IMMEDIATE COMMUNICATION

Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo

PF Sullivan¹, EJC de Geus², G Willemsen², MR James³, JH Smit⁴, T Zandbelt⁴, V Arolt⁵, BT Baune⁶, D Blackwood⁷, S Cichon⁸, WL Coventry⁹, K Domshche⁹, A Farmer¹⁰, M Fava¹¹, SD Gordon³, Q He¹, AC Heath¹², P Heutink⁴, F Holsboer¹³, WJ Hoogendijk⁴, JJ Hottenga², Y Hu¹, M Kohli¹³, D Lin¹, S Lucae¹³, DJ MacIntyre¹⁴, W Maier⁸, KA McGhee⁷, P McGuffin¹⁰, GW Montgomery³, WJ Muir⁷, WA Nolen¹⁵, MM Nöthen⁸, RH Perlis¹¹, K Pirlo¹⁰, D Posthumᵃ², M Rietschel¹⁶, P Rizzu⁴, A Schosser¹⁰, AB Smit², JW Smoller¹¹, J-Y Tzeng¹⁷, R van Dyck⁴, M Verhage⁸, FG Zitman¹⁸, NG Martin³, NR Wray³, DI Boomsma²,¹⁹ and BWJH Penninx⁴,¹⁹

¹Department of Genetics, University of North Carolina, Chapel Hill, NC, USA; ²VU University Amsterdam, Amsterdam, The Netherlands; ³Queensland Institute for Medical Research, Brisbane, QLD, Australia; ⁴VU University Medical Center Amsterdam, Amsterdam, The Netherlands; ⁵University of Münster, Münster, Germany; ⁶James Cook University, Cairns, QLD, Australia; ⁷University of Edinburgh, Edinburgh, UK; ⁸University of Bonn, Bonn, Germany; ⁹University of New England, Armidale, NSW, Australia; ¹⁰Institute of Psychiatry, London, UK; ¹¹Harvard Medical School, Cambridge, MA, USA; ¹²Washington University, St. Louis, MO, USA; ¹³Max-Planck Institute of Psychiatry, Munich, Germany; ¹⁴Royal Edinburgh Hospital, Edinburgh, UK; ¹⁵University Medical Center Groningen, Groningen, The Netherlands; ¹⁶University of Heidelberg, Heidelberg, Germany; ¹⁷North Carolina State University, Raleigh, NC, USA and ¹⁸Leiden University Medical Center, Leiden, The Netherlands

Major depressive disorder (MDD) is a common complex trait with enormous public health significance. As part of the Genetic Association Information Network initiative of the US Foundation for the National Institutes of Health, we conducted a genome-wide association study of 435,291 single nucleotide polymorphisms (SNPs) genotyped in 1738 MDD cases and 1802 controls selected to be at low liability for MDD. Of the top 200, 11 signals localized to a 167 kb region overlapping the gene piccolo (PCLO, whose protein product localizes to the cytomatrix of the presynaptic active zone and is important in monoaminergic neurotransmission in the brain) with \( P \)-values of \( 7.7 \times 10^{-7} \) for rs2715148 and \( 1.2 \times 10^{-6} \) for rs2522833. We undertook replication of SNPs in this region in five independent samples (6079 MDD independent cases and 5893 controls) but no SNP exceeded the replication significance threshold when all replication samples were analyzed together. However, there was heterogeneity in the replication samples, and secondary analysis of the original sample with the sample of greatest similarity yielded \( P = 6.4 \times 10^{-8} \) for the nonsynonymous SNP rs2522833 that gives rise to a serine to alanine substitution near a C2 calcium-binding domain of the PCLO protein. With the integrated replication effort, we present a specific hypothesis for further studies.

Keywords: major depressive disorder; genome-wide association; Netherlands study of depression and anxiety; Netherlands twin registry

Introduction

The defining features of major depressive disorder (MDD) are marked and persistent dysphoria plus additional cognitive signs and symptoms (anhedonia, sleep disturbance, weight/appetite changes, motor agitation/retardation, anergia, excessive guilt or worthlessness, poor concentration or indecisiveness, and recurrent thoughts of death or suicide).¹ MDD is distinct from normal sadness by its persistence (that is, \( \geq 2 \) weeks), additional signs and symptoms, and substantial associated impairment. The definition of MDD excludes other conditions typified by substantial depressive symptoms (other psychiatric disorders, drug/alcohol dependence and somatic diseases). The lifetime prevalence of MDD is \( \sim 15\%²–⁴ \) and is twofold higher in women⁵ with a course typified by recurrence of illness.⁶ It is associated with considerable morbidity,⁷–⁹ excess mortality from suicide and...
other causes,10–13 and substantial direct and indirect costs.14 A World Health Organization study projected MDD to be the second leading cause of disability worldwide by 2020.15

Although there is a considerable corpus of research on the epidemiology and biological correlates of MDD, little is known for certain about its etiology. An important etiological clue may be the familial tendency of MDD and its heritability of 31–42%.16 This clue led to a number of genome-wide linkage studies (Supplementary Methods) and studies of >100 theoretical or positional candidate genes. As for the use of these study designs with other biomedical disorders, their application to MDD has not been as successful as had been hoped.

It is now clear that genome-wide association studies (GWASs) can be a successful tool in the genetic dissection of complex biomedical disorders.17,18 The goal of this report is to describe a GWAS for MDD that was systematically designed to remediate a set of methodological issues common to genetic studies of MDD. Examples of these issues include small sample sizes, inhomogeneous samples in terms of ancestry and phenotyping, convenience sampling, and controls that are unaffected but not at low liability for MDD. Moreover, large-scale replication was integral to our design.

Materials and methods

This GWAS was one of the six initial Genetic Association Information Network (GAIN) studies sponsored by the Foundation for the NIH.19 Individual phenotype and genotype data are available to researchers by application to the dbGaP repository.20 We have attempted to follow published guidelines for GWAS (Chanock et al.,21 Box 1).

Subjects

The parent projects that supplied subjects for this GWASs are longitudinal studies, the Netherlands Study of Depression and Anxiety (NESDA; http://www.nesda.nl)22 and the Netherlands Twin Registry (NTR; http://www.tweelingenregister.org).23 Sampling and data collection characteristics of the GAIN–MDD study have been described in detail elsewhere.24 MDD cases were mainly from NESDA, a longitudinal cohort study designed to be representative of individuals with depressive and/or anxiety disorders. Recruitment of participants for NESDA took place from 09/2004–02/2007, and ascertainment was from outpatient specialist mental health facilities and by primary care screening. Additional cases were from the population-based cohorts NEMESIS,25 AR-IADNE26 and the NTR. Regardless of recruitment setting, similar inclusion and exclusion criteria were used to select MDD cases. Inclusion criteria were a lifetime diagnosis of DSM-IV MDD1 as diagnosed by the Composite International Diagnostic Interview psychiatric interview,27 age 18–65 years, and self-reported western European ancestry. Persons who were not fluent in Dutch and those with a primary diagnosis of schizophrenia or schizoaffective disorder, obsessive–compulsive disorder, bipolar disorder or severe substance use dependence were excluded (the etiology of MDD in these subjects may be distinct). The 1862 cases included in GAIN were recruited from mental health care organizations (N=785), primary care (N=603) and community samples (NEMESIS N=218, AR-IADNE N=96 and NTR N=160).

