

ORIGINAL ARTICLE

# Genome-wide association study of recurrent early-onset major depressive disorder

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**A genome-wide association study was carried out in 1020 case subjects with recurrent early-onset major depressive disorder (MDD) (onset before age 31) and 1636 control subjects screened to exclude lifetime MDD. Subjects were genotyped with the Affymetrix 6.0 platform. After extensive quality control procedures, 671 424 autosomal single nucleotide polymorphisms (SNPs) and 25 068 X chromosome SNPs with minor allele frequency greater than 1% were available for analysis. An additional 1 892 186 HapMap II SNPs were analyzed based on imputed genotypic data. Single-SNP logistic regression trend tests were computed, with correction for ancestry-informative principal component scores. No genome-wide significant evidence for association was observed, assuming that nominal  $P < 5 \times 10^{-8}$  approximates a 5% genome-wide significance threshold. The strongest evidence for association was observed on chromosome 18q22.1 (rs17077540,  $P = 1.83 \times 10^{-7}$ ) in a region that has produced some evidence for linkage to bipolar-I or -II disorder in several studies, within an mRNA detected in human brain tissue (BC053410) and approximately 75 kb upstream of *DSEL*. Comparing these results with those of a meta-analysis of three MDD GWAS data sets reported in a companion article, we note that among the strongest signals observed in the GenRED sample, the meta-analysis provided the greatest support (although not at a genome-wide significant level) for association of MDD to SNPs within *SP4*, a brain-specific transcription factor. Larger samples will be required to confirm the hypothesis of association between MDD (and particularly the recurrent early-onset subtype) and common SNPs.**

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## Introduction

Major depressive disorder (MDD) is a common psychiatric disorder with a lifetime prevalence of 10–15% in most large studies. Despite the availability of medication and psychotherapeutic treatments, recurrent or chronic course is common (60–80%),<sup>1</sup> often with comorbid anxiety or substance use disorders, substantial impact on family and work life and on physical health, and an approximately 4% risk of eventual suicide (higher in more severe cases).<sup>2</sup>

MDD is diagnosed when an individual experiences one or more major depressive episodes in the absence of other diagnoses such as bipolar-I or -II disorder, schizoaffective disorder, or schizophrenia. An episode is defined as two or more weeks during which the person experiences impaired functioning and five or more key symptoms (dysphoric mood, loss of enjoyment, suicidal thoughts or acts, agitated or slowed movements, guilty or self-denigrating feelings, fatigue, and disturbances of sleep, appetite, concentration).<sup>3</sup>

The heritability of MDD has been estimated at approximately 40% in population-based twin studies,<sup>4</sup> and higher in clinical samples<sup>5</sup> or with repeated assessments.<sup>6</sup> There is an approximately threefold increase in risk to first-degree relatives.<sup>4</sup> Risk is also increased by severe childhood trauma or parental

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loss,<sup>7</sup> probably interacting with genetic vulnerability.<sup>8</sup> Recurrent episodes and early onset in probands predict greater familial risk, although the size of these effects is controversial.<sup>4,9–11</sup> Women are at twofold greater risk of MDD, and there are probably both common and independent genetic factors in men and women, with similar heritability.<sup>12,13</sup> Although MDD is more frequent in relatives of probands with bipolar disorders and schizophrenia, those disorders are not more frequent in relatives of MDD probands.<sup>14</sup> The degree or nature of overlap in genetic factors underlying these disorders remains unclear.

The Genetics of Recurrent Early-Onset Depression (GenRED) project is creating a large clinical sample, based in the National Institute of Mental Health repository program, for molecular genetic studies of MDD. GenRED I recruited affected sibling pair families for linkage studies<sup>10,15,16</sup> and GenRED II is currently recruiting additional cases for association studies. We have focused on probands with recurrent MDD, early age at onset, and positive family history as indices of increased genetic risk.

We present here the results of an initial case-control genome-wide association study (GWAS) of recurrent early-onset MDD using single nucleotide polymorphism (SNP) array technology. A companion article presents a second MDD GWAS in the STAR\*D antidepressant effectiveness trial sample and a meta-analysis of the GenRED, STAR\*D and publicly available Genetic Association Information Network (GAIN-MDD) GWAS data sets.<sup>17</sup> We did not observe genome-wide significant evidence for association in the GenRED or combined analyses. The meta-analysis demonstrated increased statistical support for one of the top GenRED findings, for SNPs in the gene encoding the Sp4 transcription factor. Many of the most robust GWAS findings to date have been detected in samples of 10 000–20 000 cases (plus controls), with genotypic relative risks (GRRs) typically in the 1.1–1.2 range.<sup>18</sup> Thus, larger samples will be required to clarify whether any of our findings represent true associations, and whether recurrent, early-onset MDD is a uniquely valuable phenotype for association studies.

