



Genome-Wide Association Study of Major Depressive Disorder: New Results, Meta-Analysis, and Lessons Learned

Citation

Wray, N.R., M.L. Pergadia, D.H.R. Blackwood, B.W.J.H. Penninx, S.D. Gordon, D.R. Nyholt, S. Ripke, et al. 2012. Genome-wide association study of major depressive disorder: New results, meta-analysis, and lessons learned. *Molecular Psychiatry* 17(1): 36-48.

Published Version

doi:10.1038/mp.2010.109

Permanent link

<http://nrs.harvard.edu/urn-3:HUL.InstRepos:10304391>

Terms of Use

This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA>

Share Your Story

The Harvard community has made this article openly available.
Please share how this access benefits you. [Submit a story](#).

[Accessibility](#)

ORIGINAL ARTICLE

Genome-wide association study of major depressive disorder: new results, meta-analysis, and lessons learned

NR Wray¹, ML Pergadia², DHR Blackwood³, BWJH Penninx⁴, SD Gordon¹, DR Nyholt¹, S Ripke^{5,6}, DJ MacIntyre³, KA McGhee³, AW Maclean³, JH Smit⁴, JJ Hottenga⁴, G Willemsen⁴, CM Middeldorp⁴, EJC de Geus⁴, CM Lewis⁷, P McGuffin⁷, IB Hickie⁸, EJCG van den Oord⁹, JZ Liu¹, S Macgregor¹, BP McEvoy¹, EM Byrne¹, SE Medland¹, DJ Statham^{1,11}, AK Henders¹, AC Heath², GW Montgomery¹, NG Martin¹, DI Boomsma⁴, PAF Madden² and PF Sullivan¹⁰

¹Genetic Epidemiology, Molecular Epidemiology, Psychiatric Genetics and Queensland Statistical Genetics Laboratories, Queensland Institute of Medical Research, Brisbane, QLD, Australia; ²Department of Psychiatry, Washington University School of Medicine, St Louis, MO, USA; ³Division of Psychiatry, University of Edinburgh, Royal Edinburgh Hospital, Edinburgh, UK; ⁴Department of Biological Psychology and Medical Center, VU University, Amsterdam, The Netherlands; ⁵Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA; ⁶Broad Institute of Harvard and MIT, Cambridge, MA, USA; ⁷Department of Medical and Molecular Genetics, King's College London, MRC SGDP Centre, Institute of Psychiatry, London, UK; ⁸Clinical Research Unit, Brain and Mind Research Institute, University of Sydney, NSW, Australia; ⁹Center for Biomarker Research and Personalized Medicine, Virginia Commonwealth University, Richmond, VA, USA and ¹⁰Department of Genetics, University of North Carolina, Chapel Hill, NC, USA

Major depressive disorder (MDD) is a common complex disorder with a partly genetic etiology. We conducted a genome-wide association study of the MDD2000+ sample (2431 cases, 3673 screened controls and >1M imputed single-nucleotide polymorphisms (SNPs)). No SNPs achieved genome-wide significance either in the MDD2000+ study, or in meta-analysis with two other studies totaling 5763 cases and 6901 controls. These results imply that common variants of intermediate or large effect do not have main effects in the genetic architecture of MDD. Suggestive but notable results were (a) gene-based tests suggesting roles for adenylate cyclase 3 (*ADCY3*, 2p23.3) and galanin (*GAL*, 11q13.3); published functional evidence relates both of these to MDD and serotonergic signaling; (b) support for the bipolar disorder risk variant SNP rs1006737 in *CACNA1C* ($P=0.020$, odds ratio=1.10); and (c) lack of support for rs2251219, a SNP identified in a meta-analysis of affective disorder studies ($P=0.51$). We estimate that sample sizes 1.8- to 2.4-fold greater are needed for association studies of MDD compared with those for schizophrenia to detect variants that explain the same proportion of total variance in liability. Larger study cohorts characterized for genetic and environmental risk factors accumulated prospectively are likely to be needed to dissect more fully the etiology of MDD.

Molecular Psychiatry (2012) 17, 36–48; doi:10.1038/mp.2010.109; published online 2 November 2010

Keywords: major depressive disorder; depression; genome-wide association study; *CACNA1C*; *ADCY3*; *GAL*

Introduction

Major depressive disorder (MDD) is a common and debilitating disorder with pervasive impact on the quality of life for both sufferers and their families.

Lifetime prevalence is estimated to be ~15%,^{1,2} and is consistently estimated to be twice as common in women as men.^{3,4} MDD is associated with high morbidity, reflected in estimates of burden of disease and years lost in productivity,^{1,5} and excess mortality from suicide⁶ and other causes. Our understanding of the etiology of MDD remains fragmented, despite wide-ranging research, but is the key to effective prevention and treatment.

MDD is familial, with heritability estimated to be 0.37 (95% confidence interval (CI) 0.31–0.42) and both early age of onset and recurrence of depression are associated with higher familial aggregation.^{7,8} This implied genetic etiology has motivated studies designed to identify specific genetic variants associated with MDD. Results from genome-wide linkage,

Correspondence: Dr NR Wray, Psychiatric Genetics Laboratory, Queensland Institute of Medical Research, Herston Road, Brisbane, 4029, QLD, Australia.

E-mail: naomi.wray@qimr.edu.au or

Professor PF Sullivan, Department of Genetics, University of North Carolina, Chapel Hill, NC, USA.

E-mail: pfsulliv@med.unc.edu

¹¹Current address: Faculty of Arts and Social Sciences, University of the Sunshine Coast, Maroochydore, QLD, Australia.

Received 16 May 2010; revised 12 September 2010; accepted 27 September 2010; published online 2 November 2010

reviewed in Boomsma *et al.*,⁹ and candidate gene association studies¹⁰ have shown little consistency and hopes for new progress have spurred on a generation of genome-wide association studies (GWAS). Five GWAS for MDD have been published to date,^{11–15} each using control samples screened negative for MDD. None of these studies has identified variants that achieve genome-wide significance. Taken together, the results of these studies imply that specific genetic variants individually make very small contributions to the etiology of MDD. In this study, we present the largest GWAS for MDD to date, the MDD2000+ study comprising 2431 cases and 3673 screened controls. We compare our results with reports of the other published MDD GWAS and present a formal meta-analysis of our results with the two other largest studies (5763 cases and 6901 controls).

Materials and methods

Overview

The MDD2000+ project comprises a total of 2431 cases with MDD and 3673 screened controls from different sources and genotyped on different platforms (Tables 1 and 2). Samples were provided by the Queensland Institute of Medical Research (QIMR, Australia), The Netherlands Study of Anxiety and Depression (NESDA), The Netherlands Twin Registry (NTR), the University of Edinburgh (UK), and the Molecular Genetics of Schizophrenia study (controls only, US). Genotyping was conducted on different Illumina and Affymetrix platforms and because the overlap in genotyped single-nucleotide polymorphisms (SNPs) is limited, association analysis is based on a set of >1 M imputed SNPs. The number of SNPs for each analysis set (Table 1), represents genotyped SNPs surviving all quality control (QC) criteria that were used for imputation.