Control subjects were mainly from the NTR, which has collected longitudinal data from twins and their families since 1991 (total cohort of ~22 000 participants from 5546 families). The majority of families were recruited when the twins were adolescents or young adults through city council registrations along with alternative efforts to recruit older twins. Longitudinal phenotyping includes assessment of depressive symptoms (via multiple instruments), anxiety, neuroticism and other personality measures. Inclusion required availability of both survey data and biological samples, no report of MDD at any measurement occasion, and low genetic liability for MDD. No report of MDD was determined by the use of a factor score derived from longitudinal measures of neuroticism, anxiety and depressive symptoms28 (mean 0, s.d. 0.7); controls were required never to have scored highly (≥0.65) on this factor score. Finally, controls and their parents were required to have been born in the Netherlands or western Europe. Only one control per family was selected. There were controls (N=1703) from the NTR and additional controls from NESDA (N=133 from general practice, N=24 from AR-IADNE). NESDA controls had no lifetime diagnosis of MDD or an anxiety disorder as assessed by the CIDI and reported low depressive symptoms at baseline (K-10 score <16 and inventory of depressive symptoms score <4).29,30

Case–control matching

If there were multiple eligible NTR controls in a family, we first matched on sex and age, and used the highest number of completed questionnaires as an additional criterion. Again, only one control per family was included.

DNA sampling

Before the start of the NESDA and NTR biological sample collection, processing, and storage protocols were harmonized and DNA extraction was conducted concurrently in the same laboratory. For NESDA, blood sampling for the NESDA participants took place during the baseline visit (between 0830 and 0930 hours) and DNA was isolated using the FlexiGene DNA AGF3000 kit (Qiagen, Valencia, CA, USA) on an AutoGenFlex 3000 workstation (Autogen,
Holliston, MA, USA). For NTR, biological samples were taken in the subject’s home (between 0700 and 1000 hours) and DNA was extracted using the Puregene DNA isolation kit (Gentra, Minneapolis, MN, USA) for frozen whole blood samples. DNA concentrations were determined using the PicoGreen dsDNA Quantitation Kit (Invitrogen Corporation, Carlsbad, CA, USA). All procedures were performed according to the manufacturer’s protocols.

**Ethical issues**

The NESDA and NTR studies were approved by the Central Ethics Committee on Research Involving Human Subjects of the VU University Medical Center, Amsterdam, an Institutional Review Board certified by the US Office of Human Research Protections (IRB number IRB-2991 under Federal-wide Assurance-3703; IRB/institute codes, NESDA 03-183; NTR 03-180). All subjects provided written informed consent. As part of the GAIN application process, consent forms were specifically reviewed for suitability for the deposit of deidentified phenotype and genotype data into the controlled-access dbGaP repository.30 NESDA and NTR subjects were informed of participation in GAIN by newsletters. Only 22 NESDA respondents refused informed consent for genetic research (1.7% of all respondents approached).

**GWAS genotyping**

Individual genotyping was conducted by Perlegen Sciences (Mountain View, CA, USA) using a set of four proprietary, high-density oligonucleotide arrays. The SNPs on these arrays were selected to tag common variation in the HapMap European and Asian panels using previously described genotype data,31 tagging approach32 and methodology.33 At the beginning of GAIN, all HapMap34 samples were genotyped with the Perlegen GWAS platform. Independent review of these data by the GAIN analysis group19 showed 99.8% agreement with prior HapMap genotypes and the mean maximum $r^2$ between the Perlegen SNPs and HapMap phase II SNPs31 was 0.89 for single and 0.96 for multimarker analyses. The genotyping procedures and genotyping calling algorithms are described in the Supplementary Methods and in prior reports.35-36 Briefly, 40 × 96-well plates were sent to Perlegen for GWAS genotyping. Genotyping was conducted blind to case–control status. Cases and controls were randomly allocated to plates and to positions within plates. Each plate contained DNA samples from 93 Dutch subjects plus 3 quality control samples. The three quality control samples included: two parents of one control on that plate (40 complete trios in total); and half the plates contained the same HapMap CEU sample (used for quality control in all GAIN projects) and half had a randomly selected duplicate case sample. The total number of samples was 3840 (= 40 plates × 96 samples per plate) or 1860 cases + 1860 controls + 80 parents + 20 duplicate samples + 20 HapMap samples.

**Quality control—subjects**

Of the 3820 Dutch samples sent to Perlegen (excluding the 20 HapMap internal control samples), genotypes were delivered for 3761 samples. A total of 59 samples did not have GWAS data: 39 samples with uncertain linkage between genotype and phenotype records, 7 samples with evidence of contamination, 6 samples that failed genotyping and 7 miscellaneous failures (2 of these were excluded as chrX and chrY genotyping data were consistent with the presence of XO and XXX sex chromosome status). After further analysis, 8 subjects were removed for excessive missing genotype data (>25%), 1 case for high genome-wide homozygosity (~75%), 38 subjects whose genome-wide IBS estimates were consistent with first- or second-degree relationships and 57 additional subjects whose ancestry diverged from the remainder of the sample (see Supplementary Methods for details). After these exclusions (N = 104) and removing duplicated and trio quality control samples, there were 3540 subjects in the final analysis data set including 1738 cases and 1802 controls. The principal reason for fewer cases than controls was the higher prevalence of substantial non-European ancestry. The list of subjects in the final analyses data set is included as a Supplementary File (‘mddC.fam’).

**Quality control—SNPs**

The unfiltered data set obtained from dbGaP contained 599 156 unique SNPs. The Perlegen genotyping algorithm yielded a quality score for each individual genotype, and a more stringent quality score cutoff (>10) than that used by Perlegen was applied. The SNP quality control process is described in detail in the Supplementary Methods. Briefly, to be included in the final analysis data set, SNPs were required not to have any of the following features: gross mapping problem,37 ≥2 genotype disagreements in 40 duplicated samples, ≥2 Mendelian inheritance errors in 38 complete trio samples, minor allele frequency <0.05 or >0.05 missing genotypes in either cases or controls. A Hardy–Weinberg filter was not used as lack of fit to Hardy–Weinberg expectations can occur for valid reasons (for example, a true association)38 and given that 95.6% (= 51 592/53 994) of SNPs with $P < 0.00001$ from an exact test of Hardy–Weinberg equilibrium39 in controls were already flagged for exclusion. A total of 435 291 SNPs met these criteria and were included in the final analysis data set (included as a Supplementary File, ‘mddC.bim’). Additional quality control checks are described in the Supplementary Methods). A total of 13 controls were genotyped in a different study using the Illumina 317K platform and, of the 82 636 SNPs common to both platforms, the genotype agreement was 99.94%.