## Materials and methods

### Subjects

Genotyping was attempted for 1110 MDD cases (655 recruited by GenRED I and 455 by GenRED II). The 1636 control subjects were selected from the Molecular Genetics of Schizophrenia (MGS)<sup>19</sup> sample. All subjects were of European ancestry. Clinical characteristics are summarized in Table 1 for samples that passed all quality control (QC) filters and were included in the analyses presented here.

Cases were recruited from clinical settings and through media and Internet announcements and advertisements. After giving written informed consent, participants were interviewed by phone or in person using the Diagnostic Interview for Genetic

**Table 1** Sample characteristics

	Cases	Controls
N	1020	1636
Male	29%	56%
Age at recruitment	40.5 ± 11.9	52.5 ± 17.2
Age at onset	16.85 ± 5.4	
<i>Major depressive disorder episodes:</i>		
Recurrent	98%	
Single ≥ 3 years	2%	
Number of episodes	8.4 ± 14.6	
Longest episode (days)	931 ± 1896	
Chronic course (consensus rating)	39%	
Number of eight MDD criteria met during worst episode (plus dysphoric mood)	6.8 ± 1.1	
Comorbid anxiety disorder diagnosis (panic, agoraphobia, social phobia)	35%	

Abbreviation: MDD, major depressive disorder.

Studies<sup>20</sup> version 3 (<http://www.nimhgenetics.org>). Two independent expert reviewers achieved consensus ratings of DSM-IV mood and comorbid disorder diagnoses and associated course of illness variables, based on the Diagnostic Interview for Genetics Studies version 3.0, a narrative summary, and available treatment records and/or informant reports from the Family Interview for Genetic Studies. Eligible probands had an MDD diagnosis, two or more episodes (or one episode lasting at least 3 years), onset before age 31, at least one sibling or parent with recurrent MDD with onset before age 41, MDD independent of substance dependence (that is, no lifetime dependence, MDD before dependence, or after at least 2 years of remission from dependence), no diagnosis of bipolar or schizoaffective disorder or schizophrenia, and no suspected bipolar-I disorder in a parent or sibling.<sup>10</sup> In GenRED I, at least one affected sibling was directly interviewed. In GenRED II, MDD in a parent or sibling was documented by Family Interview for Genetic Studies with the proband, supplemented when necessary by a telephone interview with a relative. Additional family history was obtained by Family Interview for Genetic Studies. As of the excess of female probands, we replaced some female GenRED I probands with a male sibling who met proband eligibility criteria.

MGS control subjects were recruited by Knowledge Networks Inc. (Menlo Park, CA, USA), a survey research company, from a nationally representative marketing panel recruited by random digit dialing methods.<sup>19</sup> Control participants consented to anonymization and deposition of their DNA and clinical information in the NIMH repository for use in any medical research. They completed an online questionnaire including a lifetime version of the Composite International Diagnostic Interview-Short Form for common mood, anxiety, and substance use diagnoses,<sup>21</sup> supplemented by questions about schizophrenia, psychosis, or bipolar disorder. After

excluding those who endorsed or failed to answer these latter questions (or who were outliers in total number of items endorsed), MGS selected 2653 European-ancestry controls who passed SNP QC. We then excluded controls who met Composite International Diagnostic Interview-Short Form criteria for MDD, or who reported recurrent depression but missed MDD by one criterion, leaving 1636 controls for analysis.

#### *Genotyping and quality control*

Samples were genotyped with the Affymetrix 6.0 genome-wide SNP array at the Broad Institute Center for Genotyping and Analysis (Cambridge, MA, USA) in three batches: 863 controls, as part of the GAIN schizophrenia project (late 2007); GenRED cases (early 2008); and 773 controls, under MGS grant funding (mid-2008). Genotypes were called with Birdseed (version 2).<sup>22</sup> QC analyses were carried out using PLINK<sup>23</sup> supplemented by local software (see online Supplementary Methods for details). We excluded SNPs and control samples that failed either MGS or GenRED QC criteria, which were selected by determining thresholds that achieved a balance between a low genomic control  $\lambda$  value and inclusion of more data.

Criteria for included SNPs were call rate >97% for autosomes, >98% for chromosome X in females, or >99% in males; minor allele frequency >1%; Hardy-Weinberg  $P$ -value > $10^{-6}$  in controls; <3 Mendelian errors detected in 30 MGS trios; <2 discordant duplicate genotypes in GenRED duplicates or <3 in 90 MGS specimens genotyped in both the GAIN and NonGAIN experiments; case-control call rate difference <2% for autosomes or <1% for X chromosome; and passing a 1df plate-effect test (no plate differing from all others with  $P < 10^{-8}$ , or <2 plates with  $P < 10^{-4}$ ). Each SNP passed these criteria in both MGS and GenRED samples. After QC, 671 424 autosomal and 25 068 X chromosome SNPs were included.

