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ORIGINAL ARTICLE

A mega-analysis of genome-wide association studies for major depressive disorder

Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium[1]

Prior genome-wide association studies (GWAS) of major depressive disorder (MDD) have met with limited success. We sought to increase statistical power to detect disease loci by conducting a GWAS mega-analysis for MDD. In the MDD discovery phase, we analyzed more than 1.2 million autosomal and X chromosome single-nucleotide polymorphisms (SNPs) in 18 759 independent and unrelated subjects of recent European ancestry (9240 MDD cases and 9519 controls). In the MDD replication phase, we evaluated 554 SNPs in independent samples (6783 MDD cases and 50 695 controls). We also conducted a cross-disorder meta-analysis using 819 autosomal SNPs with $P < 0.0001$ for either MDD or the Psychiatric GWAS Consortium bipolar disorder (BIP) mega-analysis (9238 MDD cases/8039 controls and 6998 BIP cases/7775 controls). No SNPs achieved genome-wide significance in the MDD discovery phase, the MDD replication phase or in pre-planned secondary analyses (by sex, recurrent MDD, recurrent early-onset MDD, age of onset, pre-pubertal onset MDD or typical-like MDD from a latent class analyses of the MDD criteria). In the MDD-bipolar cross-disorder analysis, 15 SNPs exceeded genome-wide significance ($P < 5 \times 10^{-8}$), and all were in a 248 kb interval of high LD on 3p21.1 (chr3:52 425 083–53 822 102, minimum $P = 5.9 \times 10^{-9}$ at rs2535629). Although this is the largest genome-wide analysis of MDD yet conducted, its high prevalence means that the sample is still underpowered to detect genetic effects typical for complex traits. Therefore, we were unable to identify robust and replicable findings. We discuss what this means for genetic research for MDD. The 3p21.1 MDD-BIP finding should be interpreted with caution as the most significant SNP did not replicate in MDD samples, and genotyping in independent samples will be needed to resolve its status.

Introduction

Major depressive disorder (MDD) is a genetically complex trait. The lifetime prevalence of MDD is ~15%.[1,2] As a recurrent course is most common,[3] MDD is accompanied by considerable morbidity[4–6] excess mortality[5,7] and substantial costs.[8] The World Health Organization projects MDD to be the second leading cause of disability by 2020.[9]

The heritability of MDD is 31–42%,[10] although certain subsets of MDD may be more heritable (for example, recurrent, early-onset MDD or clinically ascertained MDD).[11,12] The modest heritability of MDD could reasonably be expected to complicate attempts to identify genetic loci that confer risk or protection. However, heritability is not necessarily a key determinant for the identification of strong and replicable genetic associations.[13] For example,

there have been notable successes in genome-wide searches[14] for susceptibility loci for breast cancer (heritability ~25%), lung cancer (26%), Type 2 diabetes mellitus (26%), Parkinson's disease (34%), multiple sclerosis (41%), systemic lupus erythematosus (44%) and age-related macular degeneration (46%).[15–20]

The most important determinant of success in identifying associations for complex traits is the underlying genetic architecture (that is, the number of loci and their frequencies, effect sizes, modes of action and interactions with other genetic loci and environmental factors). Heritability alone reveals little about genetic architecture. In the absence of a detailed understanding of genetic architecture, sample size and phenotypic homogeneity are the critical determinants of discovering robust and replicable genetic associations. Eight genome-wide association studies (GWAS) for MDD have been published,[21–28] with one locus of possible genome-wide significance.[26] When these studies were planned, there were few data to guide sample size requirements. Several had historically notable sample sizes and far more comprehensive genomic coverage than any prior study. However, it has become clear that the effects of

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common genetic variants for most complex human diseases are considerably smaller than many had anticipated.[14] This implies that sample sizes necessary for identification of common genetic main effects were far larger than could be attained by single-research groups or existing consortia.

Meta-analysis has thus become essential in human complex trait genetics. There are now many examples where meta-analyses combining dozens of primary data sets have illuminated the genetic architecture of complex traits such as height,[29] body mass,[30] Crohn's disease[31] and Type 2 diabetes mellitus.[32] Following this proven model, we created the Psychiatric GWAS Consortium (PGC)[33,34] to conduct field-wide combined analyses for MDD as well as ADHD,[35] bipolar disorder (BIP),[36] schizophrenia[37] and autism. Our goal was to evaluate the evidence for common genetic variation in the etiology of MDD using the largest and most comprehensively genotyped sample hitherto collected.

Materials and methods

Overview
In the discovery phase, we conducted mega-analysis for MDD using nine primary samples. All groups uploaded individual genotype and phenotype data to a central computer cluster, and the PGC Statistical Analysis Group conducted uniform quality control, imputation and association analyses. Mega-analysis and meta-analysis yield essentially identical results in theory[38] and in practice.[37] However, mega-analysis of individual phenotype and genotype data was used to allow more consistent quality control and analysis, disentangle the issue of control subjects used by multiple studies, allow conditional analyses and to enable efficient secondary analyses. In the replication phase, we evaluated the top loci in seven independent MDD samples and in the PGC BIP mega-analysis[36] given the phenotypic and genetic overlap between MDD and BIP.[39,40] Finally, we conducted exploratory analyses of MDD sub-phenotypes in an attempt to index clinical heterogeneity. Most of the primary genotype data and the results have been deposited in the NIMH Human Genetics Initiative Repository (Supplementary Methods).

Samples
Full sample details are given in the Supplementary Methods. For the discovery phase, we included all identified primary MDD samples[21–25,27,28,41] that conducted genome-wide genotyping (>200K single-nucleotide polymorphisms (SNPs)) on individual subjects of European ancestry. Cases were required to have diagnoses of DSM-IV lifetime MDD established using structured diagnostic instruments from direct interviews by trained interviewers (two studies required recurrent MDD and one recurrent, early-onset MDD) or clinician-administered DSM-IV checklists. Most studies ascertained cases from clinical sources, and most controls were randomly selected from the population and screened for lifetime history of MDD.

The sample sizes reported here differ from the primary reports due to different quality control procedures and apportioning of overlapping controls. We determined the relatedness of all pairs of individuals using genotypes of SNPs present on all platforms, and excluded one of each duplicate or closely related pair. The discovery mega-analysis consists of 18 759 independent and unrelated subjects of recent European ancestry (9240 MDD cases and 9519 controls).

