italia) populations using multidimensional scale analysis (http://hapmap.ncbi.nlm.nih.gov/) [28]. Imputation and quality control procedures were performed as described previously [21]. After the imputation, we imposed an r^2 value equal to 0.30 as the threshold to accept the imputed genotypes and retained SNPs with minor allele frequency ≥5%. Consequently, 851, 49, and 5,984 individuals and 5,539,846, 5,434,639, and 5,716,356 SNPs passed all quality control tests in the case-control design for ADNI, IMAS, and HRS (wave 8), respectively. Thus, the three cohorts had similar imputation quality and coverage within genes.

Assessment of depressive symptoms

All ADNI and IMAS participants were assessed for depressive symptoms using the short version of the Geriatric Depression Scale (GDS-15). The total score excluding the memory complaint item was used for analysis. To control for potentially confounding effects of cognitive deficits on the GDS total score in these cohorts which included participants at various stages in the AD spectrum, the CDR (Clinical Dementia Rating) Sum-of-Boxes score was included as a covariate in addition to age, gender, and education [5].

For all HRS participants, depressive symptoms were assessed using the Center for Epidemiologic Studies-Depression Scale (CES-D), consisting of eight yes/no items. To control for potentially confounding effects on the CES-D total score, we removed HRS participants with a reported diagnosis of a psychiatric condition or memory disorder. We used age, gender, and education as covariates [20].

For the definition of the phenotype for genetic analysis, we followed the approach of Arnold et al. [5]. In brief, participants were divided into those with depressive symptoms (GDS or CES-D ≥2; cases) versus those without depressive symptoms (GDS or CES-D = 0; controls), with GDS/CES-D = 1 serving as a buffer [5].

Statistical analysis

For a single SNP-based association analysis, we used PLINK with a logistic regression model and default parameters. For a gene-based association analysis, we defined genes by their official hg19 boundaries plus the 50 kb outside of the 5' and 3' UTRs in order to capture associations within regulatory regions and we used HYST, which calculated a summary *p*-value for each gene accounting for its size, linkage disequilibrium structure, and constituent GWAS SNP *p*-values, with default parameters as described previously [29]. In the gene-based analysis, 24,023 genes were tested for three cohorts. Meta-analysis

Table 1
Demographic data of participants included in the analysis

	ADNI ($n = 851$)		HRS ($n = 5,984$)		IMAS ($n = 49$)	
	Control	Case	Control	Case	Control	Case
Participants	241	610	4,126	1,858	18	31
Age, mean (SD)	74.9 (5.4)	73.3 (7.7)	68.0 (9.9)	70.1 (11.2)	70.3 (6.5)	72.4 (8.4)
Gender, M/F	129/112	368/242	1,988/2,138	705/1,153	7/11	13/18
Education, mean (SD)	16.4 (2.6)	15.7 (2.9)	13.6 (2.4)	12.6 (2.5)	17.4 (1.8)	16.5 (2.7)

ADNI, Alzheimer's Disease Neuroimaging Initiative; HRS, Health and Retirement Study; IMAS, Indiana Memory and Aging Study; Control, without depressive symptoms; Case, with depressive symptoms.

of the gene-based GWAS from each cohort was then performed using the weighted z statistic test (Stouffer's weighted z statistic) as implemented in R, with weight accounting for the sample size of each cohort. The effective sample sizes were estimated using the method [30]. Using the *p*-values for each gene obtained by meta-analysis, Metacore (Thomson Reuters; http://thomsonreuters.com/metacore/) was employed to identify pathways exhibiting enrichment of gene-based association (defined as gene-based $p < 0.05$) to depressive symptoms. Pathways were annotated based on manual curation by expert Metacore reviewers. Pathway enrichment *p*-values were calculated using overrepresentation analysis based on the Fisher's exact test statistic [31]. Metacore pathways provide high quality interactive diagrams to illustrate broader biological networks. There are many extant approaches for statistical pathway analysis but overrepresentation (as in Metacore) is one standard strategy [31]. The false discovery rate was used to correct for both gene-level and pathway-level multiple comparisons [31, 32].