Subject recruitment

QIMR. Study participants were adult twins and their families recruited through the Australian Twin Registry (<http://www.twins.org.au>). Only unrelated individuals were included in MDD2000+. All participants provided written informed consent under study protocols approved by the QIMR Human Research Ethics Committee. MDD cases were identified through psychiatric questionnaires, either the shortened Composite International Diagnostic Interview¹⁶ or the SSAGA-OZ interview instrument (a version of the Semi-Structured Assessment for the Genetics of Alcoholism¹⁷ modified for use in Australia), a comprehensive psychiatric interview designed to assess MDD and other psychiatric disorders¹⁷ according to DSM-III-R¹⁸ and DSM-IV¹⁹ criteria. Structured interviews were administered by trained telephone interviewers, closely supervised by a clinical psychologist. Briefly, from 1988 to 1990, study participants were mailed an extensive health and

lifestyle questionnaire, which included the shortened revised Eysenck personality questionnaire.²⁰ Sum scores of 12 item responses in each personality domain resulted in quantitative scores for neuroticism. Between 1992–2000, an unselected subset of these participants were interviewed by telephone using the SSAGA-OZ.²¹ Over the period 1996–9 sibling pairs that were either concordant or discordant for extreme neuroticism scores (one sibling in the top or bottom decile, the other sibling in the top or bottom quintile) were recruited to complete the Composite International Diagnostic Interview, which provides DSM-IV¹⁹ lifetime diagnoses of MDD.²² Finally, some study participants completed the SSAGA-OZ telephone questionnaire in 2003–2007 as part of alcohol and nicotine dependence studies (the nicotine addiction genetics and Inter-related Project Grant studies described in Table 2 of Hansell *et al.*²³). The Inter-related Project Grant/nicotine addiction genetics studies captured 28% of families who had already participated in earlier studies ascertaining those with either (a) large sibship size or containing a proband with either (b) nicotine dependence or (c) alcohol dependence. For this study, all cases met DSM-IV lifetime criteria for MDD. Screening items for mania were not consistent across interviews and screening items for psychosis were not included; the ability to assess accurately these less common criteria is difficult in large-scale community settings. Therefore, it is possible that a small number of individuals with a primary diagnosis of bipolar disorder or schizophrenia are included in the case group. If multiple cases were present within families then one case was selected in the following order of preference: age of onset <31 years, multiple episodes of depression, co-morbid anxiety disorders and high neuroticism score. Unrelated controls were selected as genotyped individuals from families in which no individuals qualified for diagnoses of MDD or anxiety disorders. If multiple controls were available from a family, the individual with the lowest neuroticism score was preferentially selected, otherwise an individual was selected at random.

NESDA and NTR. Additional MDD cases were selected from two parallel studies, NESDA (<http://www.nesda.nl>) and NTR (<http://www.tweelingenregister.org>); NTR also provided screened controls. These samples do not overlap with those included in a prior MDD GWAS¹² but are drawn from the same parent studies (although a small number of related individuals are included, see meta-analysis section below). Details of the data collection methods are described elsewhere.⁹ Similar inclusion and exclusion criteria were used to select MDD cases from both the NESDA and NTR studies. Inclusion criteria were a lifetime diagnosis of DSM-IV MDD as determined by the Composite International Diagnostic Interview,¹⁶ age 18–65 years, and self-reported western European ancestry. Those not fluent in Dutch or with a primary diagnosis of schizophrenia

Table 1 Analysis set statistics: numbers of samples, numbers of SNPs, ages at interview and MDD onset

Analysis set	Platform and approximate number of SNPs genotyped	Post-quality control autosomal SNPs	Post-quality control X chromosome SNPs	Sample source	N	No. of males	No. of females	No of recurrent and early onset ^a	Age at interview mean \pm s.d.	Age of onset mean \pm s.d.
I317	Illumina 317k	289 130	8722	QIMR cases ^b QIMR controls ^b	84 237	0 0	84 237	31 —	47.5 \pm 12.0 45.4 \pm 13.2	34.5 \pm 12.5 —
I370	Illumina 370k Illumina 610k	276 135	6846	QIMR case ^c QIMR controls ^c NTR controls ^f	737 795 577	325 443 269	412 352 308	264 — —	42.9 \pm 9.1 41.0 \pm 12.6 (N=794) 49.7 \pm 13.4	27.8 \pm 10.4 (N=735) — —
I610	Illumina 610k	510 092	12 822	QIMR cases ^d QIMR controls ^d	169 428	38 132	131 296	45 —	42.8 \pm 10.5 41.9 \pm 12.4	31.2 \pm 11.5 —
A6.0	Affymetrix 600k	560 631	14 447	QIMR cases ^e NESDA/NTR cases ^e UoE cases ^e MGS controls	941 127 373 1636	298 45 151 918	643 82 222 718	480 36 289 —	42.2 \pm 9.9 41.3 \pm 11.7 (N=126) 31.8 \pm 14.5 (N=352) 52.5 \pm 17.2	25.4 \pm 9.8 (N=940) 28.3 \pm 10.5 (N=92) 23.3 \pm 10.9 (N=352) —
ALL	Imputed	1 251 157	37 832 ^f	Cases Controls	2431 3673	857 1762	1574 1911	1145 —	41.1 \pm 11.4 (N=2409) 48.0 \pm 15.8 (N=3613)	26.7 \pm 10.8 (N=2372) —

Abbreviations: MAF, minor allele frequency; MDD, major depressive disorder; MGS, Molecular Genetics of Schizophrenia; NESDA, The Netherlands Study of Anxiety and Depression; NTR, The Netherlands Twin Registry; QIMR, Queensland Institute of Medical Research; SNP, single-nucleotide polymorphism; UoE, University of Edinburgh.

^aEarly onset < 31 years.

^{b,c,d,e}Samples with the same superscript had cases and controls genotyped together sometimes in multiple batches.

^fX chromosome imputed SNPs with MAF > 0.01.

The NTR controls were genotyped on the Illumina Human610-Quad but were included in the I370 analysis set to balance proportions of cases and controls in each set.

Table 2 Descriptive statistics by analysis set and sample source

Analysis set	Sample source	Female (%)	Early onset < 31 years (%)	Recurrent (%)	Recurrent and early onset (%)	Family history MDD (%)	Recurrent and/or early onset family history (%) ^a	Education level (% low, middle, high)	Partner status (% with partner)	Regular smoker (%)	Neuroticism score Mean \pm s.d. ^b
I317	QIMR cases (N=84)	100	46	48	37	25	58	40,52,9 (N=81)	73 (N=84)	20 (N=82)	0.46 \pm 1.02 (N=79)
	QIMR controls (N=237)	100				0	—	38,50,12 (N=228)	81 (N=237)	16 (N=230)	-0.58 \pm 0.80 (N=224)
I370	QIMR cases (N=737)	56	65	43	36	40	76	27,39,34 (N=724)	70 (N=737)	69 (N=729)	0.39 \pm 1.12 (N=371)
	QIMR controls (N=795)	44				0	—	28,38,34 (N=744)	81 (N=779)	59 (N=747)	-0.54 \pm 0.91 (N=524)
	NTR controls (N=577)	53				0	—	7,56,37 (N=573)	92 (N=565)	17 (N=573)	-0.43 \pm 0.76 (N=560)
I610	QIMR cases (N=169)	78	55	37	27	22	69	35,40,25 (N=161)	66 (N=169)	47 (N=163)	0.40 \pm 1.01 (N=140)
	QIMR Controls (N=428)	69				0	—	25,51,24 (N=410)	86 (N=423)	38 (N=405)	-0.29 \pm 1.00 (N=354)
A6.0	QIMR cases (N=941)	68	77	66	51	35	95	24,39,37 (N=924)	67 (N=940)	51 (N=923)	0.47 \pm 1.17 (N=708)
	NESDA/NTR Cases (N=127)	65	71	38	28	85 (N=106)	91	27,37,35 (N=124)	67 (N=106)	43 (N=126)	0.43 \pm 0.86 (N=122)
	UoE cases (N=373)	60	78	100	78	46 (N=362)	100	NA	NA	NA	NA
	MGS controls (N=1636)	44				NA	—	NA	NA	NA	NA
ALL	Cases (N=2431)	65	71	60	47	39	86				
	Controls (N=3673)	52					—				

Abbreviations: MDD, major depressive disorder; MGS, Molecular Genetics of Schizophrenia; NA, not available; NESDA, The Netherlands Study of Anxiety and Depression; NTR, The Netherlands Twin Registry; QIMR, Queensland Institute of Medical Research; UoE, University of Edinburgh.

^aFamily history for QIMR and NTR cases and controls is based on direct psychiatric interview assessment of family members; and was a screening criterion for controls. For NESDA and UoE cases family history is self-report.

^bFor QIMR cases and controls standardized residuals from regression of arcsin-transformed scores on age and sex in a population sample of > 18 000.⁶⁰ For NESDA/NTR cases, standardized scores across based on these cases and GAIN-MDD cases and controls. For NTR controls z-transformed scores based on a population sample of > 19 000.

or schizoaffective disorder, obsessive–compulsive disorder, bipolar disorder or severe substance use or dependence were excluded. All subjects provided written informed consent after study approval by the appropriate Institutional Review Boards.