**Single-marker statistical analyses**

There were three classes of SNPs—those that could be heterozygous in all subjects (chr1-22 and chrX/PAR1), those that were heterozygous in women (non-PAR
chrX) and those that were hemizygous in men (non-PAR chrX and chrY). All SNPs that passed quality control checks were tested for association with MDD using 1 d.f. Cochran-Armitage trend tests. For complex traits, it is widely believed that the contributions of individual SNPs to disease risk are often roughly additive.\(^{40}\) The Cochran-Armitage trend test can be used to detect such effects. This test is usually recommended due to its robustness to the violation of the HWE assumption.\(^{41}\) \(P\)-values from women and men for non-PAR chrX were combined using Fisher’s method.\(^{52}\)

Population stratification artifacts were assessed in two ways. As described elsewhere,\(^{30}\) including principal components as covariates in a logistic regression model can robustly control stratification effects. To do this, we identified a set of 127 688 SNPs in linkage disequilibrium with the stratification-prone SNPs used to detect such effects. This test is usually recommended due to its robustness to the violation of the HWE assumption.\(^{41}\) \(P\)-values from women and men for non-PAR chrX were combined using Fisher’s method.\(^{52}\)

For noteworthy associations, there were additional checks to ensure that an association was not due to experimental bias. These checks included: manual inspection of SNP cluster plots to ensure reasonable performance of the genotyping calling algorithm; evaluation of conformation to Hardy–Weinberg equilibrium in controls, cases and overall (discussed in the Supplementary Methods); the checks for population stratification described above; evaluation of plate-specific association results to ensure that the overall association was not driven by one or a few plates; comparison of control MAFs to the HapMap EUR panel; and evaluation of the characteristics of a SNP in high linkage disequilibrium (‘proxy association’) as a similar association with such a SNP decreases the chance of some forms of method artifacts.

Control of false discoveries
Given the \(10^5\)–\(10^7\) statistical comparisons in a GWAS, small \(P\)-values are expected by chance. To control the risk of false discoveries, \(q\)-values\(^{45,46}\) were computed for all \(P\)-values for single-marker tests of association. A \(q\)-value is an estimate of the proportion of false discoveries among all significant markers, or the false discovery rate (FDR) for the corresponding \(P\)-value. The use of \(q\)-values is preferable to more traditional multiple testing controls because \(q\)-values provide a better balance between the competing goals of finding true positives versus controlling false discoveries, allow more similar comparisons across studies because proportions of false discoveries are much less dependent on the number of tests conducted and are relatively robust against the effects of correlated tests.\(^{45,47–54}\) The \(q\)-value threshold for declaring significance was 0.10 (that is, the top 10% of the significant findings are, on average, allowed to be false discoveries).\(^{30,55}\) FDR thresholds < 0.10 result in a disproportionate drop in power to detect true effects.

Imputation
We used two imputation approaches, the SNPMStat method of Lin et al.\(^{56}\) to impute 246 additional SNPs in the piccolo (PCLO) region and Abecasis’ MACH (v1) to impute 2 037 829 autosomal SNPs with \(R^2\) > 0.5 (a cutoff that removes \(\sim 90\%\) of SNPs with unreliable imputation results while dropping 2–3% of reliably imputed SNPs). Both SNPMStat and MACH gave similar results in the PCLO region. Imputed genotypes were used in secondary analyses. The HapMap2 EUR panel\(^{31,34}\) was used as reference.

Statistical power
Quanto\(^{57,58}\) was used to approximate statistical power given the following assumptions: two-tailed \(\alpha = 1 \times 10^{-7} (= 0.05/500000)\), 1738 cases and 1802 controls, lifetime morbid risk of MDD of 0.15 and a log additive genetic model. For statistical power of 0.80 (\(\beta = 0.20\)), the minimum detectable genotypic relative risks are 1.59, 1.40 and 1.35 for minor allele frequencies of 0.10, 0.25 and 0.40.

Software
PLINK (v1.0),\(^{43}\) SAS (v9.1.3),\(^{59}\) R (v2.6.1),\(^{60}\) HAPSTAT (v3),\(^{61–63}\) MACH1, SNPMStat,\(^{56}\) HaploView,\(^{64}\) and JMP (v6)\(^{65}\) were used for data management, quality control, statistical analyses and graphics.

Bioinformatics
All genomic locations are per NCBI Build 35 \(^{56}\) (UCSC hg17).\(^{67}\) Pseudoautosomal region 1 (PAR1) is assumed to be located on chrX:1–2 692 881 and chrY:1–2 692 881 and PAR2 on chrX:154 494 747–154 824 264 and chrY:57 372 174–57 701 691.\(^{68}\) SNP annotations were per TAMAL\(^{37}\) based chiefly on UCSC genome browser files,\(^{67}\) HapMap\(^{38}\) and dbSNP.\(^{46}\)

Results
Sample description
Table 1 presents descriptive data for cases and controls. Controls had a higher proportion of men and were slightly older (and thus were farther through the period of risk for MDD). Consistent with known correlates of MDD, cases had a significantly lower educational level, less often had a partner, were more often smokers and scored much higher on the NEO-FFI neuroticism scale.

SNP description
The analysis SNP set had 435 291 SNPs including 427 049 autosomal SNPs, 7 988 SNPs on the non-PAR portions of chrX, 239 SNPs on chrXY/PAR1, 15 SNPs on chrY and 0 SNPs on PAR2. The median SNP missingness was 0.00339 (interquartile range 0.00113–0.0105) and the median minor allele frequency was 0.2422 (interquartile range 0.1375–0.3646) with similar estimates in cases and controls. The average marker density over the genome
was 1 SNP every 7069 bases (=3077088087 bases/435291 SNPs). The median intermarker distance was 2911 bases with interquartile range 966–7374 bases and a 99th percentile of 50.1 kb.

**Single-marker association tests**

We used the Cochran-Armitage trend test to test for association of the 435291 SNPs in the GWAS data set with case/control status. The estimated $z^2$ was 1.046 (similar P-value minima and $z$ were obtained using logistic regression with 10 principal components and using a stratified Cochran–Mantel–Haenszel tests based on identity-by-state clusters).\(^{43,44}\) The minimum q-value was 0.28 (that is, if these tests were called significant, over the long term, a minimum false discovery rate of ~28% would be incurred). As the prespecified q-value threshold was 0.10, no SNP reached genome-wide significance. The proportion of all SNPs without true effects ($P_l$)\(^{44}\) was conservatively estimated to be $P_l = 0.99999954$, consistent with the presence of ~2 SNPs with true effects in these GWAS data.

Figure 1a depicts the quantile–quantile plots\(^{40}\) for these analyses. The observed $P$-values do not strongly depart from the $P$-value distribution expected by chance. Figure 1b shows a plot of $–\log_{10}(P_{\text{trend}})$ by genomic location.