Criteria for DNA samples were call rate >97%; non-outlier for mean heterozygosity across all SNPs and for ancestry principal component scores (EIGENSTRAT<sup>24</sup>); pairwise identity-by-descent estimates not >0.1 with many other samples (pairs of apparent relatives were also inspected and one retained); and non-ambiguous heterozygosity values for X chromosome SNPs in females (these samples were excluded only for X chromosome analyses if they passed autosomal QC). Additional details about population substructure analyses are provided in online Supplementary Methods. Further analyses included 1020 cases and 1636 controls.

#### *Statistical analyses*

Association between single genotyped SNPs and case-control status was tested with logistic regression (trend test) using PLINK. Genotypic dosages (the estimated number of test alleles) were imputed for all HapMap II SNPs with MACH 1.0 software<sup>25</sup> for autosomal SNPs and with IMPUTE<sup>26</sup> for X chromosome SNPs, using a Hidden Markov Model algorithm

and a training data set consisting of phased HapMap CEU haplotypes. This provided an additional 1 892 186 SNPs (1 849 062 autosomal and 43 124 X chromosome SNPs) for testing in addition to the genotyped SNPs, after filtering for MAF >1% and imputation  $r^2 > 0.3$  (an estimate of expected agreement between imputed and actual genotypes). This threshold was used in four previous GWAS meta-analyses because it removed most poorly imputed SNPs but few well-imputed SNPs.<sup>27-30</sup> Association tests for imputed SNPs were carried out with local software using the same logistic regression model. For all tests, ancestry-informative principal components were included as covariates.<sup>24</sup> Each SNP was tested for all subjects, and then separately for males and for females. For the primary analysis of all subjects, a reasonable threshold for 5% genome-wide significance is a nominal  $P$ -value less than  $5 \times 10^{-8}$ , based on three estimates assuming that all common SNPs have been directly or indirectly tested.<sup>31-33</sup> The analyses of male and of female subjects were considered exploratory.

As described in online Supplementary Results, we also separately examined results for SNPs in or near 41 mood disorder candidate genes, including single-SNP tests and a permutation-based aggregate test (page S-22) of whether  $P$ -values in these genes were more significant than expected by chance.

#### *Power analysis*

Results of power analyses are shown in Supplementary Table S3. In the primary analysis, for log additive transmission, power was 78% to detect a locus with MAF of 0.25 conferring a GRR to heterozygotes of 1.45, or 45% for MAF of 0.4 and GRR of 1.35.