University of Edinburgh. MDD cases were recruited through in- and out-patient services of psychiatric hospitals in Scotland and were tertiary referrals from primary care. All patients were interviewed by an experienced psychiatrist using the Schedule for Affective Disorders and Schizophrenia-Lifetime,²⁴ supplemented by hospital case note review and information from informants. Final determination of MDD as the primary DSM-IV diagnosis was made by consensus of two psychiatrists. All cases had a lifetime history of recurrent MDD and IQ >70. The study was approved by the Central Office of Research Ethics Committees in Scotland and all subjects gave informed written consent for the collection of DNA samples for use in genetic studies.

Molecular Genetics of Schizophrenia controls. Briefly, random digit dialing was used to achieve a representative sample of individuals from the United States. Participants completed an online questionnaire including the short form Composite International Diagnostic Interview, supplemented by questions about schizophrenia, psychosis and bipolar disorder. Controls were required to never have met criteria for MDD. All subjects provided informed consent. These controls have been used in GWAS for multiple psychiatric disorders including MDD.^{13,14}

Genotyping and QC

Full details are given in the Supplementary File 1. All analysis sets were put through a common QC pipeline based on that used by the Psychiatric GWAS Consortium.²⁵ SNPs were removed based on the following criteria: minor allele frequency (MAF) <0.01, Hardy–Weinberg equilibrium test $P < 1 \times 10^{-6}$ in controls, missingness >0.01 or 0.02, missingness difference between cases and controls of 0.01 or 0.02, difference in frequency with HapMap of 0.07 or 0.15 (more stringent thresholds were applied when cases and controls were not genotyped concurrently, and were chosen to balance SNPs lost versus genomic control inflation factors). Samples were removed with missingness >0.02, if related to other samples or if identified as European ancestry outliers. Imputation was conducted in four analysis sets (I317, I370, I610, A6.0) in batches of ~300 mixed cases and controls to a common set of SNPs present in HapMap3 CEU/TSI, consisting of 410 haplotypes, using Beagle 3.04.^{26,27}

Statistical analyses

Association analysis was conducted on 2431 MDD cases and 3673 screened controls using allelic dosages for imputed SNPs passing QC. The test of association was logistic regression, including the first three ancestry principal components (PC) and

analysis set as covariates. The INFO score (ratio of observed to expected dosage variance), a measure of the quality of imputation of SNPs was used to interpret results. A second association analysis was conducted restricting cases to those with recurrent early onset MDD (REO, age of MDD onset <31 years with multiple episodes of MDD). Separate analyses were conducted by sex, as different prevalences of MDD between males and females could imply existence of sex-specific genetic risk variants.

Statistical power

Detailed power calculations are provided in the Supplementary File 1. The MDD2000+ sample affords >90% power to detect an associated variant with MAF 0.36 and genotype relative risk (GRR) 1.33 (the median values across all published complex disease/trait GWAS, with $P < 5 \times 10^{-8}$).²⁸

Comparison of association results with other studies including meta-analysis

We compared our results with those published for other GWAS of MDD. In particular, we conducted a formal genome-wide meta-analysis for autosomal SNPs from MDD2000+, GAIN–MDD¹² and UK¹⁵ studies (that is, the three largest MDD GWAS). The GAIN–MDD sample, after removing individuals related to Dutch cases or controls of MDD2000+, comprised 1696 MDD cases and 1634 screened controls and the UK sample comprised 1636 MDD cases and 1594 screened controls. Meta-analysis²⁹ on the logistic regression results from each study, restricting the imputed SNPs to those with INFO scores between 0.8 and 1.1.

Gene-based tests

To determine whether any genes harbored an excess of SNPs with small P -values, we undertook genome-wide gene-based tests which account for both gene length and linkage disequilibrium between SNPs using VEGAS.³⁰ SNPs were allocated to one or more autosomal genes using gene boundaries ± 50 kb. We investigated 183 candidate genes for MDD.^{12–15,31}

Results

Of 1,251,157 imputed SNPs, 1,079,979 (86%) had MAF >0.01 and INFO >0.8. The quantile–quantile plots of the observed versus expected $-\log(P)$ from the four (total, male, female and REO) association analyses are presented in Figure 1 and Supplementary File 1. The genomic control λ (the median χ^2 association statistic divided by the median expected under the null) shows no evidence for inflation of the test statistics. For example, for the full analysis, $\lambda = 1.05$ (and standardized to a sample size of 1000, $\lambda_{1000} = 1.02$). There were no genome-wide significant results with $P < 5 \times 10^{-8}$. The Manhattan plot of association P -values for all subjects is presented in the Supplementary File 1 and Table 3 shows associations with $< 1 \times 10^{-5}$ in any analysis.

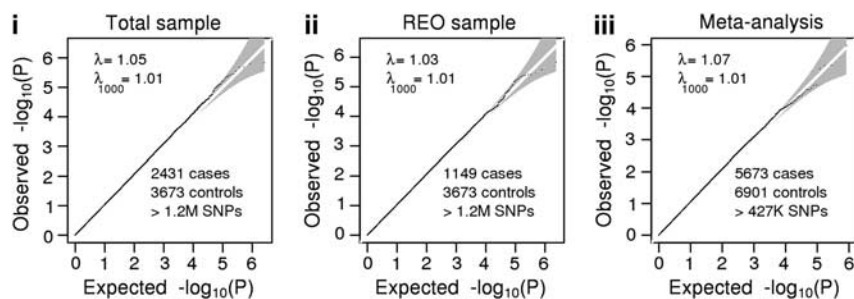


Figure 1 Quantile–quantile plots for the association analyses of (i) all cases and controls and (ii) recurrent early onset (iii) meta-analysis.

Regional association and forest plots are provided in Supplementary File 2. SNPs associated with $P < 0.001$ in any analysis are provided in Supplementary File 3.

Comparison of association results with other studies including meta-analysis

We compared the MDD2000+ results with other published studies. Muglia *et al.*¹¹ listed 27 SNPs with $P < 1 \times 10^{-5}$; 25 SNPs overlapped with our analysis but none had $P < 0.05$ and association with the same allele. Of the top 200 SNPs in one or both of their study samples, $\sim 90\%$ were analysed in our study but $\sim 5\%$ had $P < 0.05$, consistent with chance expectations. As the MDD2000+ controls were also used in GenRed¹³ and STARD,¹⁴ we did not make formal comparisons of our results, but we note that $\sim 5\%$ of their top SNPs ($P < 1 \times 10^{-5}$) had $P < 0.05$ in MDD2000+. The SNP rs2251219 identified as a mood disorder risk factor in a GWAS meta-analysis (three bipolar studies plus the GAIN–MDD study)³² but was not associated in MDD2000+ ($P = 0.51$, MAF = 0.40, odds ratio (OR) = 0.97, 95% CI 0.90–1.05). *PCLO* was the top finding in the GAIN–MDD study,¹² replicated in Australian (QIMR)¹² and Dutch³³ samples, but we found no evidence for association with *PCLO* variants (for example, $P = 0.51$ for rs2522833), despite partial overlap (562 cases and 264 controls) in the QIMR samples used here and in the original replication sample (see Supplementary File 1 for details). Meta-analysis results of the three largest MDD GWAS—427362 SNPs in MDD2000+, GAIN–MDD and the UK studies—yielded $\lambda_{1000} = 1.01$ (Figure 1iii). In all, 19 SNPs gave $P < 5 \times 10^{-5}$ compared with ~ 21 expected under the null hypothesis (ignoring linkage disequilibrium) and are listed in the Supplementary File 1.

Gene-based tests

MDD2000+ SNPs mapped to 18 454 genes. We list all genes with $P < 1 \times 10^{-4}$ and all lie within linkage regions identified in the SLEP³⁴ database of research findings for psychiatric disorders. Gene ontology annotations and names are provided for these genes (Table 4). Three are plausible candidate genes for MDD (*GAL*, *ADCY3*, *PDK4*) and others have a role in ion binding (*ZDHHC19*, *ZNF83*). None of the genes listed in Table 4 shows any evidence for association in the gene-based test applied to GAIN–MDD or the

UK MDD GWAS results, although association at $P < 0.001$ is retained for *GAL* in the gene-based test applied to the meta-analysis results.