Table 2 presents the findings for the top 25 SNPs. The quality control metrics—SNP missingness, agreement with HWE and similarity of the control MAFs to the HapMap EUR panel—for the top 200 smallest $P$-values from trend tests, logistic regression, and stratified analyses were all >0.962. Sixth, the $P$-values from trend tests, logistic regression, and stratified analyses were consistent (the Spearman’s correlations were all $>0.99$). Fifth, population stratification can cause false-positive findings if not corrected. Inspection of LD matrices excluded very high LD as the sole explanation for the results (Supplementary Figure 10), and none of the genotyped SNPs had high LD ($r^2 > 0.8$) with rs2715148 (the SNP with the most significant $P$-value in the PCLO region). Fifth, population stratification can cause false-positive findings but this did not appear to explain the PCLO association: (1) the same 11 SNPs had $P$-values among the top 200 associations in unadjusted analyses as well as with adjustment via principal components and stratified analyses; and (b) for the 57 SNPs in the PCLO region, the $P$-values across these three types of analyses were consistent (the Spearman’s correlations between $P$-values from trend tests, logistic regression and stratified analyses were all $>0.962$). Sixth, the minor allele frequencies in the control group in the PCLO region were usually quite similar to available EUR control groups suggesting that the PCLO findings were not due to an artifact of the control selection process (see below). Finally, bioinformatic investiga-

### Table 1 Descriptive data for cases with MDD and controls at low liability for MDD included in the GWAS

| Descriptor | Cases | Controls | Test | P
|------------|-------|----------|------|----------|
| Number of subjects genotyped | 1738 | 1802 | — | — |
| Mean age in years (s.d.) | 42.6 (12.6) | 45.1 (14.1) | $1.32\times 10^2 F = 31.1$, $P < 0.001$ | |
| Female (%) | 69.6 | 62.0 | $\chi^2 = 22.5$, $P < 0.001$ | — |
| Educational level (% low/middle/high) | 7.8/62.0/32.2 | 5.7/56.3/38.1 | $\chi^2 = 16.3$, $P < 0.001$ | — |
| Partner status (%) | 42.0 | 20.2 | $\chi^2 = 194.5$, $P < 0.001$ | — |
| Mean neuroticism (NEO, s.d.) | 39.3 (8.0) | 28.2 (5.5) | $1.29\times 10^2 F = 1831$, $P < 0.001$ | — |
| Smoking (current) (%) | 20.2 | — | — | — |
| Family history, recurrent MDD or early age of onset ( < 30 years) (%) | 94.8 | — | — | — |
| Recurrent MDD | 50.9 | — | — | — |
| Family history, recurrent MDD or early age of onset (<30 years) (%) | 94.8 | — | — | — |

Abbreviation: MDD, major depressive disorder.

*Focusing on piccolo* Although no association met genome-wide significance, there were clusters of SNPs in *PCLO* (Figure 2). Notably, 11 of the 200 smallest $P$-values localized to a 167 kb segment overlapping *PCLO*. Interest in *PCLO* was increased given its expression in brain, localization to the presynaptic active zone\(^{71}\) and involvement in monoamine neurotransmission, a venerable hypothesis of the etiology of MDD.\(^{72}\) Moreover, the third most significant SNP (rs2522833) codes for a non-synonymous amino-acid change (ala-4814-ser) in *PCLO* near its C2A calcium binding domain.\(^{73}\)

We investigated possible causes of spurious associations in the *PCLO* region (chr7:82032093–82436848). First, these findings were not due to plate effects as inspection of plate-specific association data for these SNPs did not show any marked outliers or systematic biases. Second, review of allelic intensity cluster plots on which genotype calls were based revealed adequate performance of the Perlegen genotype calling algorithm. Third, inspection of additional quality control metrics did not suggest systematic problems with SNPs in this region. Fourth, inspection of LD matrices excluded very high LD as the sole explanation for the results (Supplementary Figure 10), and none of the genotyped SNPs had strong LD ($r^2 > 0.8$) with rs2715148 (the SNP with the smallest $P$-value in the PCLO region). Fifth, population stratification can cause false-positive findings but this did not appear to explain the PCLO association: (1) the same 11 SNPs had $P$-values among the top 200 associations in unadjusted analyses as well as with adjustment via principal components and stratified analyses; and (b) for the 57 SNPs in the PCLO region, the $P$-values across these three types of analyses were consistent (the Spearman’s correlations between $P$-values from trend tests, logistic regression and stratified analyses were all $>0.962$). Sixth, the minor allele frequencies in the control group in the PCLO region were usually quite similar to available EUR control groups suggesting that the PCLO findings were not due to an artifact of the control selection process (see below). Finally, bioinformatic investiga-
tion did not suggest that this is a problematic region to genotype as the PCLO region is not known to be under positive selection in humans, to contain segmental duplications or common copy number variants (search of the Database of Genomic Variants yielded two rare copy number variations (CNVs) with control frequencies of 0.12 and 0.89%).

We conducted additional analyses to attempt to localize the association depicted in Figure 2. Imputation supported the directly typed SNP associations but did not yield an association P-value markedly more significant than any directly genotyped SNP (although 22 of the 25 most significant imputed associations in the genome were in this region). Haplotype analysis using three-SNP sliding windows did not improve localization. Secondary analyses by sex, case ascertainment setting and recurrent early onset MDD (reoMDD, arguably the most heritable form of MDD) suggested that most of the signals were from women and from subjects with reoMDD (Supplementary Table 11). The findings for reoMDD were often stronger than the primary analyses, particularly for the most significant SNP (rs2715148) where the P-value decreased by 1.2 orders of magnitude to 9.5 \times 10^{-8}.

PCLO replication

Although no finding met genome-wide significance, the presence of multiple possible signals in PCLO and the plausibility of a function for PCLO in the etiology of MDD led us to attempt replication in external samples. We assembled a collection of 11,972 independent subjects (6079 MDD cases and 5893 controls) from seven different groups and a total of six case–control replication samples (two German samples were combined; Supplementary Methods). As with
| SNP         | Chr | Position | Allele  | Strand | Gene    | Basic SNP data | Bioinformatics | Results | MAF | Quality control—SNP missingness | Quality control—additional checks |
|------------|-----|----------|---------|--------|---------|----------------|----------------|---------|-----|-------------------------------|----------------------------------|
| rs12471798 | 2   | 177,820  | A/G     | +      | TAMAL   | 10.12471798   | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs7301124  | 2   | 18,274    | A/G     | +      | TAMAL   | 7.12471798    | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs9203288  | 2   | 19,747    | T/C     | -      | ALK     | 1.9203288     | CNV, mutated in colon 2q | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs12621441 | 2   | 79,440    | G/A     | +      | TAMAL   | 1.2621441     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs11132168 | 4   | 18,428    | T/C     | +      | TAMAL   | 2.11132168    | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs17074631 | 4   | 18,452    | G/A     | +      | TAMAL   | 0.17074631    | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs2049823  | 6   | 131,613   | T/C     | -      | TAMAL   | 2.2049823     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs2784978  | 6   | 131,906   | C/T     | -      | TAMAL   | 2.2784978     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs1558477  | 7   | 30,807    | G/T     | +      | TAMAL   | 1.1558477     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs1779086  | 7   | 30,410    | T/C     | +      | TAMAL   | 2.1779086     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs2715148  | 8   | 20,230    | A/G     | +      | TAMAL   | 1.2715148     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs222833  | 8   | 20,998    | C/A     | -      | TAMAL   | 2.222833      | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs2252840  | 7   | 8,212     | T/C     | +      | TAMAL   | 2.2252840     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs11031676 | 11  | 32,242    | T/C     | +      | TAMAL   | 2.11031676    | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs12579771 | 12  | 44,019    | T/C     | +      | TAMAL   | 2.12579771    | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs1457266  | 8   | 24,825    | A/G     | +      | TAMAL   | 1.1457266     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs3885179  | 19  | 14,688    | A/C     | +      | TAMAL   | 2.3885179     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |
| rs928862  | 21  | 20,559    | G/A     | +      | TAMAL   | 2.928862     | Reg pot        | 0.9994 | 0.012 | 0.010                         | 0.014                            |