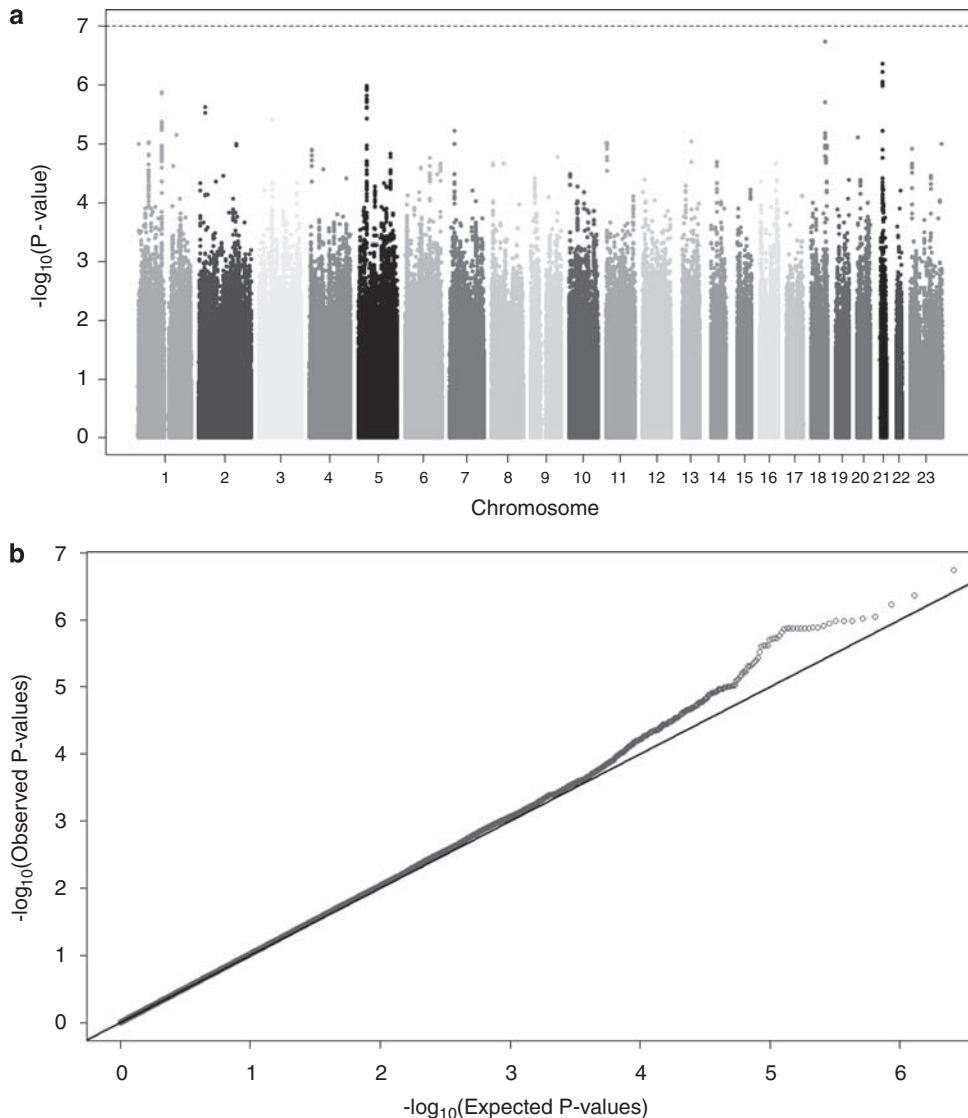
#### *Data sharing*

Genotypic and clinical data are available to qualified scientists through controlled-access repository programs: the NIMH repository program (<http://www.nimhgenetics.org>) for the GenRED sample; and dbGAP (<http://www.ncbi.nlm.nih.gov/gap>) for MGS controls.

## Results

Figure 1 illustrates results for the primary analysis and the quantile-quantile plot of observed vs expected  $\chi^2$  values for all genotyped and imputed SNPs. The genomic control  $\lambda$  value (the observed median  $\chi^2$  divided by the expected median value of 0.456 under the null hypothesis) was 1.031, indicating that there was no meaningful inflation of test statistics.<sup>34</sup>

Table 2 lists results with  $P < 10^{-5}$  for each of the three analyses. (Online file *genred\_supplementary\_data.txt* provides data for all results with  $P < 0.001$  in any analysis.) Rows report data for the 'best' SNP in independent regions (whether gene-containing or not) with  $P < 10^{-5}$  for one or more SNPs. In most regions, many SNPs were in strong linkage disequilibrium and gave similar results. Genes and other functional



**Figure 1** Genome-wide results. (a) Shows the association test result for each SNP (genotyped and imputed) as  $-\log_{10}(P\text{-value})$ , for the primary analysis of all subjects. For the same SNPs, (b) shows the quantile–quantile plot of observed vs expected  $\chi^2$  (1df) statistics. The genomic control  $\lambda$  value was 1.032. Manhattan and QQ plots are shown for males and for females separately in online Supplementary Figures S7 and S14.

elements are noted in the table if  $P$ -values less than  $10^{-5}$  were observed either within the transcribed boundaries of the gene, or within 50 kb upstream or downstream, except that the closest genes are listed for some nongenic regions. Supplementary Table S13 provides names of these genes and summarizes their known functional roles. Regions in Table 2 for which no gene or element is listed have peaks of similarity to known regulatory sequences by the ESPERR regulatory potential method available as a The University of California at Santa Cruz genome browser track.<sup>35</sup>

There were no genome-wide significant findings. Three of the top regions in the primary analysis also produced  $P$ -values less than  $10^{-5}$  in the meta-analyses that are presented in a companion article.<sup>17</sup> (Low meta-analytic  $P$ -values required a consistent allelic direction of association across samples.) In the

primary meta-analysis (Broad phenotype) that included all MDD cases from GenRED, STAR\*D, and GAIN-MDD, SNP rs17144465 in SP4 (7p14.3) had  $P=8.38 \times 10^{-7}$  lower than that for GenRED alone ( $P=5.97 \times 10^{-6}$ ). Another two of the top GenRED regions (18q22.1 and 5p13.2) yielded  $P < 10^{-5}$  in the Narrow meta-analysis (recurrent early-onset MDD in all samples). For 18q22.1 (rs17077540), the meta-analysis ( $P=7.55 \times 10^{-7}$ ) did not provide stronger support than GenRED alone ( $1.83 \times 10^{-7}$ ). For 5p13.2, the meta-analysis  $P$ -value ( $1.68 \times 10^{-6}$ ) was slightly smaller than for GenRED alone ( $P=2.49 \times 10^{-6}$ ). Please see the companion article for further details.