Of the 183 candidate genes investigated in other GWAS for MDD, we could test for association with 180 (Supplementary File 4). None were associated with $P < 0.00028$ ($0.05/180$). The six most associated were *IL10*, *OPRM1*, *HTT*, *HTR1B*, *GRIN1* and *CACNA1C*. Of these, *OPRM1* was reported to have $P < 0.05$ in the GAIN–MDD gene-based test and *CACNA1C* was top ranked in the GenRed¹³ study; the other studies all used a gene test based on best single associated SNP corrected for gene length. In this study, *CACNA1C* ranked 808 out of 18 454 (4th percentile). Our top associated SNP in *CACNA1C* is rs98545 ($P = 0.0019$, OR = 0.83, MAF = 0.15) which is 387 kb from, and in near linkage equilibrium ($r^2 = 0.06$) with, rs1006737 ($P = 0.020$, OR = 1.10, MAF = 0.35), the SNP first identified in genome-wide association studies of bipolar disorder;³⁵ an interaction between these SNPs was not significant ($P = 0.21$). The 3rd ranked of the candidate genes (*Huntingtin*, *HTT*) was included in the candidate list because mood disorders are characteristic of presymptomatic carriers of the Huntington's disease trinucleotide repeat polymorphisms.³¹ *HTT* ranked in the top 0.7% of all genes; the SNP showing highest individual association within the gene is rs363099 (also known as rs4690074, $P = 4.7 \text{ E} - 04$, OR = 0.86, MAF = 0.31) located in exon 29, 85 kb from the exon 1 CAG repeat.

Discussion

Study results

The MDD2000+ study is the largest GWAS for MDD reported to date, comprising 2431 cases with MDD and 3673 screened controls. Our analysis was of $> 1 \text{ M}$ SNPs but none achieved genome-wide significance. Indeed, the quantile–quantile plots (Figure 1) show that the distribution of observed associations closely follows that expected under the null hypothesis. These results are consistent with other GWAS of MDD.^{11–15} Furthermore, comparison of our results with these other studies including formal meta-analysis of results from MDD2000+ and the next two largest studies^{12,15} (a total of 5763 cases and 6901

Table 3 Regions containing at least one SNP with a P -value of $P < 10^{-5}$ in any one of the four association analyses—total sample (all), males only, females only and recurrent early onset (REO) MDD ordered by chromosomal position

Asso- ciation	SNP	Chromo- some band	Position	MAF	Minor allele	Other allele	All		Male		Female		REO		Gene
							Odds	P	Odds	P	Odds	P	Odds	P	
All	rs182358	1p21	97 235 738	0.50	C	T	0.83	8.9E-06	0.78	3.3E-04	0.86	7.6E-03	0.86	9.4E-03	
All	rs7551221	1q23	160 267 022	0.21	T	C	1.25	5.8E-06	1.13	1.2E-01	1.31	3.5E-05	1.19	6.2E-03	OLFML2B, NOS1AP
All	rs2384061	2p23.3	24 989 124	0.42	A	G	1.20	6.2E-06	1.24	7.0E-04	1.18	1.8E-03	1.21	2.4E-04	ADCY3, RBJCENPO
All	rs3732293	2p13.1	74 503 442	0.08	C	A	1.41	1.5E-06	1.45	1.0E-03	1.38	6.3E-04	1.38	5.4E-04	WDR54, ZNHIT4, WBP1, RTKN, MRPL53, GCS1, DCTN1
All	rs625588	2q36.1	224 310 891	0.05	T	C	0.64	7.9E-06	0.69	1.7E-02	0.61	2.1E-04	0.67	1.7E-03	AP1S3
All	rs9394026	6p21.3	31 090 523	0.23	A	G	1.24	5.0E-06	1.24	2.9E-03	1.24	6.1E-04	1.28	5.2E-05	MUC21
All	rs805284	6p21.3	31 790 008	0.05	A	G	1.49	8.2E-06	1.32	4.3E-02	1.62	6.9E-05	1.51	3.3E-04	LY6G6D + 12 more
All	rs10815615	9p24.1	7 475 343	0.46	G	A	0.84	6.3E-06	0.90	7.9E-02	0.78	4.2E-06	0.84	6.7E-04	
All	rs2031616	10q23-q33	94 726 947	0.39	A	G	0.84	9.1E-06	0.79	2.7E-04	0.86	4.1E-03	0.85	3.1E-03	EXOC6
All	rs2638463	12q22-23	88 193 916	0.31	A	G	1.22	2.9E-06	1.22	2.9E-03	1.21	5.2E-04	1.25	7.4E-05	
All	rs17226852	20p12.1	13 921 760	0.08	C	T	1.41	1.5E-06	1.52	2.6E-04	1.36	1.2E-03	1.35	1.3E-03	MACROD2, SEL1L2 = AL137678
Male	rs1526285	2q23.3	150 216 771	0.10	A	C	0.86	1.6E-02	0.58	3.4E-06	1.01	9.4E-01	0.91	2.6E-01	LOC642340
Male	rs268624	7q35-q36	146 689 924	0.46	G	A	0.93	5.6E-02	0.76	9.2E-06	1.07	2.2E-01	0.95	3.2E-01	CNTNAP2
Male	rs1536723	13q12	22 696 431	0.46	A	C	0.90	6.7E-03	0.74	2.6E-06	1.03	5.8E-01	0.86	4.6E-03	SGCG
Male	rs7318876	13q14.13	45 077 228	0.11	A	G	0.87	2.9E-02	0.61	1.8E-06	1.13	1.5E-01	0.88	1.2E-01	FLJ32682
Male	rs9538386	13q21.2	58 794 051	0.17	G	A	1.15	1.1E-02	1.46	7.1E-06	0.97	6.7E-01	1.18	2.1E-02	
Male	rs7490744	13q31.1	83 874 970	0.24	T	G	0.84	1.3E-04	0.70	1.5E-06	0.96	4.9E-01	0.84	3.6E-03	
Female	rs695884	3q28	192 630 774	0.17	G	A	0.85	2.2E-03	1.05	5.3E-01	0.73	7.9E-06	0.85	2.3E-02	POP2, CCDC50
Female	rs11938628	4q26	115 677 617	0.17	T	C	1.19	1.1E-03	0.95	5.7E-01	1.39	2.2E-06	1.14	5.8E-02	
Female	rs797729	7q32	129 097 328	0.17	C	T	1.20	2.9E-04	1.04	6.1E-01	1.35	9.2E-06	1.26	4.8E-04	NRF1
Female	rs10815615	9p24.1	7 475 343	0.46	G	A	0.84	6.3E-06	0.90	7.9E-02	0.78	4.2E-06	0.84	6.7E-04	
Female	rs1762444	10q26	131 140 655	0.38	A	G	0.88	1.8E-03	1.01	9.0E-01	0.79	7.5E-06	0.93	1.5E-01	MGMT
Female	rs9564791	13q22	70 696 044	0.41	T	C	0.86	1.6E-04	1.02	7.4E-01	0.76	4.1E-07	0.89	3.5E-02	
Female	rs2253168	14q23	68 120 218	0.40	A	G	0.86	2.9E-04	0.98	7.3E-01	0.78	5.2E-06	0.89	3.6E-02	RAD51L1
Female	rs725308	Xq23	112 717 434	0.45	T	C	0.91	5.3E-03	1.02	6.6E-01	0.78	4.6E-07	0.88	3.1E-03	
REO	rs2352834	1q21.1	145 831 644	0.12	G	A	0.80	4.7E-04	0.85	9.3E-02	0.78	3.4E-03	0.68	5.3E-06	GPR89, GJA8
REO	rs1430306	2q13	109 174 317	0.42	G	A	1.15	3.8E-04	1.15	2.6E-02	1.15	7.5E-03	1.27	3.8E-06	SH3MD4 = SH3RFB3
REO	rs4478239	4q35.1	188 428 300	0.10	C	A	1.26	2.7E-04	1.18	1.1E-01	1.31	1.0E-03	1.45	3.9E-06	
REO	rs17400379	7q21.3	94 047 868	0.15	G	T	1.26	6.3E-05	1.24	2.1E-02	1.23	5.2E-03	1.39	9.0E-06	CASD1, SGCE
REO	rs715217	20p13	1 420 408	0.08	T	C	1.21	5.0E-03	1.34	7.0E-03	1.12	2.1E-01	1.50	2.7E-06	NSFL1C, SIRPB2, SIRPD

Abbreviations: LD, linkage disequilibrium; MAF, minor allele frequency; MDD, major depressive disorder; SNP, single-nucleotide polymorphism.