There were two sets of analyses conducted on additional samples. For MDD replication, we used meta-analysis to combine the autosomal discovery results (554 SNPs with $P < 0.001$) with summary association results from independent samples[42–48] (6783 MDD cases and 50 695 controls). The discovery SNP results were grouped into regions defined by linkage disequilibrium using an iterative process after ranking all SNPs by association P-value: for SNPs with $r^2 > 0.2$ in a 1 Mb window (based on HapMap3 CEU + TSI), the most strongly associated SNP was retained. In addition, given the close genetic and phenotypic relationships between MDD and BIP, we combined the MDD discovery sample and the PGC BIP mega-analysis[36] to evaluate 819 autosomal SNPs with $P < 0.0001$ in either of the separate analyses. (See Sklar et al.[36] for complete description). In effect, we tested for associations with a more broadly defined mood disorder phenotype. After resolving overlapping control samples, there were 32 050 independent subjects (9238 MDD cases/8039 controls and 6998 BIP cases/7775 controls).

SNP genotyping
SNP genotyping is described in the Supplementary Methods and summarized in Supplementary Table S2. Briefly, all samples were genotyped with SNP arrays intending to provide genome-wide coverage of common variation. Imputation was performed within each study in batches of 300 individuals. Batches were randomly assigned to keep the same case–control ratios as in the primary studies. We used Beagle 3.0.4 [ref. 49] with the CEU + TSI HapMap3 data as reference (410 phased haplotypes)[50] to impute 1 235 109 autosomal SNP allele dosages. We had previously evaluated this approach by masking and then imputing genotyped loci and found a high correlation between the genotyped and imputed allele dosages (Pearson $r > 0.999$).[37]

Quality control
Genotyping coordinates are given in NCBI Build 36/UCSC hg18. For the discovery phase, quality control was conducted separately for each resolved sample. SNPs were removed for missingness ≥ 0.02, case–control difference in SNP missingness ≥ 0.02, SNP frequency difference from HapMap3 [ref. 50] ≥ 0.15, or exact Hardy–Weinberg equilibrium test in controls $< 1 \times 10^{-6}$. Subjects were removed for excessive missingness (≥ 0.02), identical or closely related to any subject in any sample ($\hat{\pi} > 0.2$ based on common autosomal SNPs) and if there was evidence for

diverging ancestry. Ancestry was estimated using multidimensional scaling applied to 8549 SNPs directly genotyped in all samples and in approximate linkage equilibrium.

Statistical analysis

We used logistic regression to test the association of MDD diagnosis with imputed SNP dosages under an additive model. This test has correct type 1 error with imputed data.[51] Covariates included study indicators and five principal components reflecting ancestry. For the MDD replication samples, the top SNP in each region was tested for association, and fixed-effect meta-analysis was used for the replication samples, and for the combination of PGC discovery and replication data.

Chromosome X

Female sex is an established risk factor for MDD, and analysis of chromosome X is particularly salient (although not included in many GWAS). Imputation using HapMap3 reference genotypes (as in the primary analysis) was not possible due to persisting difficulties with the phased chromosome X data, but we were able to impute using 1000 Genomes Project data.[52] Chromosome X imputation was conducted for subjects passing QC for the autosomal analysis and with SNP call rates > 0.95 for chrX SNPs. SNPs with missingness $\geqslant 0.05$ or HWE $P < 10^{-6}$ (females) were excluded. Phasing was conducted using MACH[53] in female subjects. Imputation was performed separately for males and females using MINIMAC with haplotypes from 381 European samples from the 1000 Genomes Project as reference (1.45 million chrX SNPs, but many were monomorphic in our sample). Chromosome X SNPs in HapMap2 and HapMap3 with $r^2 \geqslant 0.3$ were carried forward for further analysis (122 602 SNPs). Association was tested under an additive logistic regression model implemented in PLINK (meta-analysis of male and female association results) using the same covariates as for the autosomal analysis.

Secondary analyses

MDD is suspected to have important phenotypic heterogeneity, and association analyses might yield clearer findings if clinical features are incorporated into genetic analyses. Thus, we conducted pre-defined secondary analyses intended to index plausible sources of phenotypic heterogeneity in MDD cases. (a) *Sex.* As the lifetime prevalence of MDD is approximately two times greater in females,[54,55] we conducted association analyses separately in males and females to evaluate sex-specific genetic risk variants. (b) *Recurrence and age of onset.* As recurrence and age of onset may index heterogeneity in MDD,[10,56] we analyzed early-onset MDD ($\leqslant 30$ years), recurrent MDD ($\geqslant 2$ episodes), pre-pubertal onset MDD ($\leqslant 12$ years, see Weissman et al.[57]) and age of onset of MDD as a quantitative trait. (c) *Symptoms.* As MDD is phenotypically heterogeneous, we obtained MDD symptom data from 88% of all MDD cases (the nine DSM-IV 'A' criteria disaggregated to code increase and decrease in appetite, weight, sleep and energy level). Latent class cluster models were fit to binary responses for these MDD 'A' criteria, and identified three latent classes in MDD cases characterized by weight loss/insomnia, weight gain/insomnia and hypersomnia (see Supplementary Methods for more details). The predominant latent class was consistent with 'typical' MDD[58,59] and we analyzed cases indexed by this class.

Results

In the discovery stage, we conducted a GWAS mega-analysis for MDD in 18 759 independent and unrelated subjects of recent European ancestry (9240 MDD cases and 9519 controls, Table 1). There were considerable similarities across samples: all subjects were of European ancestry, all cases were assessed with validated methods and met DSM-IV criteria for lifetime MDD, and most controls were ascertained from community samples and screened to remove individuals with lifetime MDD (Supplementary Methods and Supplementary Figures S6–S9).