Figure 2 includes genome browser plots showing association  $P$ -values and relevant genomic information for three regions: 18q22.1 that produced the lowest GenRED  $P$ -value (all subjects); SP4/7p15.3,



**Table 2** Strongest association findings (all, male, or female subjects)

Band	SNP	BP	Alleles	Frq	R <sup>2</sup>	All subjects		Males		Females		Annotation
						OR	P	OR	P	OR	P	
<i>P</i> < 10 <sup>-5</sup> in all subjects												
18q22.1 <sup>a</sup>	rs17077540	63436259	A/G	0.11	0.86	<b>1.61</b>	<b>1.83E-07</b>	1.67	6.02E-04	1.57	2.62E-04	mRNA BC053410 (brain; LOC643542); <i>DSEL</i> (up). (Figure 2)
21q21.2	rs2828520	24064990	A/G	0.31	1.00	1.35	4.33E-07	1.52	2.94E-05	1.28	1.45E-03	
5p13.2 <sup>b</sup>	rs270545	38087350	A/G	0.69	1.00	1.37	1.03E-06	1.35	5.97E-03	1.42	2.89E-05	<i>GDNF</i> (217 kb up)
1p13.3	rs6537837	109921255	C/T	0.17	0.99	1.43	1.31E-06	1.35	1.55E-02	1.40	5.85E-04	<i>GNAT2</i> ; <i>GNAI3</i> ; <i>AMPD2</i> (up) (Figure 2)
2p23.2	rs882632	29134265	C/T	0.29	1.00	1.34	2.41E-06	1.25	3.07E-02	1.53	4.04E-07	<i>FAM179A</i> (LOC165186) (+ 5665); <i>C2orf71</i> (+ 3796)
3p14.2	rs10514718	61388854	C/G	0.94	0.82	2.12	3.96E-06	1.72	4.49E-02	2.46	1.85E-05	<i>FHIT</i> (176 kb up); <i>PTPRG</i> (133 kb up)
1p13.3	rs12049330	109832711	G/T	0.14	0.95	1.44	5.87E-06	1.56	1.05E-03	1.34	4.46E-03	<i>ATXN7L2</i> ; <i>SYPL2</i> (dwn); <i>CYB561D1</i> (up) (Figure 2)
7p15.3	rs17144465	21470952	A/G	0.04	1.00	1.82	5.97E-06	1.39	1.48E-01	2.11	4.44E-05	<i>SP4</i> (Figure 2)
13q21.33	rs9572423	69744204	A/G	0.88	0.96	1.54	9.25E-06	1.94	3.14E-04	1.46	2.53E-03	
1p33	rs1167264	49705632	C/T	0.20	0.99	1.35	9.51E-06	1.22	7.53E-02	1.41	1.26E-04	<i>AGBL4</i>
11p15.4	rs2898938	3788833	G/T	0.72	1.00	1.34	9.60E-06	1.27	3.57E-02	1.40	9.31E-05	<i>FRAG1</i> ; <i>NUP98</i> (up); <i>STIM1</i> (up)
11p15.5	rs11024034	2746739	C/T	0.09	0.99	1.45	9.64E-06	1.34	3.63E-02	1.53	2.03E-04	<i>KCNQ1</i> (including one SNP at exon-intron boundary)
<i>P</i> < 10 <sup>-5</sup> in males												
16q12.2	rs2631522	53782584	C/T	0.71	1.00	1.13	7.05E-02	<b>1.86</b>	<b>2.50E-07</b>	0.87	7.54E-02	Enhancer element_26 (ends at 53781784 bp)
12p12.3	rs12581840	19725418	C/T	0.57	1.00	1.18	3.42E-03	1.70	3.47E-07	1.02	8.16E-01	
3p14.2	rs12633494	61120090	C/T	0.04	1.00	1.36	2.25E-02	2.75	4.63E-07	0.84	3.65E-01	<i>FHIT</i>
1q24.3	rs17657363	170068039	C/T	0.18	1.00	1.26	1.05E-03	1.79	5.19E-07	1.01	8.48E-01	<i>MYOC</i> (up); <i>VAMP4</i> (in, up); <i>KIAA0859</i> ; <i>DNM3</i> (in, up)
<i>P</i> < 10 <sup>-5</sup> in females												
2p23.2	rs882632	29134265	C/T	0.29	1.00	1.34	2.41E-06	1.25	3.07E-02	<b>1.53</b>	<b>4.04E-07</b>	<i>FAM179A</i> (LOC165186) (dwn); <i>C2orf71</i> (dwn)
18q21.2	rs1676089	47448507	C/A	0.88	1.00	1.38	9.67E-04	0.87	3.56E-01	1.89	1.35E-06	
8p23.2	rs2616976	4465647	C/G	0.41	0.97	1.28	2.35E-05	1.06	5.25E-01	1.43	3.60E-06	<i>CSMD1</i>
10p14	rs11254981	7148786	C/G	0.43	0.98	1.28	3.62E-05	1.08	4.57E-01	1.44	4.08E-06	mRNA AK096400 (6.55-7.22 Mb)
11q22.3	rs1430871	102650380	C/T	0.76	0.99	1.29	3.07E-04	1.05	6.67E-01	1.53	4.93E-06	<i>DYNC2H1</i>
3p14.2	rs11918585	61403222	C/T	0.93	0.74	1.86	6.12E-05	1.19	4.79E01-	2.58	5.80E-06	<i>FHIT</i> (191 kb up); <i>PTPRG</i> (118 kb up)