For SNPs with INFO > 0.8. If multiple SNPs in high LD qualified for inclusion in the table, the one with the smallest P is listed. Regional association plots are provide for all regions with a SNP $P < 10^{-5}$ in Supplementary File 2.

Table 4 Top ten ranked genes from gene-based test for genes ± 50 kb from start and stop positions

Rank	Band	Gene ID	No. of SNPs	Length (kb)	Start (bp) of gene	Stop (bp) of gene	Gene test P	SNP with minimum P-value	SNP stats ^a	Gene name	Molecular function from GO annotation
2	2p23.3	<i>ADCY3</i>	a. 84 b. 169 c. 29 d. 28	100	24 895 541	24 995 559	a. 4.3E-05 b. 6.3E-01 c. 7.3E-01 d. 3.1E-01	rs2384061	6.3E-06 A 0.42 1.20	<i>Adenylate cyclase 3</i>	Nucleotide binding; magnesium ion binding; calmodulin binding; ATP binding; calcium- and calmodulin-responsive adenylylate cyclase activity
8	5q35	<i>NPM1</i>	a. 52 b. 52 c. 16 d. 15	23	170 747 402	170 770 493	a. 3.3E-04 b. 6.6E-01 c. 9.6E-01 d. 1.7E-01	rs11134697	2.7E-04 G 0.47 1.16	<i>Nucleophosmin</i>	Nucleic acid binding; DNA binding; RNA binding; protein binding; rRNA binding; ribosomal large subunit binding; ribosomal small subunit binding
3	6q27	<i>UNC93A1</i>	a. 95 b. 147 c. 31 d. 29	25	167 624 890	167 649 491	a. 4.4E-05 b. 2.3E-01 c. 6.1E-01 d. 4.9-01	rs2076008	1.3E-04 G 0.26 1.19	<i>Unc-93 homolog A</i>	—
5	6q27	<i>TTL21</i>	a. 84 b. 131 c. 27 d. 25	18	167 658 563	167 676 167	a. 1.1E-04 b. 3.5E-01 c. 5.6E-01 d. 5.2E-01	rs2076008	1.3E-04 G 0.26 1.19	<i>Tubulin tyrosine ligase-like family, member 2</i>	Catalytic activity; tubulin-tyrosine ligase activity; ATP binding; ligase activity
7	7q21.3	<i>PDK4²</i>	a. 77 b. 133 c. 29 d. 28	13	95 050 744	95 063 861	a. 2.1E-04 b. 6.1E-01 c. 1.8E-01 d. 3.5E-01	rs11531570	3.4E-05 A 0.41 1.18	<i>Pyruvate dehydrogenase kinase, isozyme 4</i>	Two-component sensor activity; nucleotide binding; pyruvate dehydrogenase (acetyl transferring) kinase activity; ATP-binding; transferase activity
10	7q21-q22	<i>ASB4²</i>	a. 104 b. 182 c. 46 d. 46	52	94 953 219	95 005 007	a. 3.6E-04 b. 6.5E-01 c. 4.5E-01 d. 5.6E-01	rs11531570	3.4E-05 A 0.41 1.18	<i>Ankyrin repeat and SOCS box-containing 4</i>	— SOCS = suppressor of cytokine signalling
4	9q31.1	<i>TEX10</i>	a. 51 b. 90 c. 17 d. 14	51	102 104 190	102 154 995	a. 4.7E-05 b. 1.8E-01 c. 8.5E-01 d. 1.1E-01	rs1930243	2.7E-04 A 0.49 1.15	<i>Testis expressed 10</i>	Binding
6	11q13.3	<i>GAL</i>	a. 48 b. 52 c. 11 d. 11	7	68 208 558	68 215 219	a. 1.5E-04 b. 2.4E-01 c. 7.8E-01 d. 2.4E-03	rs2156464	2.7E-05 A 0.19 1.24	<i>Galatinin</i>	Neuropeptide hormone activity
9	15q22.3	<i>USP3</i>	a. 74 b. 121 c. 23 d. 23	87	61 583 862	61 670 716	a. 3.5E-04 b. 9.8E-01 c. 8.5E-01 d. 5.8E-02	rs7183892	4.5E-04 C 0.15 1.20	<i>Ubiquitin-specific peptidase 3</i>	Ubiquitin thiolesterase activity; ubiquitin-specific protease activity; peptidase activity; cysteine-type peptidase activity; zinc ion binding; metal ion binding
1	20p12.1	<i>SEL1L2</i> = <i>AL137678</i>	a. 98 b. 209 c. 46 d. 42	141	13 778 049	13 919 262	a. 1.1E-05 b. 9.8E-01 c. 7.5E-01 d. 3.9E-01	rs17226852	1.5E-06 C 0.08 1.41	<i>Sel-1 suppressor of lin-12-like 2</i>	Binding

Abbreviations: ATP, adenosine triphosphate; GO, gene ontology; MAF, minor allele frequency; MDD, major depressive disorder; SNP, single-nucleotide polymorphism; rRNA, ribosomal RNA.

a, MDD2000+; b, GAIN-MDD; c, UK GWAS; d, meta-analysis. ¹The ± 50 kb regions of *UNC93A* and *TTL2* overlap. ²The ± 50 kb regions of *PDK4* and *ASB4* overlap. $P < 1 \times 10^{-2}$ in bold. Top associated SNPs lie in region ± 50 kb from the listed start and stop positions. Regional association plots for *ADCY3*, *GAL* and *CACNA1C* are in Supplementary File 2. SNP stats: P-value, minor allele, MAF, odds ratio.

controls) showed little evidence for replication between studies.

To determine whether any genes harbored an excess of low-associated variants, we undertook gene-based tests which account for both gene length and linkage disequilibrium between SNPs. Our top associated genes were not replicated in the gene-based test when applied to the GAIN–MDD or UK MDD results, although some genes retained low association *P*-values when applied to the meta-analysis results (Table 4). Despite this there are some suggestive results worthy of note. Among the top ranked genes is *GAL*, which encodes the neuropeptide galanin, proposed by Weiss *et al.*³⁶ as having an important role in MDD. Their hypothesis, based on animal models is that *GAL* released in the ventral tegmentum inhibits the activity of dopaminergic cells resulting in decreased motor activity and anhedonia. *GAL* is a regulator of brain serotonin and 5-HT_{1A} receptor-mediated transmission,³⁷ agonists of *GAL* receptors have been proposed as potential drug targets for MDD.³⁸ *GAL* also has an important role in the hippocampal processing of cognition.³⁹ Our most associated SNP in *GAL* (rs2156464, $P=2.7 \times 10^{-5}$, OR=1.24, CI=1.12–1.37, MAF=0.19) lies in the same haplotype block as an association reported for panic disorder.⁴⁰ Another top ranked gene (*ADCY3*) encodes the enzyme adenylate cyclase 3 that catalyses synthesis of cyclic adenosine monophosphate, the association underpinned by SNP rs2384061 ($P=6.3 \times 10^{-6}$, OR=1.20, CI=1.11–1.29, MAF=0.42, Table 3). *ADCY3* is a plausible candidate gene because depressed patients display reduction in platelet *ADCY* activity.⁴¹ Perhaps most interesting is that *ADCY* and *GAL* activity are inter-related⁴² and *ADCY3* binds *CACNA1C* (calcium channel, voltage dependent, L type, α -1C subunit).⁴³ A SNP (rs1006737, intron 3) within the gene *CACNA1C* is associated with bipolar disorder⁴⁴ schizophrenia and MDD.⁴⁵ In this study, *CACNA1C* ranked in the top 4% of genes and we replicated the association with rs1006737 ($P=0.020$, OR=1.10, CI=1.01–1.19, MAF=0.34), but the top associated SNP in *CACNA1C* was rs98545 ($P=0.0019$, OR=0.83, CI 0.75–0.94, MAF=0.15, intron 29). rs98545 lies 387 kb from rs1006737; they are in near linkage equilibrium ($r^2=0.06$) and in our data their effects are additive in a logistic regression. Neither rs1006737 nor rs98545 were genotyped in the UK MDD or GAIN–MDD studies; imputation of GAIN–MDD genotypes showed weak association with rs1006737 ($P=0.070$, OR=1.10, CI=0.99–1.21, MAF=0.32) but not with rs98545 ($P=0.52$). We cannot exclude that the association of rs98545 has occurred by chance in our sample. Regional association plots for *ADCY3*, *GAL* and *CACNA1C* can be found in Supplementary File 2.