RESULTS

In the analysis, we used participants from ADNI-1 and ADNI-GO/2. Initially, there were 1,250, 69, and 12,507 participants for ADNI, IMAS, and HRS (wave 8), respectively. After standard sample and SNP quality control and population stratification procedures and additional quality control steps such as removal of siblings, we retained 851, 49, and 5,984 participants from ADNI, IMAS, and HRS, respectively. A total of 6,884 non-Hispanic Caucasian participants had genotype, phenotype, and covariate data available for analysis. Sample characteristics are presented in Table 1. For ADNI, IMAS, and HRS, respectively, 72%, 63%, and 31% of participants were positive for depressive symptoms as defined in the Methods. More participants with depressive symptoms were found in ADNI and IMAS, which were observational but clinical trial-like samples including participants with MCI and clinical AD, as compared to HRS, which was a population-based sample of older Americans.

From the gene-based GWAS (Fig. 1 for the SNP-based and gene-based Q-Q plots), the ten most significant genes are summarized in Table 2. Four genes (glutamate receptor, metabotropic 7-antisense RNA 3 (*GRM7-AS3*), angiopoietin 4 (*ANGPT4*), family with sequence similarity 110, member A (*FAM110A*), and leucine rich repeat and fibronectin type III domain containing 5 gene (*LRFN5*)) achieved genome-wide significant association with presence of depressive symptoms (q-value <0.05).

Pathway analysis based on meta-analytic *p*-values revealed enrichment in 105 pathways within q-value <0.05. The top 20 pathways based on false discovery rate correction are presented in Table 3 and include multiple pathways related to Extracellular Signal-regulated Kinase/Mitogen-Activated Protein Kinase (ERK/MAPK) signaling, glycogen synthase kinase 3 (GSK3) signaling, cell development, and immune activation and inflammation, among others.

DISCUSSION

Using complementary genome-wide gene- and pathway-based analysis in three independent cohorts, we identified four genome-wide gene-based associations and 105 pathway-based associations to the presence of depressive symptoms in older adults.

GRM7-AS3 (glutamate receptor, metabotropic 7-antisense RNA 3) is a RNA gene which is complementary to a functional RNA. *GRM7* is one of the Group III glutamate metabotropic receptors. Chang et al. recently identified GRM7 as among the important proteins involved in neuronal signaling and cellular structure in major depressive disorder [33]. Knockout mouse studies of *mGluR7*, the analog of the human *GRM7* gene, have revealed the importance of