<sup>a</sup>*P* = 6.04 × 10<sup>-7</sup> in the meta-analysis of Narrow cases (GenRED, STAR\*D, and GAIN) in a companion paper.<sup>17</sup>

<sup>b</sup>*P* = 1.88 × 10<sup>-6</sup> in the Narrow meta-analysis.

The single nucleotide polymorphism (SNP) with the lowest *P*-value is listed for all genes or non-genic regions with at least one SNP with *P* < 10<sup>-5</sup>, separately for the analyses of all subjects (primary analysis; top section of table, with box around results for all subjects), males (middle section and box), and females (lower section and box). The annotation column lists all genes in the region with one or more SNPs with *P* < 10<sup>-5</sup>, either within the gene or within 50 kb upstream (up) or downstream (dwn), unless a longer distance is listed. Other functional elements in a region are as noted. Non-genic regions all contain peaks of bioinformatically predicted high homology to known regulatory sequences.<sup>35</sup>

Odds ratio (OR) for the tested allele, indicated in bold font in the alleles column.

Frequency (Frq) of the tested allele in controls. (Case-control frequencies for all subjects findings are available in online Supplementary Table S11.)

*R*<sup>2</sup> indicates the *R*<sup>2</sup> predicted (by MACH 1.0) between imputed and actual genotypes; *R*<sup>2</sup> = 1 indicates that the SNP was genotyped.

Note that many of these regions contained multiple SNPs with low *P*-values, see online file genred\_supplementary\_data.txt.

the best finding that received increased support in the meta-analysis; and 1p13.3, which was not supported in the meta-analysis, but contained the largest number of SNPs with the lowest *P*-values in two large linkage disequilibrium blocks in the GenRED analysis, and which spans a set of interesting candidate genes. Genotyping cluster plots for top

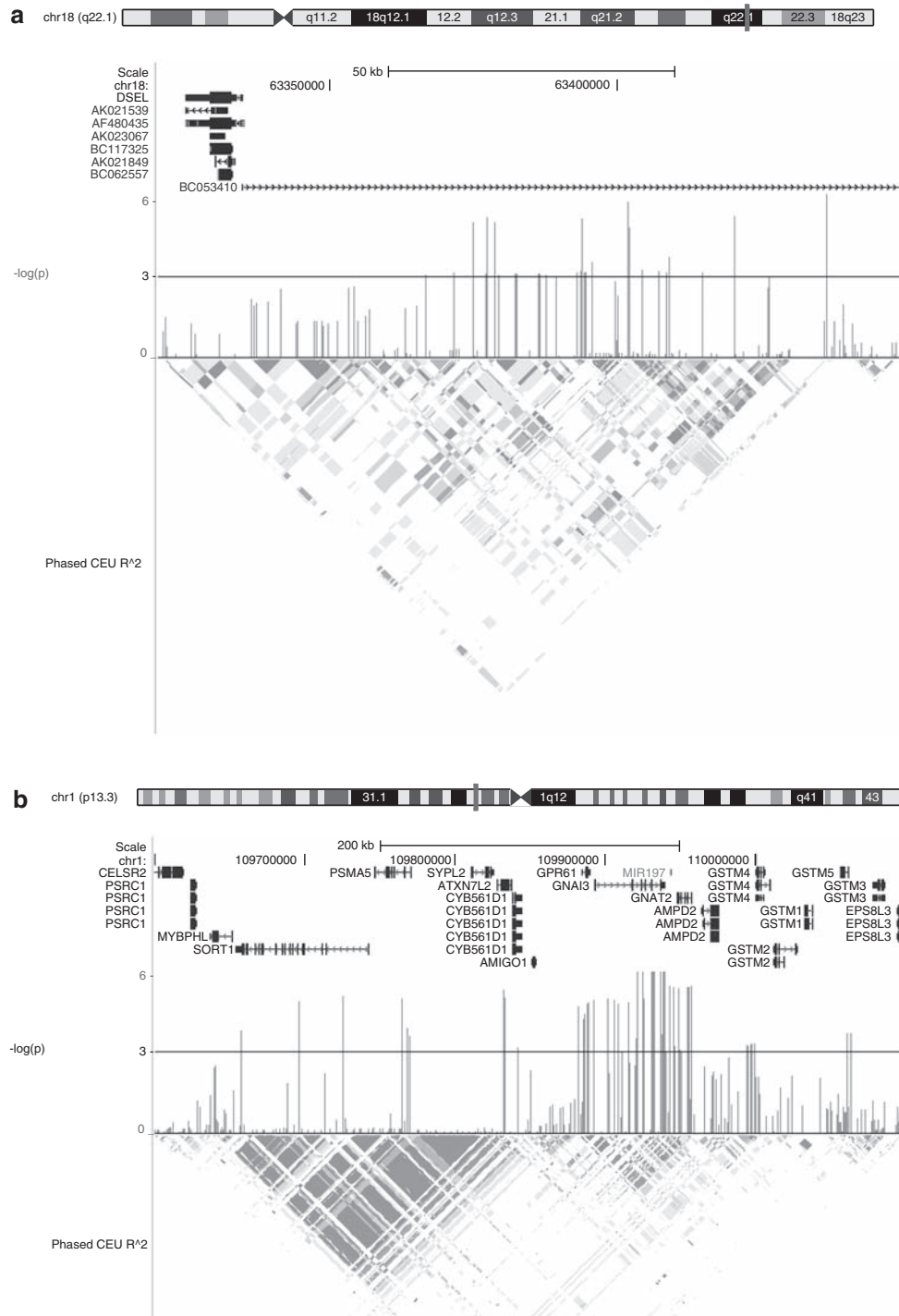
SNPs with *P* < 10<sup>-6</sup> (Table 2) or tags for those SNPs are provided in online file SNP\_intensity\_cluster\_plots.pdf.