Notes: Sorted by location. All locations per NCBI Build 35 (UCSC hg17). Alleles are given as minor/major. OR (CI), odds ratio (95% confidence interval). P-asymptotic, P-value from Trend test. P-empirical, pointwise P-value from adaptive permutation method in PLINK. For P-value see text. P-empirical, genome-wide empirical P-value by traditional permutation testing (5000 replicates). MAF, minor allele frequency. HapMap MAFs have been converted to the reference allele of the MDD sample. P-missing tests the difference in missingness between cases and controls. For noteworthy associations, the four flags refer to acceptable cluster plots, conformation to Hardy–Weinberg equilibrium, absence of plate-specific association outliers and the presence of a "proxy" SNP in high linkage disequilibrium with the primary SNP.

TAMAL codes. Bioinformatic flag possibilities: coding SNP (cSNP), SNP in segmental duplication, known copy number variant (CNV), conserved (Cons), miRNA target site, region of regulatory potential (reg pot), predicted promoter, transactor binding site, enhancer, exon, splice site, mRNA expression QTL (lymphocytes or cortex). Only positive flags are shown.

BIP Sullivan Lab Evidence Project (http://bip.samp.org) a compendium of genetic findings from the literature. Sources (PubMed IDs): CNVs from Database of Genomic Variation (PMID 15286789), breast and colon cancer mutations (17932254), MDD genome-wide linkage studies (12612864, 14582139, 17427203), SCZ genome-wide linkage meta-analysis (12802786) and bipolar disorder (BIP) GWAS (17554300).
NESDA cases, all replication cases were adults of European ancestry on whom a structured clinical interview was used to substantiate the lifetime diagnosis of DSM-IV MDD,\(^1\) and all studies excluded common MDD phenocopies (for example, depressive symptoms due to another psychiatric disorder or a general medical condition). As with NTR controls, all replication controls were adults of European ancestry ascertained from the population, and individuals reporting MDD symptoms were excluded. We estimated statistical power using Quanto\(^5\) (assumptions: log-additive genetic model, MDD lifetime risk 0.15, MAF = 0.45 (similar to rs2522833), a genotypic relative risk of 1.14 (‘shrunk’ down from the observed GRR of 1.26 for rs2522833 to account for the ‘Winner’s Curse’ phenomenon))\(^7\) and a conservative two-tailed type 1 error rate of 0.00167 ( = 0.05/30 replication SNPs). Statistical power was 97.2% for replication for the two SNPs genotyped in all samples (\(N = 11,972\)) and 90.4% for the remaining SNPs (\(N = 9278\)). Five replication samples were genotyped for 30 SNPs using the same Sequenom iPLEX SNP pool (15 SNPs were in the primary GWAS and 15 were selected to tag common variation in Europeans)\(^8\) and one sample was successfully genotyped for two SNPs using TaqMan. The SNP selection strategy effectively cast a broad net over the region showing association in Figure 2. For the NESDA/NTR samples, agreement between the initial Perlegen genotypes in this region and independent re-genotyping was high (0.9987).

The single SNP results for MDD are depicted in Figure 3 and Table 4a. Our analytic plan dictated the combined analysis of all replication samples with the use of a one-tailed directional test. No association in the replication sample reached statistical significance after correction for multiple comparisons and SNP nonindependence due to LD (ninth column in Table 4a). Similarly, haplotype analyses did not reveal significantly associated regions (Supplementary Figure 16). There were four \(P\)-values < 0.05 in the replication sample but only rs10954694 also had \(Z\)-scores of the same sign in both samples. Table 4b shows the results for reoMDD, and no single SNP was significant after correction for multiple comparisons. When we repeated the MDD analyses restricted to female subjects, the observed significance levels did not become markedly stronger in any of the replication samples in contrast to the initial NESDA/NTR sample. Thus, results from analyses of all replication samples did not reach the \(a\ priori\) criterion for replication evidence for the involvement of \(PCLO\) in the etiology of MDD.

**Unanticipated heterogeneity in cases**

However, we observed, \(a\ posteriori\), that there was potentially important heterogeneity in the replication samples for eight SNPs that were strongly associated in the original sample (\(I^2 > 0.4\), ninth column in Table 4a). In investigating this further (Supplementary Methods), we determined that there was little evidence for genetic heterogeneity in the genotyped region for controls but, unexpectedly, there was significant heterogeneity in the cases. Principal components analysis and inspection of Table 4a and the forest plots in Figure 3 indicated that the outlier

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**Figure 2.** Plot of the piccolo (\(PCLO\)) region (NCBI build 35, UCSC hg17, chr7:82 000 000–82 500 000). \(P\)-values in this figure are all from SNPMeta. The x axis is chromosomal position, the left y axis is \(-\log_{10}(P)\) for genotyped SNPs (colored diamonds) and imputed SNPs (grey diamonds), and the right y axis is the recombination rate from the HapMap EUR panel (light blue curve). The color of the genotyped single nucleotide polymorphisms (SNPs) corresponds to LD with the SNP with smallest \(P\)-value (rs2715148); red \(0.8 < r^2 < 1.0\), orange \(0.5 < r^2 < 0.8\), yellow \(0.2 < r^2 < 0.5\) and white \(r^2 < 0.2\). The significant and extent of all three-SNP haplotypes with \(P < 0.0001\) in this region are colored light green. The transcripts for two \(PCLO\) isoforms are shown in dark green at the bottom. Graph adapted from an \(R\) function by the Broad DGI group.
## Table 3  Clustering of SNPs with low P-values