Despite the large sample size of MDD2000+, a number of limitations may reduce the realized power for detection of association. First, our study combines data sets genotyped on different primary platforms,

and the power of our sample will vary between SNPs depending on the linkage disequilibrium structure between genotyped and imputed SNPs. Second, some of our cases and controls were genotyped separately because of economic constraints. We were fortunate that we had >300 QIMR cases genotyped on both the Affymetrix and Illumina platforms, which we used extensively for QC. Third, when combining cases and controls genotyped as separate sets, it was necessary to impose more stringent QC constraints than for cases and controls genotyped on the same platform. These constraints were successful in removing artefactual differences between the case–control sets, but could also have eliminated true associations. Fourth, in MDD2000+ with the aim of maximizing sample size we have combined the clinical cases from the University of Edinburgh cohort with the community samples from QIMR and NTR. The extent to which this is important depends on the unknown genetic etiology of MDD. Lastly, 26% of both cases and controls contributed by the QIMR sample (which makes the largest contribution in this study) were from families ascertained on the basis of a sibling with nicotine dependence, as reflected by the high proportion of smokers in the QIMR samples. Smoking is associated with depression and if some genes are involved in smoking behavior and MDD, then the power to detect genetic variants for MDD may be reduced.

Implications

Our analysis and meta-analysis represent the largest and most powerful investigation into the genetic architecture of MDD to date. The lack of clear-cut evidence for association allows us to exclude genetic main effects that are common (MAF>0.1) with moderate to strong effect sizes (GRR>1.4). As noted earlier, the MDD2000+ sample alone possessed >90% power to detect the median MAF (0.36) and GRR (1.33) of GWAS associations published to date with $P<5 \times 10^{-8}$. Moreover, as we would expect to detect >20% of variants with OR 1.2 and MAF 0.2–0.7 (Supplementary File 1), and having detected none that are genome-wide significant, we can probably draw an even more stringent boundary on the risk allele architecture under a main effect model. These results are informative for researchers planning association studies of MDD.

These results are unsurprising in the light of results for a range of complex genetic disorders published since this study was conceived in 2007.⁴⁶ Results from GWAS of psychiatric disorders⁴⁷ have, on the whole, yielded replicated associations only for low prevalence/high heritability disorders, that is, autism, bipolar disorder and schizophrenia, with tobacco dependence being the exception.⁴⁸ As a high prevalence/low heritability disorder, the challenge for MDD was always going to be harder. From Yang *et al.*,⁴⁹ we estimate that sample sizes 2.4-fold greater are needed for GWAS of MDD (prevalence 0.15) compared with schizophrenia (prevalence 0.007) to

identify a variant that explains the same proportion of phenotypic variance to liability of these disorders (Supplementary File 1). This result reflects the smaller mean difference in phenotypic liability between cases and controls for MDD compared with schizophrenia. Hospital-based MDD cohorts may represent a more extreme phenotype, with lower prevalence and higher heritability.⁵⁰ Using a prevalence for such clinical samples to be 0.06 (the average across sexes)⁵¹ still requires a sample size ~1.8 times greater for a case-control study of MDD compared with one for schizophrenia.

Further, for main effect models, as the heritability on the liability scale (h^2) is a function of the number of variants (n), their frequencies (q_i) and their effect sizes (a_i),

$$h^2 = 2 \sum_{i=1}^n q_i(1 - q_i)a_i^2 \quad (1)$$

(with total variance of liability of 1), then the lower heritability of MDD (~0.37, although this may be higher in clinical samples)⁵¹ compared to schizophrenia (~0.81), must be explained by fewer risk alleles, lower risk allele frequencies and/or smaller effect sizes. It seems most plausible that MDD might have smaller effect sizes compared with schizophrenia (except in the unlikely event that the number of loci for MDD is substantially fewer than for schizophrenia). We estimate that sample sizes 4–5 times those needed for schizophrenia studies may be needed for MDD to detect variants that explain an equal proportion of the known *genetic* variance (Supplementary File 1).

Study designs

It is clear that larger sample sizes are needed to achieve the power required to detect common variants of smaller effect, and that larger sample sizes will be needed for MDD compared with less prevalent but more heritable disorders. The prize is major: any replicated hit provides a priceless window into the etiology of this idiopathic disorder. However, alternative and complementary strategies are possible, particularly those that consider a broader range of genetic models and relax further assumptions of etiological homogeneity.

First, recognizing that effect size is the average effect of a variant across all genetic and environmental backgrounds,⁵² it may be possible to forgo larger sample sizes to concentrate on more homogeneous subsets in which larger effect sizes may be detectable. There has been much debate about the genetic heterogeneity of MDD. Some genetic heterogeneity is consistent with the polygenic model; each affected individual could potentially harbor a different combination of risk variants which could imply a spectrum of phenotypic symptoms, with those presenting similar symptom profiles carrying more similar profiles of risk alleles, consistent with symptom sharing of closely related individuals.⁵³ For this reason, most GWAS for MDD have prioritized

genotyping of the less prevalent and more heritable recurrent early onset MDD, but even so genome-wide significant associations have been elusive. Stratification by sex has also not yielded consistent results. Other possibilities certainly exist but phenotypes may be unavailable (for example, accurate delineation of typical versus atypical MDD)⁵⁴ or prohibitively expensive (for example, magnetic resonance imaging). Use of quantitative scores of severity and reliability may be the best strategy to balance sample size with phenotype definition,⁵⁵ as MDD symptom profiles are consistent with a quantitative rather than dichotomous liability.⁵⁶

Second, in addition to achieving a more homogeneous genetic background, larger estimated effect sizes may come from selecting more homogeneous environmental backgrounds. The low heritability of liability for MDD implies an important role for environmental risk factors. Although genotype \times environment interaction cannot explain the so-called ‘missing heritability’,⁵² it can contribute to small effect sizes. Although genotype \times environment studies are conceptually attractive, the lessons learned from the most studied genotype \times environment hypothesis for MDD (5HTTLPR and stressful life event) are sobering.⁵⁷ However, this broad conclusion has been challenged^{58,59} with Uher and McGuffin⁵⁸ arguing that replication has been achieved when stressful life event were recorded objectively, temporally and before the onset to MDD. More mileage might be gained by considering MDD in the context of more clearly defined exposures, such as pregnancy, in which women with perinatal and post-partum MDD have been exposed to a similar event.