In the 41 mood disorder candidate genes (see Supplementary Tables S5 and S6), the lowest *P*-value (0.000067) was observed in an intron of *CACNA1C* (calcium channel, voltage-dependent, L type), a gene

with strong evidence for association to bipolar disorder.<sup>36</sup> For further details, see Supplementary Table S6. The aggregate analysis did not yield evidence that the distribution of *P*-values in these genes was more significant than expected by chance.

## Discussion

A GWAS of 1020 recurrent early-onset MDD cases and 1636 screened controls did not detect genome-wide significant evidence of association. This is consistent



**Figure 2** Results for selected chromosomal regions. Shown are plots of genomic information in three of the top regions as noted in the text and in Table 2: (a) 18q22.1, (b) 1p13.3, and (c) 7p15.3. From top to bottom of each plot are chromosome ideogram (plotted region marked with vertical red bar); genomic information (RefSeq genes with direction of transcription; and in panel a, mRNAs); association test results ( $-\log_{10}[P]$ ) in the primary analysis of all subjects; and HapMap linkage disequilibrium ( $r^2$ ) information. (The color reproduction of this figure is available on the html full text version of the manuscript.)

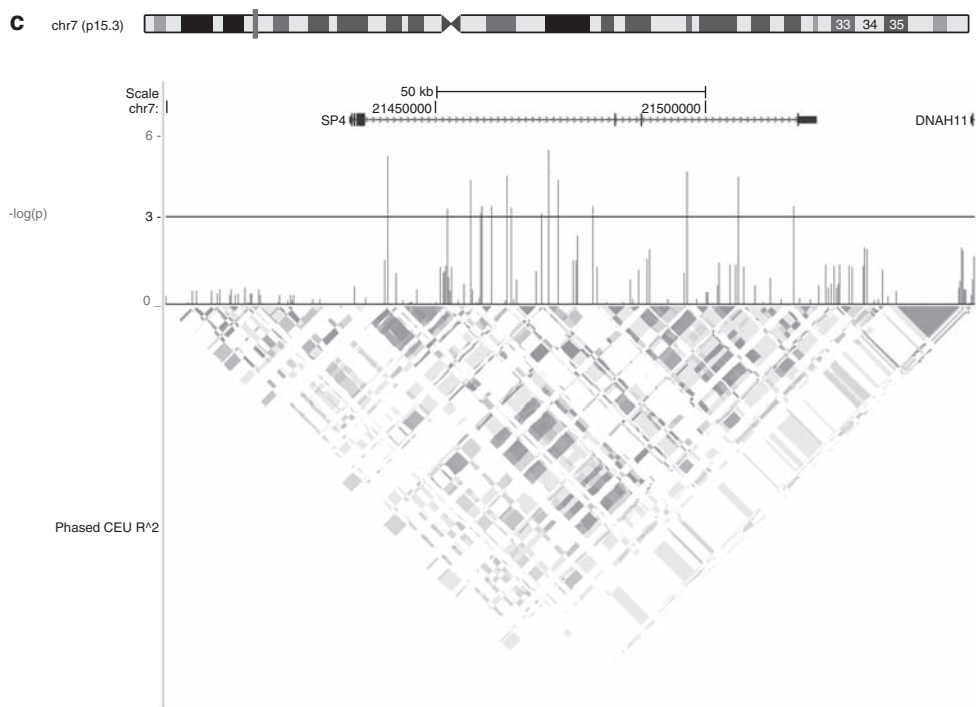


Figure 2 Continued.