| Rank | Chr | Start  | End    | Nsnps | Expressed in brain? | Genes                            | Gene products                                                                 |
|------|-----|--------|--------|-------|---------------------|----------------------------------|--------------------------------------------------------------------------------|
| 1    | 7   | 30928587 | 30931521 | 3     | Yes                 | ADCYAP1R1                        | Adenylate cyclase activating polypeptide 1 [putative]; 1 receptor type I         |
| 2    | 7   | 82041576 | 82208167 | 10    | Yes                 | PCLO                             | Piccolo (presynaptic cytomatrix protein)                                        |
| 4    | 6   | 14388932 | 14399068 | 2     | Yes                 | LAPTMAA                          | Lyosomal-associated protein transmembrane 4x                                    |
| 5    | 2   | 20177020 | 20183313 | 2     | Yes                 | CREBBP/FPSO inhibitor 1/SP308     | Interacting inhibitor of differentiation 1 (railike protein)/SHC                 |
| 9    | 2   | 201794446 | 201880818 | 2     | Yes                 | AJ487678/AJ487679/AK125384/AY690681/CASP10 CFLAR/NDUFb3 | Caspase 10/caspase 10/PRO3089/caspase 10 splice variant 5/caspase 10, apoptosis-related cysteine peptidase/CASP8 and FADD- like apoptosis regulator/NADH dehydrogenase (ubiquinones) 5f subcomplex, 3, 12kDa |
| 10   | 1   | 48985973 | 49039120 | 10    | Yes                 | CRI1/EID1/CKLF/SHC4               | Neuroactive ligand/receptor interaction                                           |
| 14   | 20  | 39724220 | 39742644 | 5     | Yes                 | C9H12                            | Cadherin 12, type 2 [N-cadherin 2]                                               |
| 15   | 6   | 14386148 | 14397091 | 3     | Yes                 | ANPEP/MESP2                      | Alanyl (membrane) aminopeptidase (aminopeptidase N, aminopeptidase M, microsomal aminopeptidase, CD13, p150)/mesoderm posterior 2 homolog (mouses) |
| 16   | 4   | 184652456| 184658003| 3     | Yes                 | CDH12                            | Cadherin 12, type 2 [N-cadherin 2]                                               |
| 17   | 5   | 117174763| 117282687| 4     | Yes                 | AK128371/CHRNA2/PTK2B            | Hypothetical protein PL46514/cholinergic receptor, nicotinic, α2 (neuronal)/PTK2B protein tyrosine kinase 2β/Neuropeptide precursor-activated receptor |
| 29   | 3   | 12453817 | 12459985 | 2     | Yes                 | PPARG                            | Peroxisome proliferator-activated receptor                                        |
| 32   | 3   | 99975021 | 100183009| 2     | Yes                 | DCBLD2/ST3GAL6                   | Discoidin, CUB and LDL domain containing 2/ST3G galactose α2,3sialyltransferase 6 |
| 34   | 3   | 70451852 | 70476913 | 2     | Yes                 | GZMK                             | Granzyme K [procarboxypeptidase K, k (lysosomal)]                                |
| 38   | 2   | 7424008  | 7440754  | 3     | Yes                 | GZMK                             | Granzyme K [procarboxypeptidase K, k (lysosomal)]                                |
| 41   | 5   | 54352635 | 54383712 | 3     | Yes                 | NEF3/NEFM                        | Neurofilament 3 (150 kDa medium)/neurofilament, medium polypeptide 150kDa       |
| 43   | 13  | 111889283| 111902203| 2     | Yes                 | NEF3/NEFM                        | Neurofilament 3 (150 kDa medium)/neurofilament, medium polypeptide 150kDa       |
| 44   | 4   | 211470329| 211508991| 4     | Yes                 | NEF3/NEFM                        | Neurofilament 3 (150 kDa medium)/neurofilament, medium polypeptide 150kDa       |
| 46   | 8   | 24784576 | 24825085 | 2     | Yes                 | NEF3/NEFM                        | Neurofilament 3 (150 kDa medium)/neurofilament, medium polypeptide 150kDa       |

Abbreviation: MDD, Major depressive disorder.

*SLEP, Sullivan Lab Evidence Project (http://slep.unc.edu) a compendium of genetic findings from the literature.
was the Australian QIMR sample. Notably, the original and QIMR samples were particularly similar in that both studies included population-based cases and controls were selected to be at low liability for MDD based on longitudinal assessments. Of the nine SNPs with \( P < 0.05 \) in the QIMR sample, eight had both low \( P \)-values and \( Z \)-scores with the same sign as in the NESDA/NTR sample. As an exploratory analysis, we analyzed the original and QIMR samples jointly, and the minimum \( P \)-value was \( 6.4 \times 10^{-8} \) at the nonsynonymous SNP rs2522833 that gives rise to a serine to alanine substitution near the C2A calcium-binding domain of the PCLO protein.

Secondary analyses
We conducted additional analyses of the NESDA/NTR GWAS data set that were specified \textit{a priori} but which should be considered exploratory.

(1) The network of proteins with which \textit{PCLO} interacts in its function at the presynaptic cytoskeletal matrix is relatively well characterized, and we reasoned that genes encoding these proteins might harbor risk or protective variants. We assessed this hypothesis by testing for association conditioning on the \textit{PCLO} nsSNP rs2522833 (that is, investigating whether controlling statistically for the effect of rs2522833 increases the salience of other SNP associations), assessing the minimum \( P \)-value per gene, and then comparing this list to a list of 54 genes that make proteins that interact with \textit{PCLO}. This analysis did not reveal any SNPs or genes whose significance was markedly lower than without including rs2522833 in the logistic regression model. Moreover, no known \textit{PCLO} interacting protein was notable on this list.

(2) We imputed genotypes for 2,037,829 autosomal SNPs using MACH with reference to HapMap CEU genotypes. The resulting \( \lambda \) was 1.048, and the minimum \( P \)-value was \( 1.21 \times 10^{-7} \). As noted above, 22 of the 25 most significant imputed associations were in the \textit{PCLO} region. Investigation of SNP clustering that accounted for LD yielded results similar to those shown in Table 3.

(3) We assembled a list of 103 candidate genes that had been studied for association with MDD in the literature. A total of 19 of these genes had no SNPs
within its transcript and another 9 genes had inadequate coverage (>1 SNP per 15 kb; Supplementary Table 17). Of the remaining 75 genes, only neuronal nitric oxide synthase (NOS1, \( P = 0.0006 \)) had \( P < 0.001 \). However, NOS1 (as with most genes in Supplementary Table 16) is quite large and there is a possibility of a potential influence on these results.

(4) We compared the GWAS association results to a meta-analysis of gene expression data from 12 studies of postmortem brain tissue in MDD cases compared with controls (10 frontal cortex and 2 cerebellum studies). These data are available via the Stanley Foundation (http://www.stanleygenomic.org). There were five genes with GWAS \( P < 0.05 \) (all had gene expression changes significant at \( P 0.0004–0.007 \)). The genes were: SGC9 (sarcoglycan), CALD1 (caldesmon 1), EEF1A1 (eukaryotic translation elongation factor 1α1), CFLAR (CASP8 and FADD-like apoptosis regulator) and TP73L (tumor protein p73-like). There is no overlap of this list with the PCLO interactors or MDD candidate genes from the literature.