Table 1 Cases and controls used in discovery and replication phases

Phase	Sample	Subjects	MDD case	Control
Discovery	GAIN	3461	1696	1765
	GenRED	2283	1030	1253
	GSK	1751	887	864
	MDD2000-QIMR_610	1184	433	751
	MDD2000-QIMR_317	1977	1017	960
	MPIP	913	376	537
	RADIANT + Bonn/ Mannheim	2225	935	1290
	RADIANT	3213	1625	1588
	STAR*D	1752	1241	511
MDD replication	deCODE	34 229	1067	33 162
	GenPod/NEWMEDS	5939	477	5462
	Harvard i2b2	902	460	442
	PsyCoLaus	2794	1303	1491
	SHIP-LEGEND	1806	313	1493
	TwinGene	9562	1861	7701
	GenRED2/ DepGenesNetworks	2246	1302	944
MDD-BIP cross-disorder	PGC MDD	17 277	9238	8039
	PGC BIP	14 773	6998	7775
Totals	Discovery	18 759	9240	9519
	MDD replication	57 478	6783	50 695
	MDD-BIP cross-disorder	32 050	16 236	15 814

Abbreviations: BIP, bipolar disorder; MDD, major depressive disorder; PGC, Psychiatric GWAS Consortium.
Sample acronyms are defined in the Supplementary Methods. Sample sizes differ from the primary publications due to varying quality control procedures and re-allocation of controls that were used in multiple studies.
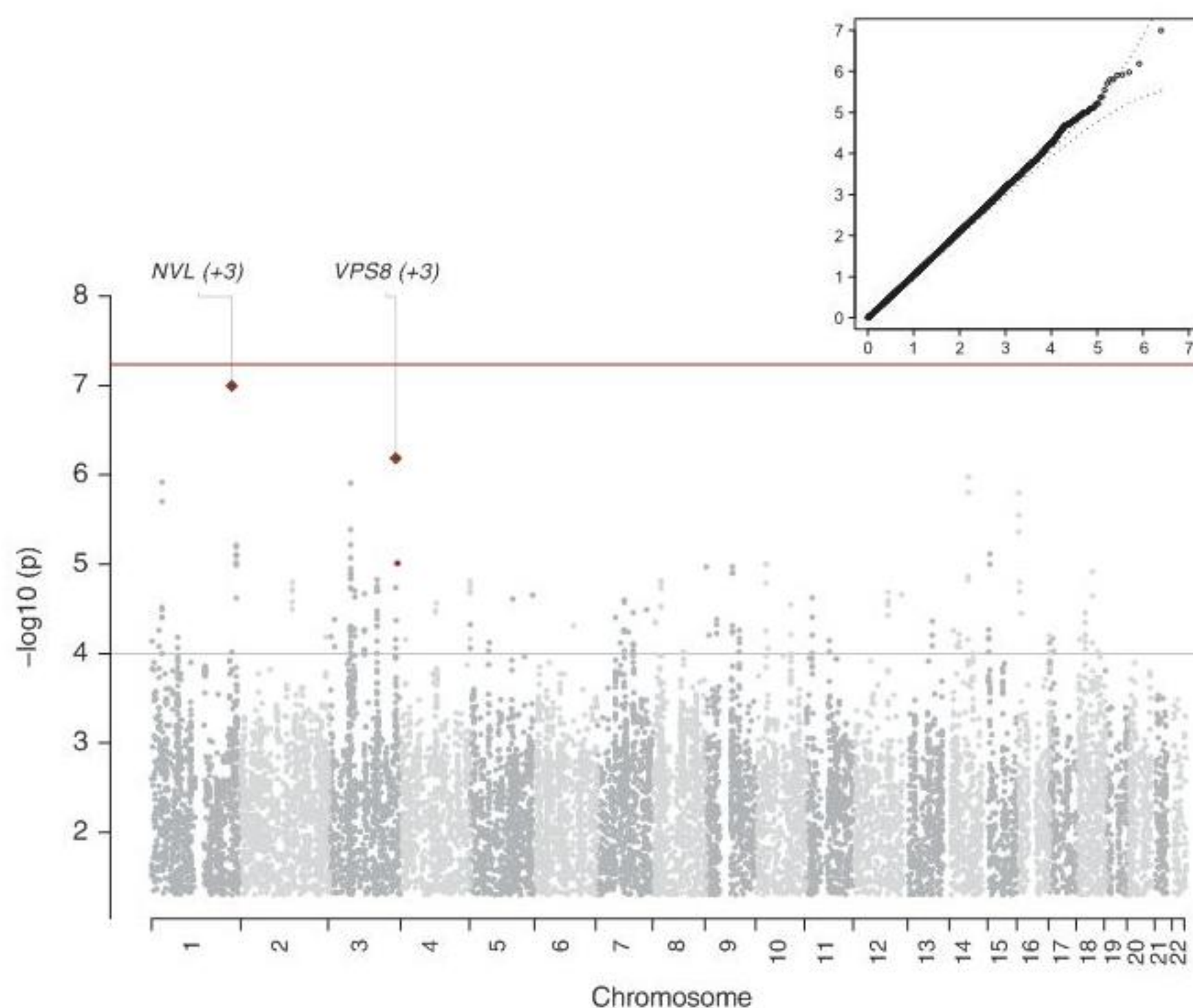
Figure 1 Overview of results from the discovery genome-wide association study mega-analysis for major depressive disorder. The inset shows the quantile–quantile plot (observed by expected P-values on the $-\log_{10}$ scale) showing conformity of the observed results to expectations under the null. The main part of the figure shows the Manhattan plot ($-\log_{10}$ of the P-value by genomic location) of the association results in genomic context. No region exceeded genome-wide significance in the discovery sample.

An overview of the results is in Figure 1. The quantile–quantile plot shows conformity of the observed results to those expected by chance. The overall λ [ref. 60] (the ratio of the observed median χ^2 to that expected by chance) was 1.056 and λ_{1000} was 1.006 (that, λ rescaled to a sample size of 1000 cases and 1000 controls).[61] The Manhattan plot depicts the association results in genomic context, and no region exceeded genome-wide significance ($P < 5 \times 10^{-8}$).[62] We conducted imputation with HapMap2 [ref. 63] and 1000 Genomes Project data[52] in addition to HapMap3 and obtained similar genome-wide association results.