Whichever way we look at it, and whether risk variants are common or rare, it seems that the challenge for MDD will be much harder than for the less prevalent more heritable psychiatric disorders. Larger samples are required whether we attempt to identify associated variants with small effect across average backgrounds or attempt to enhance detectable effects sizes by selection of homogeneity of genetic or environmental background. In the long-term, a greater understanding of the etiology of MDD will require large prospective, longitudinal, uniformly and broadly phenotyped and genotyped cohorts that allow the joint dissection of the genetic and environmental factors underlying MDD.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

We thank the twins and their families registered at the Australian Twin Registry for their participation in the many studies that have contributed to this research. We thank Dixie Statham (sample collection); Leanne Wallace, Anthony Caracella and staff of the Molecular Epidemiology Laboratory (DNA processing); David

Smyth, Harry Beeby, and Daniel Park (IT support). Funding was provided by the Australian National Health and Medical Research Council (241944, 339462, 389927, 389875, 389891, 389892, 389938, 442915, 442981, 496675, 496739, 552485, 552498, 613608), the FP-5 GenomeEUtwin Project (QLG2-CT-2002-01254), and the US National Institutes of Health (NIH grants AA07535, AA10248, AA13320, AA13321, AA13326, AA14041, MH66206, DA12854, DA019951). A portion of the genotyping on which this study was based (Illumina 370K scans on 4300 individuals) was carried out at the Center for Inherited Disease Research, Baltimore through an access award to our late colleague Dr Richard Todd (Psychiatry, Washington University School of Medicine, St Louis). Statistical analyses were partly conducted at the Genetic Cluster Computer (<http://www.geneticcluster.org>), which is financially supported by the Netherlands Scientific Organization (NWO 480-05-003). SEM, SM and GWM are supported by the National Health and Medical Research Council Fellowship Scheme. NRW and DRN are supported by the Australian Research Council Future Fellowship Scheme.

Funding support was provided by the Netherlands Scientific Organization (904-61-090, 904-61-193, 480-04-004, 400-05-717, Centre for Medical Systems Biology (NWO Genomics), the Neuroscience Campus Amsterdam and the EMGO+ institute; the European Union (EU/WLRT-2001-01254), ZonMW (Geestkracht program, 10-000-1002), NIMH (RO1 MH059160) and matching funds from participating institutes in NESDA and NTR. The NTR controls were genotyped in the Genomics platform (certified service provider (CSPRO(R)) for Illumina) at the LIFE and BRAIN Center Bonn (funded by NWO-SPI 56-464-1419).

We would like to record our appreciation for much practical support from our late colleague Walter J Muir, Professor of Developmental Psychiatry at the University of Edinburgh and especially for his many stimulating ideas for genetic studies in depression. We thank Margaret Van Beck for sample collection and Lee Murphy, Wellcome Trust Clinical Research Facility, Western General Hospital, Crewe Road South, Edinburgh, for sample management. The collection of the Edinburgh cohort was supported by grants from The Wellcome Trust, London and the Chief Scientist Office of the Scottish Government.

Additional funding was provided by National Institute of Mental Health Schizophrenia (NIMH) grant MH080403 to EJCGvdO and PFS. Control subjects from the NIMH Genetics Initiative, data and biomaterials are being collected by the 'Molecular Genetics of Schizophrenia II' collaboration. The investigators and coinvestigators are: ENH/Northwestern University, Evanston, IL, MH059571, Pablo V Gejman, MD (Collaboration Coordinator; PI), Alan R Sanders, MD; Emory University School of Medicine, Atlanta, GA, MH59587, Farooq Amin, MD (PI); Louisiana State University Health Sciences Center; New Orleans, Louisiana, MH067257, Nancy Buccola

APRN, BC, MSN (PI); University of California-Irvine, Irvine, CA, MH60870, William Byerley, MD (PI); Washington University, St Louis, MO, U01, MH060879, C Robert Cloninger, MD (PI); University of Iowa, Iowa, IA, MH59566, Raymond Crowe, MD (PI), Donald Black, MD; University of Colorado, Denver, CO, MH059565, Robert Freedman, MD (PI); University of Pennsylvania, Philadelphia, PA, MH061675, Douglas Levinson, MD (PI); University of Queensland, Queensland, Australia, MH059588, Bryan Mowry, MD (PI); Mt. Sinai School of Medicine, New York, NY, MH59586, Jeremy Silverman, PhD (PI).

Author contributions

Funding: PFS, EJCGvdO, PAFM, MLP, DIB, NGM, GWM, ACH, DHB, BWJHP, IBH
QIMR sample: Phenotype: MLP, PAFM, DS, ACH, NGM; Biobank: GWM, AKH
NTR sample: DIB, JJH, GW, CM, EJCG
NESDA sample: BWJHP, JHS
University of Edinburgh sample: DHRB, DMM, KAMcG, AWM
UK GWAS: CML, PMcG
Analysis Set QC: DRN, SDG, NRW, BPMcE, SEM
Joint analysis set QC, imputation, association analysis and regional plots of MDD2000+: SR
Post-association analysis analyses: NRW, SDG, EMB, JZL, SM
Manuscript preparation: NRW, PFS and all other authors

References

- 1 Australian Bureau of Statistics 4326.0—National Survey of Mental Health and Wellbeing: Summary of Results, 2007 <http://www.abs.gov.au/AUSSTATS/abs@nsf/mf/43260>.
- 2 Kessler RC, Berglund P, Demler O, Jin R, Walters EE. Lifetime prevalence and age-of-onset distributions of DSM-IV disorders in the national comorbidity survey replication. *Arch Gen Psychiatry* 2005; **62**: 593–602.
- 3 Weissman MM, Bland R, Joyce PR, Newman S, Wells JE, Wittchen HU. Sex-differences in rates of depression—cross-national perspectives. *J Affect Disord* 1993; **29**: 77–84.
- 4 Wilhelm K, Mitchell P, Slade T, Brownhill S, Andrews G. Prevalence and correlates of DSM-IV major depression in an Australian national survey. *J Affect Disord* 2003; **75**: 155–162.
- 5 Craddock N, Forty L. Genetics of affective (mood) disorders. *Eur J Hum Genet* 2006; **14**: 660–668.
- 6 Fairweather-Schmidt AK, Anstey KJ, Mackinnon AJ. Is suicidality distinguishable from depression? Evidence from a community-based sample. *Aust N Z J Psychiatry* 2009; **43**: 208–215.
- 7 Sullivan PF, Neale MC, Kendler KS. Genetic epidemiology of major depression: review and meta-analysis. *Am J Psychiatry* 2000; **157**: 1552–1562.
- 8 Kendler KS, Gatz M, Gardner CO, Pedersen NL. Age at onset and familial risk for major depression in a Swedish national twin sample. *Psychol Med* 2005; **35**: 1573–1579.
- 9 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**: 335–342.
- 10 Lopez-Leon S, Janssens A, Ladd A, Del-Favero J, Claes SJ, Oostra BA et al. Meta-analyses of genetic studies on major depressive disorder. *Mol Psychiatry* 2008; **13**: 772–785.