(5) Alternative models, filters and phenotypes: (i) For reoMDD, the minimum \( P \)-value over all GWAS SNPs was at the PCLO region SNP rs2715148 \((8.4 \times 10^{-8})\) which ranked second of all SNPs using the trend test (Table 2). (ii) rs2715148 also had the smallest \( P \)-value under a dominant model of SNP action \((6.2 \times 10^{-8})\). (iii) Given the female predominance in MDD, we analyzed data from women and men separately. For female cases and controls, rs2715148 had the smallest \( P \)-value \((4.0 \times 10^{-7})\) and multiple other PCLO SNPs had \( P \)-values in the \( 10^{-5}–10^{-6} \) range. For men, most PCLO SNPs had \( P > 0.05 \) and the minimum was in the SLC9A9 SNP rs4839627 \((9.1 \times 10^{-7})\). (iv) Again, given sex differences in MDD prevalence, we investigated SNPs on chrX and chrY more closely. The minimum \( P \)-value in chrX pseudoautosomal region 1 was 0.02. For the non-PAR regions of chrX in women, the SNPs with the smallest \( P \)-values were rs11094388 \((P = 0.0003,\) intergenic), rs5971108 \((P = 0.0003,\) PTCHD1), rs5930667 \((P = 0.0004,\) intergenic), rs4618863 \((P = 0.0005,\) intergenic), rs2207796 \((P = 0.0005,\) in the very large gene DMD) and rs5936428 \((P = 0.0009,\) FMR2). For men, the minimum \( P \)-value on chrY was at rs10521594 \((P = 5.4 \times 10^{-5},\) intergenic) and 0.22 on chrY.

Discussion

Overview

MDD is a common complex trait of enormous public health significance. As part of the GAIN initiative of the US Foundation for the NIH,\(^9\) we conducted a GWAS of 435 291 SNPs genotyped in 1738 MDD cases and 1802 controls selected to be at low liability for MDD. Our study had numerous positive attributes including its historically large sample size, its largely population-based and longitudinal design, and relatively unbiased and dense genome-wide genotyping designed to capture common variation in subjects of European ancestry.

According to our primary analysis plan, no SNP–MDD phenotype association reached genome-wide significance as the minimum \( q \)-value was 0.28, greater than the pre-defined \( q \)-value threshold of 0.10. This result was not unexpected. For example, type 2 diabetes mellitus has arguably reaped the greatest harvest from GWAS\(^4\) and yet two of the initial T2DM GWAS were unremarkable when analyzed independently.\(^8,9\) One of the key lessons of the GWAS era is the importance of meta-analysis where its application to the primary GWAS can uncover positive findings that replicate well across studies.\(^18,85\)

Is PCLO a causal risk factor for MDD?

Although no locus exceeded the genome-wide threshold after correction for multiple comparisons, 11 of the top 200 signals localized to a 167 kb region overlapping the gene PCLO. The protein product of PCLO localizes to the presynaptic active zone and is important in brain monoaminergic neurotransmission,\(^8\) clearly intersecting with a venerable hypothesis of the etiology of mood disorders.\(^8\) Moreover, the third most significant association was a common nonsynonymous SNP near its critical C2A binding domain in PCLO.\(^8,80\) Although it is an obvious candidate gene, we are not aware of any prior association studies of PCLO and mood disorders (PCLO is in a region of 7q implicated by linkage in autism and one autism association study has been published).\(^90\)

We judged the intersection of this GWAS result with prior knowledge sufficient to trigger a large-scale replication effort by genotyping PCLO SNPs in 6079 MDD-independent cases and 5893 controls. Statistical power to replicate exceeded 90% even after accounting for the ‘Winner’s Curse’ phenomenon (a form of regression to the mean whereby the true genotypic relative risk is overestimated in the initial study).\(^91,92\) However, in spite of the apparent \textit{a priori} strength of a hypothesis of genetic variation in PCLO in the etiology of MDD, no SNP analyzed in the replication sample met appropriately rigorous criteria for replication.\(^21\) Therefore, unlike GWAS for many nonpsychiatric biomedical disorders, our GWAS and replication efforts did not yield ‘proof beyond a reasonable doubt’ level of evidence for an association between genetic variation in PCLO and MDD.

Investigation of the sources of heterogeneity in the replication samples indicated that controls were genetically similar to the original sample in the PCLO region but that cases were dissimilar. We observed, \textit{a posteriori}, that both principal components derived from PCLO region genotypes in QIMR cases and effect size estimates in the QIMR replication sample tended to be similar to the original sample. This is notable because, of all the replication samples, ascertainment of QIMR subjects was most similar to the primary NESDA/NTR sample in that cases were identified from population-based sources (100% for QIMR and 60% for NESDA) rather than tertiary sources as for the other replication samples. MDD cases from clinical
samples may differ from population-based cases due to selection bias. Berkson’s bias, differing referral filters or even a different genetic basis with respect to genetic variation in the PCLO region.

Joint analysis of the NESDA/NTR and QIMR samples yielded \( P = 6.4 \times 10^{-8} \) (uncorrected for multiple hypothesis testing) for the nonsynonymous SNP rs2522833. This result suggests a specific hypothesis for future studies: an association between genetic variation in PCLO and MDD may be detected only in population-based cases. Thus, it would be premature to exclude PCLO from a function in the etiology of some forms of MDD.

The heterogeneous nature of MDD

Interpretation of the PCLO replication efforts is consistent with two broad possibilities. The first possibility is that genetic variation in PCLO is truly not associated with MDD. This interpretation is supported by the replication analyses (specified a priori) in which no SNP was significantly associated after correction for multiple comparisons and SNP dependence due to LD. This strict interpretation is generally viewed as ‘best practice’ in human genetics but implicitly assumes etiological homogeneity for MDD in the PCLO region. The second possibility invokes a less parsimonious model involving heterogeneity, that genetic variation in PCLO is etiologically causal to some subtypes of MDD. This interpretation is an a posteriori hypothesis consistent with the empirical results particularly in the notable differences in associations between samples, case ascertainment strategies, and indications from principal components analysis that NESDA and QIMR cases are more similar than the clinically ascertained subjects.

It is notable that the control samples from each site were considerably more similar than cases from the same sites.

The tension between null a priori results and plausible a posteriori hypotheses is a core issue in psychiatric genetics. Important phenotypes like MDD are defined reliably and with reference to diagnostic schema developed principally for clinical purposes. Heterogeneous etiology of MDD is widely suspected but there are no proven ways to identify heterogeneity (indeed, a prominent rationale for genetics studies is improve differential diagnosis).

Our results are consistent with prior observations of the heterogeneous nature of MDD, particularly with regard to ascertainment. Individuals who meet MDD criteria from community or primary care sources may have a more inclusive and less comorbid form of MDD whereas tertiary ascertainment may yield subjects with greater comorbidity and perhaps distinctive etiology. In particular, it is formally possible (but unproven) that the PCLO results are accurate—genetic variation in PCLO might be causal to the types of MDD seen in community samples but other loci contribute to a distinctive type of MDD seen in tertiary care samples.