The minimum P-values for the main analysis were at rs11579964 (chr1: 222 605 563 bp, $P = 1.0 \times 10^{-7}$) and rs7647854 (chr3:186 359 477 bp, $P = 6.5 \times 10^{-7}$; Supplementary Tables S16 and S17). Bioinformatic analyses of 201 SNPs with $P < 0.0001$ and the 1655 SNPs in moderate linkage disequilibrium (LD, $r^2 > 0.5$) showed no overlap with literature findings in the NHGRI GWAS catalog,[14] with transcripts differentially expressed in post-mortem brain samples of individuals with MDD,[64] or with SNPs that were genome-wide significant or notable in the PGC association analyses of ADHD, BIP, or schizophrenia. We noted that a few of these 201 SNPs were ± 20 kb of genes previously studied in MDD (*ADCY9* and *PDLIM5*),[65] or notable in prior hypotheses of the etiology of psychiatric disorders (*GRM7*, *HTR7* and *RELN*).

In the analyses of chrX, no SNP achieved genome-wide significance in analysis of all samples or in separate analyses of females and males. The most significant SNP across all analyses was rs12837650 in the female-only analysis ($P = 5.6 \times 10^{-6}$).

In the MDD replication phase, 554 SNPs with $P < 0.001$ from the discovery mega-analysis were evaluated in independent samples totaling 6783 MDD cases and 50 695 controls (Table 1). For these SNPs, the replication samples did not produce logistic regression β coefficients in the same directions as the discovery analysis more frequently than expected by chance (sign test, $P = 0.05$). No SNP exceeded genome-wide significance for a joint analysis of the discovery and replication samples (Supplementary Table S18). The minimum P-value was for rs1969253 ($P = 4.8 \times 10^{-6}$, chr3:185 359 206), located in an intron of the disheveled 3 gene (*DVL3*). Given the probable etiological heterogeneity of MDD, we also conducted replication analyses of subtypes of MDD. For analyses restricted to female cases and controls, the direction of effects tended to be consistent between the discovery and replication samples (sign test, $P = 0.006$) although no SNP neared genome-wide significance (minimum $P = 4.8 \times 10^{-6}$ at rs1969253, chr3: 185 359 206). For male cases and controls, the sign test was not significant ($P = 0.17$), and no SNP was genome-wide significant (minimum $P = 3.8 \times 10^{-7}$ at rs2498828, chr14:91 491 028). For recurrent MDD, there was

greater evidence of consistency of effects between the discovery and replication samples (sign test, $P = 0.006$), and the minimum P-value was 1.0×10^{-6} at rs2668193 (chr3:185 419 374).

In the MDD-BIP cross-disorder analyses, we evaluated support for a broader mood disorders phenotype. Due to the need to resolve overlapping subjects, the sample sizes and P-values differ from the numbers given above. There were 32 050 independent subjects (9238 MDD cases/8039 controls and 6998 BIP cases/ 7775 controls), 160 SNPs with $P < 0.0001$ in the MDD discovery phase and 659 SNPs in the BIP discovery phase (no SNP had $P < 0.0001$ for both MDD and BIP). First, in aggregate, SNPs selected from the BIP discovery phase showed evidence of replication in MDD (65 of 100 independent SNPs had logistic regression β-coefficients in the same direction in both BIP and MDD, sign test, $P = 0.0018$). However, the reverse comparison was near chance level (46 of 76 independent SNPs selected from MDD analyses had consistent effects in BIP, sign test $P = 0.042$). Second, in the combined analysis of these 819 SNPs, 15 exceeded genome-wide significance ($P < 5 \times 10^{-8}$) and all were in a 248 kb interval of high LD on 3p21.1 (chr3:52 425 083–53 822 102, minimum $P = 5.9 \times 10^{-9}$ at rs2535629; Supplementary Table S19, Supplementary Figure S20). The 116 SNPs in this region were all selected from the BIP sample ($P < 0.0001$), and none from the MDD sample. The region of strongest signal contained 84 SNPs from rs2878628 to rs2535629 (chr3:52 559 755–52 808 259). This region contains multiple genes: PBRM1 (chromatin remodeling and renal cell cancer), GNL3 (stem cell maintenance and tumorgenesis), GLT8D1, SPCS1, NEK4, the ITIH1-ITIH3-ITIH4 gene cluster (possibly involved in cancer), four micro-RNA and three small nucleolar RNA genes. This region had genome-wide significant findings in three prior GWAS: rs1042779 (chr3:52 796 051) for BIP,[66] rs736408 (chr3:52 810 394) for a combined BIP-schizophrenia phenotype[36] and rs2251219 (chr3:52 559 827) for a combined MDD-BIP phenotype[67] (although a reanalysis suggested most of the signal arose from the BIP group).[68] The PGC analyses include nearly all subjects in the prior reports, and thus cannot be considered independent evidence. As discussed below, we advise caution in interpreting this result.

We conducted a set of pre-planned secondary analyses using the discovery samples. These analyses presume that observable clinical features allow the ability to index etiological genetic heterogeneity. The clinical features we chose—sex, age of onset, recurrence and typicality—had a rationale from genetic epidemiological studies, and were comparably assessed in most of the discovery samples (Supplementary Methods). The results are summarized in Table 2, and detail on regions with $P < 1 \times 10^{-5}$ provided in Supplementary Table S21. Parallel analyses of chrX SNPs for these secondary phenotypes also failed to identify convincing associations. Given the level of resolution afforded by our sample size and genotyping, none of these clinical features successfully indexed the clinical heterogeneity of MDD (all λ_{1000} values were small and no P-value approached genome-wide significance). However, we note that the total samples available for these analyses were small for a GWAS of a complex and modestly heritable trait. Moreover, as described above, SNPs identified in analyses by sex and for recurrent MDD did not yield genome-wide significance in replication in external samples.

Finally, under the assumptions that MDD is highly polygenic and that power is not optimal,[69,70] we conducted risk profile analyses using the MDD

Table 2 Summary of secondary analyses

Secondary analysis	Cases	Controls	λ_{1000}	Best finding
Primary analyses as reference				
Discovery phase	9240	9519	1.008	rs11579964, chr1:222,605,563, $P = 1.0 \times 10^{-7}$
Combined discovery plus replication	16 023	60 214	NA	rs1969253, chr3:185,359,206, $P = 3.4 \times 10^{-6}$
(a) By sex				
Females	6118	5366	1.005	rs1969253, chr3:185,359,206, $P = 1.0 \times 10^{-7}$
Males	3122	4153	0.999	rs7296288, chr12:47,766,235, $P = 2.3 \times 10^{-7}$
(b) Onset and recurrence				
Recurrent	6743	9519	1.006	rs4478239, chr4:188,428,300, $P = 4.7 \times 10^{-7}$
Recurrent early onset ($\leqslant$ 30 years)	4710	9519	1.007	rs1276324, chr18:19,172,417, $P = 6.7 \times 10^{-7}$
Childhood onset ($\leqslant$ 12 years)	774	6077	1.015	rs4358615, chr6:27,106,546, $P = 2.3 \times 10^{-6}$
Age of onset as a continuous trait	8920	—	0.998	rs16948388, chr17:45,242,175, $P = 1.0 \times 10^{-6}$
(c) Sub-type analysis				
Latent class 1 (weight loss and insomnia)	3814	9519	1.007	rs9830950, chr3:61,097,358, $P = 1.0 \times 10^{-7}$

λ_{1000} is the genomic inflation factor scaled to a constant sample size of 1000 cases and 1000 controls. Age of onset analyzed using a square root transformation.