- 11 Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ *et al*. Genome-wide association study of recurrent major depressive disorder in two European case-control cohorts. *Mol Psychiatry* 2010; **15**: 589–601.
- 12 Sullivan PF, de Geus EJC, Willemsen G, James MR, Smit JH, Zandbelt T *et al*. Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. *Mol Psychiatry* 2009; **14**: 359–375.
- 13 Shi J, Potash JB, Knowles JA, Weissman MM, Coryell W, Scheftner WA *et al*. Genome-wide association study of recurrent early-onset major depressive disorder. *Mol Psychiatry* 2 February 2010; e-pub ahead of print.
- 14 Shyn SI, Shi J, Kraft JB, Potash JB, Knowles JA, Weissman MM *et al*. Novel loci for major depression identified by genome-wide association study of sequenced treatment alternatives to relieve depression and meta-analysis of three studies. *Mol Psychiatry* 29 December 2009; e-pub ahead of print.
- 15 Lewis CM, Ng MY, Bulter AW, Cohen-Woods S, Uher R, Pirlo K *et al*. Genome-wide association study of major depression in the UK population. *Am J Psychiatry* 2010; **167**: 949–957.
- 16 World Health Organisation. *Composite International Diagnostic Interview Version 2.1* World Health Organization: Geneva 1997.
- 17 Bucholz KK, Cloninger CR, Dinwiddie DH, Hesselbrock VM, Nurnberger JL, Reich T *et al*. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report of the reliability of the SSAGA. *J Stud Alcoholism* 1994; **55**: 149–158.
- 18 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, revised 3rd edn*. American Psychiatric Association: Washington, DC, 1987.
- 19 American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders, revised 4th edn*. American Psychiatric Association: Washington DC, 1994.
- 20 Eysenck SBG, Eysenck HJ, Barrett P. A revised version of the psychoticism scale. *Pers Individ Dif* 1985; **6**: 21–30.
- 21 Bierut LJ, Heath AC, Bucholz KK, Dinwiddie SH, Madden PA, Statham DJ *et al*. Major depressive disorder in a community-based twin sample: are there different genetic and environmental contributions for men and women? *Arch Gen Psychiatry* 1999; **56**: 557–563.
- 22 Kirk KM, Birley AJ, Statham DJ, Haddon B, Lake RI, Andrews JG *et al*. Anxiety and depression in twin and sib pairs extremely discordant and concordant for neuroticism: prodromus to a linkage study. *Twin Res* 2000; **3**: 299–309.
- 23 Hansell NK, Agrawal A, Whitfield JB, Morley KI, Zhu G, Lind PA *et al*. Long-term stability and heritability of telephone interview measures of alcohol consumption and dependence. *Twin Res Hum Genet* 2008; **11**: 287–305.
- 24 Endicott J, Spitzer RL. A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* 1978; **35**: 837–844.
- 25 The Psychiatric GWAS Consortium. Genome-wide association studies: history, rationale and prospects for psychiatric disorders. *Am J Psychiatry* 2009; **166**: 540–556.
- 26 Browning BL, Browning SR. A unified approach to genotype imputation and haplotype-phase inference for large data sets of trios and unrelated individuals. *Am J Hum Genet* 2009; **84**: 210–223.
- 27 Browning SR, Browning BL. Rapid and accurate haplotype phasing and missing-data inference for whole-genome association studies by use of localized haplotype clustering. *Am J Hum Genet* 2007; **81**: 1084–1097.
- 28 Hindorf LA, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS *et al*. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci USA* 2009; **106**: 9362–9367.
- 29 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559–575.
- 30 Liu JZ, McRae AF, Nyholt DR, Medland SE, Wray NR, Brown KM *et al*. A versatile gene-based test for genome-wide association studies. *Am J Hum Genet* 2010; **87**: 139–145.
- 31 Perlis RH, Smoller JW, Mysore J, Sun M, Gillis T, Purcell S *et al*. Prevalence of incompletely Penetrant Huntington's disease alleles among individuals with major depressive disorder. *Am J Psychiatry* 2010; **167**: 574–579.
- 32 McMahon FJ, Akula N, Schulze TG, Muglia P, Tozzi F, Detera-Wadleigh SD *et al*. Meta-analysis of genome-wide association data identifies a risk locus for major mood disorders on 3p21.1. *Nat Genet* 2010; **42**: 128–131.
- 33 Hek K, Mulder CL, Luijckendijk HJ, van Duijn CM, Hofman A, Uitterlinden AG *et al*. The PCLO gene and depressive disorders: replication in a population-based study. *Hum Mol Genet* 2010; **19**: 731–734.
- 34 Konneker T, Barnes T, Furberg H, Losh M, Bulik CM, Sullivan PF. Rapid publication—a searchable database of genetic evidence for psychiatric disorders. *Am J Med Genet B Neuropsychiatr Genet* 2008; **147B**: 671–675.
- 35 Sklar P, Smoller JW, Fan J, Ferreira MAR, Perlis RH, Chambert K *et al*. Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008; **13**: 558–569.
- 36 Weiss JM, Bonsall RW, Demetrikopoulos MK, Emery MS, West CH. Galanin: a significant role in depression? *Ann N Y Acad Sci* 1998; **863**: 364–382.
- 37 Ogren SO, Schott PA, Kehr J, Yoshitake T, Misane I, Mannstrom P *et al*. Modulation of acetylcholine and serotonin transmission by galanin. Relationship to spatial and aversive learning. *Ann N Y Acad Sci* 1998; **863**: 342–363.
- 38 Ogren SO, Kuteeva E, Hokfelt T, Kehr J. Galanin receptor antagonists—a potential novel pharmacological treatment for mood disorders. *CNS Drugs* 2006; **20**: 633–654.
- 39 Ogren SO, Kuteeva E, Elvander-Tottie E, Hokfelt T. Neuropeptides in learning and memory processes with focus on galanin. *Eur J Pharmacol* 2010; **626**: 9–17.
- 40 Unschuld PG, Ising M, Erhardt A, Lucae S, Kohli M, Kloiber S *et al*. Polymorphisms in the galanin gene are associated with symptomseverity in female patients suffering from panic disorder. *J Affect Disord* 2008; **105**: 177–184.
- 41 Hines LM, Tabakoff B, Trait WISS. Platelet adenylyl cyclase activity: a biological marker for major depression and recent drug use. *Biol Psychiatry* 2005; **58**: 955–962.
- 42 Karelson E, Langel U. Galaninergic signalling and adenylyl cyclase. *Neuropeptides* 1998; **32**: 197–210.
- 43 Ingenuity knowledge base <http://www.ingenuity.com> 2010.
- 44 Ferreira MAR, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L *et al*. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet* 2008; **40**: 1056–1058.
- 45 Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S *et al*. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry* 2010; **15**: 1016–1022.
- 46 Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorf LA, Hunter DJ *et al*. Finding the missing heritability of complex diseases. *Nature* 2009; **461**: 747–753.
- 47 O'Donovan MC, Craddock NJ, Owen MJ. Genetics of psychosis: insights from views across the genome. *Hum Genet* 2009; **126**: 3–12.
- 48 Saccone SF, Hinrichs AL, Saccone NL, Chase GA, Konvicka K, Madden PAF *et al*. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet* 2007; **16**: 36–49.
- 49 Yang J, Wray NR, Visscher PM. Comparing apples and oranges: equating the power of case-control and quantitative trait association studies. *Genet Epidemiol* 2010; **34**: 254–257.
- 50 McGuffin P, Cohen S, Knight J. Homing in on depression genes. *Am J Psychiatry* 2007; **164**: 195–197.
- 51 McGuffin P, Katz R, Watkins S, Rutherford J. A hospital-based twin register of the heritability of DSM-IV unipolar depression. *Arch Gen Psychiatry* 1996; **53**: 129–136.
- 52 Falconer D, Mackay T. *Introduction to Quantitative Genetics*, 4th edn. Longman: England, 1996, 464pp.
- 53 Korszun A, Moskvina V, Brewster S, Craddock N, Ferrero F, Gill M *et al*. Familiality of symptom dimensions in depression. *Arch Gen Psychiatry* 2004; **61**: 468–474.
- 54 Sullivan PF, Kessler RC, Kendler KS. Latent class analysis of lifetime depressive symptoms in the National Comorbidity Survey. *Am J Psychiatry* 1998; **155**: 1398–1406.

- 55 Hettema JM, Neale MC, Myers JM, Prescott CA, Kendler KS. A population-based twin study of the relationship between neuroticism and internalizing disorders. *Am J Psychiatry* 2006; **163**: 857–864.
- 56 Rice JP, Rochberg N, Endicott J, Lavori PW, Miller C. Stability of psychiatric diagnoses—an application to the affective disorders. *Arch Gen Psychiatry* 1992; **49**: 824–830.
- 57 Risch N, Herrell R, Lehner T, Liang KY, Eaves L, Hoh J *et al*. Interaction between the serotonin transporter gene (5-HTTLPR), stressful life events, and risk of depression a meta-analysis. *JAMA* 2009; **301**: 2462–2471.
- 58 Uher R, McGuffin P. The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update. *Mole Psychiatry* 2010; **15**: 18–22.
- 59 Rutter M, Thapar A, Pickles A. Gene-environment interactions biologically valid pathway or artifact? *Arch Gen Psychiatry* 2009; **66**: 1287–1289.
- 60 Wray NR, Birley AJ, Sullivan PF, Visscher PM, Martin NG. Genetic and phenotypic stability of measures of neuroticism over 22 years. *Twin Res Hum Genet* 2007; **10**: 695–702.



This work is licensed under the Creative Commons Attribution-NonCommercial-No Derivative Works 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-nd/3.0/>

Supplementary Information accompanies the paper on the Molecular Psychiatry website (<http://www.nature.com/mp>)