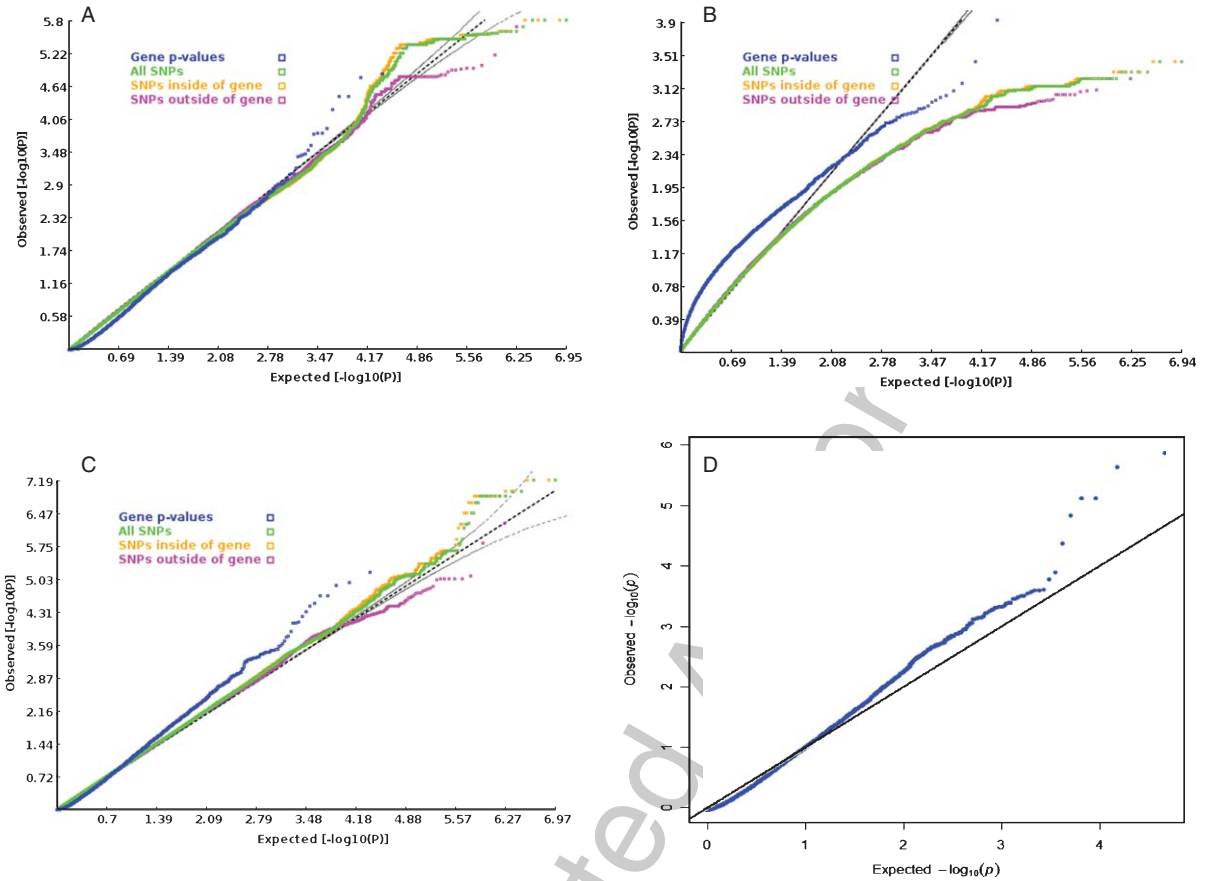

Fig. 1. Quantile-Quantile plots of SNP-based and gene-based *p*-values calculated by PLINK and HYST in three cohorts and meta-analysis. A) Alzheimer's Disease Neuroimaging Initiative ($n = 851$); B) Indiana Memory and Aging Study ($n = 49$); C) Health and Retirement Study ($n = 5{,}984$); and D) gene-based meta-analysis.

Table 2
Meta-analysis *p*-values of top 10 genes for depressive symptoms in older adults from gene-based GWAS analysis

Gene	Start Position	Length	SNPs in ADNI	HRS *p*-value	ADNI *p*-value	IMAS *p*-value	Meta-analysis *p*-value	*q*-value
ANGPT4	853296	43665	496	0.000013	0.0432	0.0396	1.24E-06	0.026
FAM110A	814339	12584	433	0.000169	0.0024	0.0937	2.15E-06	0.026
GRM7-AS3	6674044	173093	859	0.000007	0.2280	0.0960	6.97E-06	0.042
LRFN5	42076763	296990	671	0.000022	0.0563	0.4700	7.04E-06	0.042
SCN10A	38738836	96666	447	0.000394	0.0118	0.0393	1.35E-05	0.064
FAM214A	52873517	70731	248	0.000083	0.2450	0.0061	3.97E-05	0.159
HPYR1	133572744	983	178	0.002500	0.0098	0.1100	1.17E-04	0.380
ARPP19	52839431	21783	227	0.000087	0.4440	0.0339	1.54E-04	0.380
GTF2E2	39436939	79709	246	0.005220	0.0022	0.6140	2.26E-04	0.380
SHISA8	42305557	5115	222	0.003930	0.0169	0.0511	2.32E-04	0.380

this encoded protein in neurotransmitter release [34] and neuronal plasticity in the hippocampus [34–36]. Absence of *mGluR7* in mice leads to the reduction of anxiety and changes in handling behaviors, thought due to its putative roles in anxiety and depression pathogenic pathways [37, 38]. *GRM7* may also modulate synaptic activity when glutamate rises to high levels in the synapse [39]. Epidemiologic studies have identified associations between variation in *GRM7* and depression, anxiety, schizophrenia, bipolar disorder, and epilepsy [11, 40–42]. Our new finding taken in the context of other recent studies highlights the potential role of *GRM7* in risk for depressive symptoms and also as a potential therapeutic target [43, 44].