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discovery phase samples. We split these samples into two sets and used 80% to develop a risk profile to predict case–control status in the remaining 20% of the samples (Supplementary Methods). These analyses showed a modest ($R^2 = 0.6\%$) but highly significant ($P < 10^{-6}$) predictive capacity.

Discussion

This is the largest and most comprehensive genetic study of MDD. There were 18 759 subjects in the MDD discovery phase, 57 478 subjects in the MDD replication phase and 32 050 subjects in cross-disorder analyses of MDD and BIP. Analyses included the primary phenotype of MDD, three sets of autosomal imputation data (HapMap3, HapMap2 and 1000 Genomes), analysis of chrX, and multiple sub-phenotypes selected based on prior epidemiological and genetic epidemiological studies (Table 2).

The primary finding of this paper is that no locus reached genome-wide significance in the combined discovery and replication analysis of MDD. Our results are consistent with null results from other MDD meta-analyses using subsets of the present sample.[22,23,25,28] The risk profile analyses are consistent with the presence of genetic effects, which our analysis was underpowered to detect. Although not significant, several analyses (that is, MDD, females-only and recurrent MDD) pointed at a region on chr3:185.3 Mb near the gene (*DVL3*) encoding the Wnt-signaling phosphoprotein disheveled 3. *DVL3* transcripts are decreased in the nucleus accumbens of individuals with MDD[71] and are overexpressed in the leukocytes of individuals reporting social isolation,[72] and the DVL3 protein product is upregulated in rats after treatment with antipsychotics.[73,74] The chr3:185.3 Mb region also contains several serotonin receptors (*HTR3D*, *HTR3C* and *HTR3E*). However, none of these analyses were strongly compelling.

We advise caution in interpreting the evidence for association of SNPs on 3p21.1 with a broad mood disorder phenotype based on the combined PGC MDD and BIP discovery samples (minimum $P = 5.9 \times 10^{-9}$ at rs2535629, chr3:52808259). Evidence to date suggests that this locus is associated with BIP[66] and schizophrenia,[36] and an even broader association was suggested by a PGC meta-analysis of MDD, BIP, schizophrenia, ADHD and autism. This separate PGC analysis included nearly all of the samples reported here, and the top finding was again for rs2535629 ($P = 2.5 \times 10^{-12}$).[75] The BIP sample made the strongest contribution to the combined analysis (OR = 1.15) followed by schizophrenia (OR = 1.10), MDD (OR = 1.10), ADHD (OR = 1.05) and autism (OR = 1.05). Although a five-disorder model was statistically the most likely and significant heterogeneity of ORs across disorders was not detected, the MDD replication data reported here raise some questions whether MDD also has an association in this region. We obtained MDD replication data for two SNPs on 3p21.1 (Supplementary Table S18), and

observed no additional support for association for rs2535629 (discovery $P = 0.0001$, replication $P = 0.56$, combined $P = 0.002$) or rs3773729 (discovery $P = 0.00022$, replication $P = 0.022$ with different direction of association, combined $P = 0.0095$). Similarly, replication samples for the PGC BIP study[36] provided little additional evidence for two SNPs in this region (rs736408 and rs3774609). In contrast, stronger evidence for association was observed in the PGC SCZ study after adding data from replication samples (rs2239547, chr3:52 830 269; discovery $P = 2.2 \times 10^{-6}$, replication $P = 0.003$, combined $P = 6 \times 10^{-8}$).[37] The PGC analyses reported here include most samples used in previous reports of genome-wide significant association in this region for BIP,[66] BIP-SCZ[36] and MDD-BIP,[67] underscoring the need for analysis of independent samples.

Thus, this locus has produced genome-wide significant evidence for association to BIP,[66] with evidence for broader set of associated phenotypes (especially SCZ).[36,75] The inconsistency of results in large MDD and BIP replication samples suggests that the current finding should be viewed with caution. If specific genetic variants can be identified that underlie the BIP association in this region, it will be possible to evaluate their degree of association with other phenotypes including MDD. A continuing challenge in this field is the differentiation between true pleiotropy (genetic risk factors associated with distinct phenotypes) versus diagnostic misclassification (phenotypic overlap in cases with different genetic risk factors, leading to diagnostic 'error'). There is a robust and evolving literature in psychiatric genetic epidemiology regarding the degree of independence versus co-segregation of current diagnostic categories, as well as the occurrence and familial risks of cases with mixed syndromes and changes in clinical syndromes over time. It is likely that analyses of large-scale genomic data will provide new perspectives on these issues.

On the whole, these results for MDD are in sharp contrast to the now substantial experience with GWAS for other complex human traits. GWAS has been a widely applied (> 860 studies) and remarkably successful technology in the identification of > 2200 strong associations for a wide range of biomedical diseases and traits.[14] The vast majority of GWAS with sample sizes > 18 000 found at least one genome-wide significant finding (178/189 studies, 94.2%),[14] and yet we found no such associations for MDD. What implications do these null results have for research into the genetics of MDD? Why might the results have turned out this way? We frame our discussion around a series of implications and hypotheses for future research.

Caveat: genome coverage
The genotyping chips used by the primary studies had good coverage of common variation across the genome. It is possible that genetic variation important in the etiology of MDD was missed if LD was

insufficient with genotyped variants. In particular, we had suboptimal or poor coverage of uncommon variation (MAF 0.005–0.05), and we have not yet analyzed copy number variation (PGC analyses of copy number variants are underway). In addition, the discovery studies used eight genotyping platforms, and it is possible that causal common variation was missed because not all platforms had good coverage in the same regions. However, these caveats should be interpreted in the context of the many successful GWAS meta-analyses that faced similar limitations.