Other hypotheses

There were two MDD cases who may have had unrecognized genomic disorders (possible Turner’s and Klinefelter’s syndromes). We speculate that small numbers of cases with MDD will have CNV-related genomic disorders that are plausibly causal to MDD. Clarification of the function of such rare variants will require larger samples.

Most of the additional exploratory analyses were unrevealing, including examination of proteins known to interact with PCLO, genotype imputation, comparison of GWAS findings with MDD candidate genes from the literature and gene expression changes in the brain in cases with MDD, and alternative genetic models, phenotype definitions and sex-specific analyses.

We searched the Sullivan Lab Evidence Project (SLEP) compendium of psychiatric genetics findings in an attempt to discover overlap of our findings with those reported in the literature. First, with reference to a meta-analysis of microarray studies on the Stanley brain bank MDD and control samples, expression of CFLAR and MARCH3 were increased and LST1 and HLA-B were decreased in MDD postmortem frontal cortex. These regions ranked 9, 232, 267 and 432 in the NESDA/NTR GWAS. Second, we looked for convergence of our findings with other GWAS of psychiatric disorders. Notable genomic locations of overlap of the top 480 regions in the present GWAS were found with GWAS for ADHD (ITIH1; S Faroane, personal communication), the Wellcome Trust Case-Control Consortium GWAS for bipolar disorder (SHFM1 and UGT2B4) and a bipolar GWAS that used DNA pooling (GRM7 and DGKH). Third, we looked at the minimum \( P \)-values in our study for genes that met or nearly achieved genome-wide significance: the minimum \( P \)-values in our study for \( MAMDC1 \) were 0.004, 0.03 for \( ZNF804A \), 0.002 for \( ANK3 \) and 0.03 for \( CACNA1C \). These overlaps are intriguing (although the possibility of chance cannot be excluded), and will be formally investigated as part of our participation in the Psychiatric GWAS Consortium analyses.

Limitations

(1) Although statistical power has been systematically underestimated in psychiatric genetics, when we began this study in Q3 2006, it was believed that statistical power would be reasonable to detect realistic genetic effects. However, the definition of ‘realistic’ has shifted considerably since 2006 and it may be important to design studies that can detect genotypic relative risks \(< 1.10 \). (2) When this study began, the coverage and performance of the Perlegen GWAS platform were among the better options available. The technology and pricing have evolved rapidly and superior platforms are now available. A key limitation of the Perlegen platform is its inability to assess CNV that may be particularly salient for psychiatric disorders. More generally, the GWAS platform might not be sufficiently ‘genome-wide’ and
| SNP ID   | rs76540436 | 82 048 395 | 3655 | 2.87 | 0.0044 | 0.0056 | 1087 | 1.87 | 0.90 | 0.59 | 3.91 | 9.1E-05 | 9269 | 1.38 | 0.17 | 0.92 | 0.52 | 2004 | 2.88 | 0.0039 | 0.076 | 0.19 | 0.03 | 0.77 | 0.92 | 0.52 | 2004 |
|----------|-------------|------------|------|------|-------|--------|-------|-------|------|------|------|--------|-------|------|------|------|------|------|-------|-------|------|------|------|------|------|------|------|------|
| rs6979066 | 82 061 588 | 3656 | 3.94 | 8.1E-06 | 9257 | 0.67 | 0.51 | 1 | 0.00 | 2005 | 0.88 | 0.38 | 1 | 1893 | 0.15 | 0.02 | 0.82 | 1.53 | 0.13 | 0.00 | 1902 | 1.59 | 0.11 | 0.79 | 1307 | 0.70 | 0.49 | 1 | 2487 |
| rs2715148 | 82 094 686 | 3651 | 5.05 | 4.4E-07 | 11 850 | 0.23 | 0.82 | 1 | 0.40 | 2003 | 0.88 | 0.38 | 1 | 1893 | 0.41 | 0.68 | 1 | 710 | 1.15 | 0.23 | 0.95 | 1307 | 0.70 | 0.49 | 1 | 2487 |
| rs2371367 | 82 163 042 | 3656 | 2.87 | 0.00406 | 9257 | 0.67 | 0.51 | 1 | 0.00 | 2004 | 0.88 | 0.38 | 1 | 1893 | 0.15 | 0.02 | 0.82 | 1.53 | 0.13 | 0.00 | 1902 | 1.59 | 0.11 | 0.79 | 1307 | 0.70 | 0.49 | 1 | 2487 |
| rs9690648 | 82 205 975 | 3658 | 0.52 | 0.60 | 9278 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| rs6959723 | 82 206 991 | 3656 | 0.42 | 0.67 | 1 | 826 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| rs12669254 | 82 217 749 | 3656 | 2.31 | 0.02077 | 9274 | 1.04 | 0.30 | 1 | 0.00 | 1254 | 0.41 | 0.68 | 1 | 710 | 0.20 | 0.84 | 1 | 1548 | 0.21 | 0.83 | 1 | 710 | 1.00 | 0.32 | 0.99 | - | - |
| rs17282875 | 82 048 576 | 2368 | 4.48 | 7.5E-06 | 4983 | 0.10 | 0.92 | 1 | 0.00 | 1254 | 0.41 | 0.68 | 1 | 710 | 0.20 | 0.84 | 1 | 1548 | 0.21 | 0.83 | 1 | 710 | 1.00 | 0.32 | 0.99 | - | - |
| rs10954694 | 82 201 812 | 2367 | 1.91 | 0.06 | 6406 | 2.51 | 0.01 | 0.19 | 0.00 | 1256 | 1.72 | 0.09 | 0.71 | 1310 | 0.48 | 0.63 | 1 | 710 | 0.20 | 0.84 | 1 | 1548 | 0.21 | 0.83 | 1 | 710 | 1.00 | 0.32 | 0.99 | - | - |

Abbreviations: N = total sample size for an analysis, Z = logistic regression beta divided by its standard error, P = asymptotic P-value from Wald χ²-test (1 df) uncorrected for multiple comparisons, Pcorr = empirical P-value for accounting for multiple comparisons and LD structure (50K permutations), and P2corr = an index of heterogeneity of logistic regression parameter estimates.

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unbiased: the platform may have had inadequate coverage in an etiologically important region of the genome, SNPs are only one type of genetic variation, and important non-SNP genetic variation might not have been sufficiently well captured. (3) There was an imbalance in the proportion of men in cases and controls. Although it is unclear whether and how this might bias the results, it may have lead to some degree of bias. (d) Finally, GWASs are predicated upon the crucial assumption that the predominant diagnostic criteria are valid with respect to the fundamental architecture of the disorder.

Conclusions
We describe here a large effort to identify DNA sequence variation fundamental to MDD. Although our initial GWAS results for the PCLO region were intriguing, this highly plausible hypothesis did not find support in a large-scale replication attempt. Our hypothesis about a function of genetic variation in PCLO for MDD in population but not clinical settings emphasizes the importance of knowing the epidemiological sampling frame for a study. Finally, we hope that the model we used in this study—a cooperative international effort—will be adopted by groups studying other psychiatric disorders in order to maximize progress.

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Conflict of interest/disclosure (past 3 years)
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