Table 3
List of top canonical pathways for depressive symptoms in older adults

Pathway maps	Set size[a]	Uncorrected *p*-value	*q*-value
Apoptosis and survival HTR1A signaling	11 (50)	2.62E-06	1.75E-03
Neurophysiological process/Constitute and regulated NMDA receptor trafficking	12 (63)	4.61E-06	1.75E-03
Regulation of CFTR activity (normal and CF)	11 (62)	2.33E-05	3.69E-03
Cytoskeleton remodeling/TGF, WNT and cytoskeletal remodeling	15 (111)	2.49E-05	3.69E-03
ENaC regulation in normal and CF airways	10 (53)	3.15E-05	3.69E-03
G-protein signaling/K-RAS regulation pathway	7 (25)	3.41E-05	3.69E-03
Signal transduction/Erk Interactions:Inhibition of Erk	8 (34)	3.71E-05	3.69E-03
PGE2 pathways in cancer	10 (55)	4.41E-05	3.69E-03
Role of Tissue factor in cancer independent of coagulation protease signaling	8 (35)	4.65E-05	3.69E-03
Development/Ligand-independent activation of ESR1 and ESR2	9 (45)	4.85E-05	3.69E-03
G-protein signaling/H-RAS regulation pathway	8 (37)	7.13E-05	4.51E-03
Development/Beta-adrenergic receptors transactivation of EGFR	8 (37)	7.13E-05	4.51E-03
Development/EGFR signaling pathway	11 (71)	8.59E-05	4.97E-03
Development/Thromboxane A2 signaling pathway	9 (49)	9.80E-05	4.97E-03
Transcription/CREB pathway	9 (49)	9.80E-05	4.97E-03
Cell adhesion/PLAU signaling	8 (39)	1.06E-04	5.04E-03
Immune response/Histamine signaling in dendritic cells	9 (50)	1.16E-04	5.13E-03
G-protein signaling/Rap1A regulation pathway	8 (40)	1.28E-04	5.13E-03
Reproduction/Progesterone-mediated oocyte maturation	8 (40)	1.28E-04	5.13E-03
Main growth factor signaling cascades in multiple myeloma cells	8 (41)	1.54E-04	5.85E-03

[a]Number of genes from study data (number of genes in the pathway).

ANGPT4 (angiopoietin 4) encodes a protein involved in angiogenesis and has been associated with cases of mixed AD/vascular dementia in family-based studies [45]. Meta-analysis results summarizing prior studies has indicated that past diagnosis of depression confers heightened risk for AD later in life [46]. Multiple mechanisms have been suggested including immune related changes. For example, depressive symptoms might induce disregulation of the cytokine network linked to vascular disease and increasing emotional and cognitive disturbances [47, 48].

LRFN5 (leucine rich repeat and fibronectin type III domain containing 5) encodes a cell adhesion molecule that is highly expressed in the dentate gyrus among other brain regions (OMIM 612811) and has a role in synapse formation and maintenance [49]. Successful anti-depressant treatment of an experimental model of depression showed that sustained usage of the drug had effects on the stability of synaptic changes [50].

Pathway analysis also identified additional associations with depressive symptoms. A recent genetic study proposed the possibility of a link between variants in genes for apoptotic proteins and major depression, suggesting individuals with these variants may have accelerated cell death in susceptible brain regions [51]. The NMDA glutamatergic receptor is the major ion channel that participates in neuronal development and synaptic plasticity [52]. The NMDA receptor is thought to play an important role in the neurobiology and treatment of major depression [53]. Cytoskeletal proteins undergo post-translational modifications to define their structure and function. In depression, disrupted post-translational modifications may result in altered cytoskeletal functions [54]. The ERK/MAPK signaling pathway plays a role in cellular plasticity and cellular process such as proliferation, differentiation, survival, and apoptosis [55, 56]. Activation of MAP kinases and expression of ERK1/2 significantly change in major depression [55, 57], indicating that this signaling pathway may be vital for preserving structural plasticity and synaptic remodeling to prevent the onset of depressive symptoms. Meanwhile, glycogen synthase kinase 3 (*GSK3*) regulates cytokine and interleukin production to modulate inflammatory processes important in depression pathogenesis [58, 59]. Adjunct *GSK3* inhibitors such as lithium and recently ketamine have been used as mood stabilizing antidepressants [60, 61]. We also observed enrichment of association with depressive symptoms within pathways related to intracellular signaling, cell development, immune activation and inflammation, and lipid metabolism.

A limitation of the present report is that we performed association analyses of depressive symptoms on a dichotomous variable instead of a continuous

phenotypic scale. Another limitation includes the absence of sufficient data for analysis of potential confounding factors such as history of depression, the use of antidepressant and sleep medications, and behavioral therapy. It is noteworthy in this context that HRS was population-based by design whereas ADNI and IMAS were designed to recruit older adults who are typical of participants at various clinical stages along the continuum from normal aging to AD.

In conclusion, our results using gene- and pathway-based analyses with increased statistical power for discovery identified novel associations with depressive symptoms that warrant further investigation in independent and larger cohorts. At a broader level, this study adds to the growing understanding of the genetics and pathobiology of depressive symptoms in aging and neurodegenerative disorders and nominates novel potential targets for diagnostic and therapeutic approaches to combat depressive symptoms in older adults.

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