Implication: exclusions
For the phenotype of MDD, we can exclude combinations of MAF and effect size with 90% power. The exclusionary regions are genotypic relative risks (GRRs) $\geqslant 1.16$ for MAF 0.30–0.50, $\geqslant 1.18$ for MAF 0.20–0.25, $\geqslant 1.21$ for MAF 0.15, $\geqslant 1.25$ for MAF 0.10 and $\geqslant 1.36$ for MAF 0.05. The technologies we used for genotyping probably captured the more common variation well, but were progressively less comprehensive at lower MAF. These exclusion GRRs equate to a variance in liability of $\sim 0.5\%$. Since this study was conceived, we have gained considerable knowledge about the likely effect sizes of variants contributing to common complex disease. Therefore, these exclusion architectures are not unexpected.

Implication: future sample sizes
Association studies in psychiatry have traditionally had small sample sizes (< 1000 total subjects). For even a modest amount of genotyping in a candidate gene (10 SNPs), 90% power to detect a genotypic relative risk of 1.16 at MAF 0.30 requires 3600 cases and 3600 controls. It is possible to speculate that larger genetic effects exist at smaller MAF (0.005–0.05). Investigators, reviewers and editors need to be cognizant of these requirements, as smaller samples may be difficult to interpret due to inadequate power.

Hypothesis: suboptimal phenotype
MDD is defined descriptively without reference to any underlying biology, biomarker or pathophysiology.[76,77] Genetic epidemiological studies have suggested that subtypes of MDD might be more familial or have higher heritability (for example, recurrent MDD,[10] recurrent early-onset MDD[11] and clinically ascertained MDD[12]). It is possible that well-powered genetic studies of these less common and arguably more heritable forms of MDD would have greater success. However, a sizable fraction of our cases were from hospital sources and our analyses of recurrent MDD and recurrent early-onset MDD were unrevealing, although these observations are qualified by the smaller sample sizes. The selection of a phenotype for genetic studies presents a dilemma for MDD researchers: larger samples which are more representative of the population can be achieved for broadly defined MDD, whereas restricted phenotypes may be more familial but are more difficult to recruit in large numbers from the population. Some other forms of MDD can only be defined using methods that are difficult to operationalize in large samples (for example, extensive clinical interviews, biological assays like repeated hormone measures or brain imaging).

Hypothesis: MDD is particularly heterogeneous
An early criticism of GWAS meta-analysis was that combining samples from multiple sites to increase sample size would introduce crippling heterogeneity. This concern was not borne out by experience. Indeed, the number of significant associations has increased as more individual studies have been combined using meta-analysis for other heterogeneous diseases such as Type 2 diabetes mellitus,[32] inflammatory bowel disease[78] and multiple cancers[79,80] along with anthropometric traits like height[29] and body mass.[30] It is possible that MDD might be exceptional, and have greater clinical and etiological heterogeneity, as well as non-genetic phenocopies. The different endorsement rates of the MDD criteria between cohorts may support this conjecture (Supplementary Table S12). Higher heterogeneity implies reduced statistical power as the genetic effect size distribution will be diluted. Higher heterogeneity— that is, many different 'types' of MDD—would suggest that identifying more optimal MDD-related phenotypes may be a practical step forward if adequate sample sizes could be achieved.

Hypothesis: MDD has a divergent genetic architecture
The unquestionable success of GWAS in identifying strong and replicable associations for so many human diseases is intriguing given that the additive logistic regression model generally used is rudimentary. The dependent variable is disease status (1 = yes, 0 = no), the continuous independent variable is a SNP genotype (coded as the number of copies of the minor allele or as the imputed allelic dosage, 0–2), plus covariates like principal components to adjust for ancestry. It is possible that MDD is distinctive, and that the additive logistic model is not an adequate approximation of the genetic architecture of MDD (see Kohli *et al.*[26]). There are numerous alternative genetic architectures, although many are at least partly detectable using an additive model.

There has been considerable speculation that gene–environment interactions are particularly salient for MDD. It is possible that MDD can only be understood if genetic and environmental risk factors are modeled simultaneously. The most prominent example for MDD is the moderation of environmental stress by genetic variation in a functional polymorphism near the serotonin transporter (5-HTTLPR).[81] As in the initial report in 2003, some evidence has supported this GxE interaction[82,83] other analyses have not[84,85] and the original finding (from a longitudinal study in Dunedin, New Zealand) did not replicate in an independent longitudinal study in Christchurch, New Zealand.[86] A practical issue is again the tradeoff between relatively inexpensive, cross-sectional assessments of MDD case and control status and the

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detailed longitudinal data required to accurately characterize environmental stressors.

Hypothesis: insufficient power
Although this is one of the largest GWAS analyses ever conducted in psychiatry (second only to the PGC schizophrenia study),[37] the sample size may still have been too small. The very small but highly significant variance explained in the polygenic risk score analyses ($P < 10^{-6}$ testing one hypothesis) is consistent with a hypothesis of insufficient power in this study.

The overlapping hypotheses listed above imply that an association study for MDD has less power than for studies of many other complex genetic disorders. However, even if the hypotheses listed above were not the contributing factors, we may still conclude that insufficient power underpins the dearth of results from this mega-analysis by considering the epidemiology of MDD. MDD is highly prevalent in the population, implying that cases are less extreme in the population compared with the controls and therefore larger sample sizes are required. For example, we have calculated that sample sizes 2.4 times larger are needed for GWAS of MDD (prevalence 0.15) compared with schizophrenia (prevalence 0.007).[25,87] Furthermore, if we assume as a first approximation that the number and frequency distribution of risk alleles is the same for MDD and schizophrenia, then samples sizes five times larger are needed to account for the lower heritability of MDD (0.37)[10] compared with schizophrenia (0.81),[88] implying lower effect sizes at each locus (see Wray *et al.*[25] and Yang *et al.*[87] for details). Obtaining a total sample size on the order of 100 000 MDD cases plus controls would require a significant investment for ascertainment, phenotyping, DNA collection and genotyping, but could be accomplished using national registers or via electronic medical records of large health care organizations. Such sample sizes have been achieved in studies of quantitative traits and yielded large numbers of genome-wide significant results.[29,30]

Conclusion

This report contributes important new data about the nature of MDD.[33] Unlike a large number of other GWAS that provide precious etiological clues, our analyses are more informative about what MDD is not. The path to progress is likely to be more difficult for MDD, but there are a number of rational next steps. We have offered some ideas about how progress might be achieved. The PGC is conducting GWAS meta-analyses across ADHD, autism, BIP, MDD and schizophrenia, and these very large analyses could identify genetic variants that predispose or protect to psychiatric disorders in general, and thus provide key initial findings that could be used to disentangle the etiology of MDD. Analysis of copy number variation has provided important leads for autism and schizophrenia, and might prove informative for MDD.

Conflict of interest

Acknowledgments

Author contributions

All collaborators reviewed and approved the final version of the manuscript. Overall coordination: Patrick F Sullivan. Statistical analysis: Mark J Daly (lead), Stephan Ripke, Cathryn M Lewis, Dan-Yu Lin, Naomi R Wray, Benjamin Neale, Douglas F Levinson, Gerome Breen, Enda M Byrne. Phenotype analysis: Naomi R Wray (lead), Douglas F Levinson, Marcella Rietschel, Witte Hoogendijk, Stephan Ripke. Writing committee: Patrick F Sullivan (lead), Steven P Hamilton, Douglas F Levinson, Cathryn M Lewis, Stephan Ripke, Myrna M Weissman, Naomi R Wray. MDD discovery sample data contributed by: Bonn/Mannheim study—René Breuer, Sven Cichon, Franziska Degenhardt, Josef Frank, Magdalena Gross, Stefan Herms, Susanne Hoefels, Wolfgang Maier, Manuel Mattheisen, Markus M Nöethen, Marcella Rietschel, Thomas G Schulze, Michael Steffens, Jens Treutlein; GAIN MDD study—Dorret I Boomsma, Eco J De Geus, Witte Hoogendijk, Jouke Jan Hottenga, Tzeng Jung-Ying, Dan-Yu Lin, Christel M Middeldorp, Willem A Nolen, Brenda P Penninx, Johannes H Smit, Patrick F Sullivan, Gerard van Grootheest, Gonneke Willemsen, Frans G Zitman; GenRED study—William H Coryell, James A Knowles, William B Lawson, Douglas F Levinson, James B Potash, William A Scheftner, Jianxin Shi, Myrna M Weissman; GlaxoSmithKline study—Florian Holsboer, Pierandrea Muglia, Federica Tozzi; MDD2000 study—Douglas HR Blackwood, Dorret I Boomsma, Eco J De Geus, Jouke Jan Hottenga, Donald J MacIntyre, Andrew McIntosh, Alan McLean, Christel M Middeldorp, Willem A Nolen, Brenda P Penninx, Stephan Ripke, Johannes H Smit, Patrick F Sullivan, Gerard van Grootheest, Gonneke Willemsen, Frans G Zitman, Edwin JCG van den Oord; Max Planck Institute of Psychiatry Study—Florian Holsboer, Susanne Lucae, Elisabeth Binder, Bertram Müller-Myhsok, Stephan Ripke, Darina Czamara, Martin A Kohli, Marcus Ising, Manfred Uhr, Thomas Bettecken; RADIANT study—Michael R Barnes, Gerome Breen, Ian W Craig, Anne E Farmer, Cathryn M Lewis, Peter McGuffin, Pierandrea Muglia; Queensland Institute for Medical Research study—Enda Byrne, Scott D Gordon, Andrew C Heath, Anjali K Henders, Ian B Hickie, Pamela AF Madden, Nicholas G Martin, Grant M Montgomery, Dale R Nyholt, Michele L Pergadia, Naomi R Wray; and STAR*D study—Steven P Hamilton, Patrick J McGrath, Stanley I Shyn, Susan L Slager.

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

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mpip	Prof	Bertram	Müller-Myhsok	Max Planck Institute of Psychiatry	Germany	Collaborator
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SHIP-LEGEND	Dr	Alexander	Teumer	University of Greifswald	Germany	Genetic analyses
TwinGene		Alexander	Viktorin	Karolinska Institutet	Sweden	Genetic analyses
radiant	Dr	Michael R	Barnes	GlaxoSmithKline	UK	Co-investigator
mpip	Dr	Thomas	Bettecken	Max Planck Institute of Psychiatry	Germany	Collaborator
mpip	Dr	Elisabeth B	Binder	Max Planck Institute of Psychiatry	Germany	Collaborator
boma		René	Breuer	Central Inst Mental Health, University of Heidelberg	Germany	Collaborator
Harvard i2b2		Victor M	Castro	Partners HealthCare System	USA	Bioinformatician

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Study	Title	First name	Last name	Affiliation	Country	Role on primary study
Harvard i2b2	Dr	Susanne E	Churchill	Partners HealthCare System	USA	Co-investigator
genred, GenRED2	Prof	William H	Coryell	University of Iowa	USA	PI
radiant	Prof	Nick	Craddock	Cardiff University	UK	Co-investigator
radiant	Prof	Ian W	Craig	Institute of Psychiatry, King's College London	UK	Co-investigator
mpip	Dr	Darina	Czamara	Max Planck Institute of Psychiatry	Germany	Collaborator
gain, mdd2000	Prof	Eco J	De Geus	VU University, Amsterdam	The Netherlands	Co-investigator GAIN, co-PI NTR
boma		Franziska	Degenhardt	University of Bonn	Germany	Collaborator
radiant	Prof	Anne E	Farmer	Institute of Psychiatry, King's College London	UK	Co-investigator
Harvard i2b2	Prof	Maurizio	Fava	Massachusetts General Hospital	USA	Co-investigator
boma		Josef	Frank	Central Inst Mental Health, University of Heidelberg	Germany	Collaborator
Harvard i2b2		Vivian S	Gainer	Partners HealthCare System	USA	Bioinformatician
Harvard i2b2		Patience J	Gallagher	Massachusetts General Hospital	USA	Research Coordinator
QIMR, mdd2000	Dr	Scott D	Gordon	Queensland Institute of Medical Research	Australia	Genetic analyses
Harvard i2b2		Sergey	Goryachev	Partners HealthCare System	USA	Bioinformatician
boma		Magdalena	Gross	University of Bonn	Germany	Collaborator
genpod	Dr	Michel	Guipponi	University of Geneva	Switzerland	Co-investigator
QIMR, mdd2000		Anjali K	Henders	Queensland Institute of Medical Research	Australia	QIMR project manager
boma		Stefan	Herms	University of Bonn	Germany	Collaborator
QIMR, mdd2000	Prof	Ian B	Hickie	University of Sydney, Sydney	Australia	QIMR funding
boma		Susanne	Hoefels	University of Bonn	Germany	Collaborator
gain	Prof	Witte	Hoogendijk	Erasmus Medical Center	The Netherlands	Co-investigator
gain, mdd2000	Dr	Jouke Jan	Hottenga	VU University, Amsterdam	The Netherlands	Genetic analyses
Harvard i2b2	Assoc Prof	Dan V	Iosifescu	Massachusetts General Hospital	USA	Co-investigator
mpip	Dr	Marcus	Ising	Max Planck Institute of Psychiatry	Germany	Collaborator
radiant	Dr	Ian	Jones	Cardiff University	UK	Co-investigator
radiant	Dr	Lisa	Jones	University of Birmingham	UK	Co-investigator
gain	Assoc Prof	Tzeng	Jung-Ying	North Carolina State University	USA	Statistical geneticist
genred, GenRED2	Prof	James A	Knowles	University of Southern California	USA	Co-PI
Harvard i2b2	Prof	Isaac S	Kohane	Brigham and Women's Hospital	USA	Co-investigator
mpip	Dr	Martin A	Kohli	Max Planck Institute of Psychiatry	Germany	Collaborator
radiant	Dr	Ania	Korszun	Queen Mary University of London	UK	Co-investigator
TwinGene	Dr	Mikael	Landen	Karolinska Institutet	Sweden	Co-investigator
genred, GenRED2	Prof	William B	Lawson	Howard University	USA	PI
genpod	Prof	Glyn	Lewis	University of Bristol	UK	PI
mdd2000	Dr	Donald	MacIntyre	University of Edinburgh	UK	Co-investigator
boma	Prof	Wolfgang	Maier	University of Bonn	Germany	Collaborator
boma	Dr	Manuel	Mattheisen	University of Bonn	Germany	Collaborator
stard	Prof	Patrick J	McGrath	Columbia University	USA	Co-PI
mdd2000	Dr	Andrew	McIntosh	University of Edinburgh	UK	Co-investigator
mdd2000	Dr	Alan	McLean	University of Edinburgh	UK	Co-investigator
gain, mdd2000	Dr	Christel M	Middeldorp	VU University, Amsterdam	The Netherlands	Phenotype collection
radiant	Dr	Lefkos	Middleton	Imperial College	UK	Co-investigator
QIMR, mdd2000	Prof	Grant M	Montgomery	Queensland Institute of Medical Research	Australia	QIMR sample and funding
Harvard i2b2	Asst Prof	Shawn N	Murphy	Massachusetts General Hospital	USA	Co-investigator
SHIP-LEGEND	Prof	Matthias	Nauck	University of Greifswald	Germany	Biobanking
gain, mdd2000	Prof	Willem A	Nolen	Groningen University Medical Center	The Netherlands	Site PI NESDA
QIMR, mdd2000	Dr	Dale R	Nyholt	Queensland Institute of Medical Research	Australia	Genetic analyses

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Study	Title	First name	Last name	Affiliation	Country	Role on primary study
genpod	Prof	Michael	O'Donovan	Cardiff University	UK	Co-investigator
deCODE	Dr	Högni	Oskarsson	Therapeia, University Hospital	Iceland	Phenotype collection
TwinGene	Dr	Nancy	Pedersen	Karolinska Institutet	Sweden	Co-investigator
genred, GenRED2	Prof	William A	Scheftner	Rush University Medical Center	USA	PI
SHIP-LEGEND		Andrea	Schulz	University of Greifswald	Germany	Phenotype collection
boma	Prof	Thomas G	Schulze	University of Goettingen	USA	Collaborator
stard	Asst Prof	Stanley I	Shyn	University of Washington	USA	Collaborator
deCODE	Dr	Engilbert	Sigurdsson	Landspitali University Hospital	Iceland	Phenotype collection
stard	Assoc Prof	Susan L	Slager	Mayo Clinic	USA	Co-PI
gain, mdd2000	Prof	Johannes H	Smit	VU University Medical Center, Amsterdam	The Netherlands	Co-investigator GAIN, phenotype collection NESDA
deCODE	Dr	Hreinn	Stefansson	deCODE Genetics	Iceland	Co-investigator
boma	Dr	Michael	Steffens	University of Bonn	Germany	Collaborator
deCODE	Dr	Thorgeir	Thorgeirsson	deCODE Genetics	Iceland	Co-investigator
gsk	Dr	Federica	Tozzi	GlaxoSmithKline	Italy	Co-investigator
boma	Dr	Jens	Treutlein	Central Inst Mental Health, University of Heidelberg	Germany	Collaborator
mpip	Dr	Manfred	Uhr	Max Planck Institute of Psychiatry	Germany	Collaborator
mdd2000	Prof	Edwin JCG	van den Oord	Virginia Commonwealth University	USA	Funding
gain, mdd2000		Gerard	Van Grootheest	VU University Medical Center, Amsterdam	The Netherlands	Phenotype collection
SHIP-LEGEND	Prof	Henry	Völzke	University of Greifswald	Germany	PI
Harvard i2b2	Dr	Jeffrey B	Weilburg	Massachusetts General Hospital	USA	Co-investigator
gain, mdd2000	Dr	Gonneke	Willemsen	VU University, Amsterdam	The Netherlands	Phenotype collection
gain, mdd2000	Prof	Frans G	Zitman	Leiden University Medical Center, Leiden	The Netherlands	Site PI NESDA
statistical genetics	Dr	Benjamin	Neale	Harvard University/Broad Institute	USA	Collaborator
statistical genetics	Assoc Prof	Mark	Daly	Harvard University/Broad Institute	USA	Statistical geneticist
genred, GenRED2, DepGenesNetworks	Prof	Douglas F	Levinson	Stanford University	USA	PI
gain, mdd2000	Prof	Patrick F	Sullivan	University of North Carolina	USA	PI