with other GWAS results for common, genetically complex diseases<sup>37,38</sup>; the GRRs of significant findings have typically been in the range of 1.1–1.2, often requiring samples 10 000–20 000 cases obtained by combining multiple samples (in each of which the evidence for association can be quite modest). The odds ratios listed in Table 2 are much higher. They could represent a combination of false positive results and of true associations whose GRRs have been over-estimated by selecting the best results in one study (the ‘winner’s curse’ effect), and particularly with an underpowered sample. A companion paper<sup>17</sup> provides details of meta-analyses combining the GenRED, STAR\*D, and GAIN-MDD samples. We are also participating in an effort to carry out larger meta-analyses of MDD GWAS data through the Psychiatric GWAS Consortium.<sup>38,39</sup> However, because findings with the strongest statistical evidence for association in each study are most likely to be true positive results, we briefly review here several interesting findings.

Chromosome 18q22.1 (Figure 2) has produced suggestive evidence for linkage to bipolar disorder in several studies<sup>40–46</sup> (although not in the largest combined analysis<sup>47</sup>) and particularly in families with multiple bipolar-II (BP-II) cases<sup>42,43</sup> (characterized by recurrent depression plus hypomania). Several studies have also reported suggestive linkage to MDD or related personality traits in the same region.<sup>48</sup> Study of a family with diverse mood disorders<sup>45</sup> identified *DSEL* (dermatan sulfate epimerase-like), a brain-expressed gene in which two non-synonymous mutations were observed in cases but not in

controls.<sup>49</sup> Dermatan sulfate epimerase is involved in D-glucuronic acid metabolism and tumor rejection. The most strongly associated SNPs in this study are upstream of *DSEL*, in regions with possible regulatory functions, and within an mRNA (BC053410), identified in pooled human brain tissue, that encodes a hypothetical protein (LOC643542) of unknown function. Familial co-aggregation of MDD and BP-II disorder has been inadequately studied. BP-II was an exclusion criterion for GenRED probands, although in GenRED I, BP-II was diagnosed in 4.2% of the siblings selected for interview because of a history of depression, so the prevalence among all siblings was lower.

On chromosome 1p13.3 (Figure 2), low *P*-values spanned two broad linkage disequilibrium blocks that include many genes, including those encoding two G proteins (*GNAI3*, *GNAT2*), a G-protein coupled receptor related to biogenic amine receptors (*GPR61*), a transcription factor gene resembling those involved in neurodegenerative syndromes (*ATXN7L2*), and genes involved in neuronal growth and plasticity (*AMIGO1*) and neuronal apoptosis (*SORT1*). Current hypotheses suggest that both G-protein coupled signaling and mechanisms of neuronal plasticity are relevant to the pathophysiology of MDD and the actions of antidepressant drugs.<sup>50</sup>

The signal on chromosome 7p15.3 is within *SP4* (Figure 2), and comes almost entirely from females ( $P=4.44E-05$  vs 0.148 in males). *Sp4* is specific to neurons and expressed primarily early in development,<sup>51–53</sup> forms complexes with estrogen receptors that influence regulation of many genes,<sup>54</sup> and could have a role in the mediation of neuroprotective

enzymes and in glutamate-induced neurotoxicity.<sup>55,56</sup> Zhou *et al.*<sup>53</sup> have reported that reduced expression of Sp4 in mice leads to hippocampal vacuolization, age-dependent reduced expression of neurotrophin 3, and deficits in sensorimotor gating and contextual memory,<sup>53</sup> possibly mediated by defects in the development of dentate gyrus cells.<sup>57</sup> There is also evidence for a possible association between bipolar disorder and SNPs in the promoter of *ADRBK2* ( $\beta$  adrenergic receptor kinase 2, previously *GRK3*), which disrupt an Sp1/Sp4 binding site.<sup>58</sup> Thus, there are several mechanisms by which *SP4* could have a role in psychiatric disorders.

In conclusion, we carried out a GWAS of recurrent early-onset MDD in 1020 cases and 1636 controls of European ancestry. No genome-wide significant evidence for association was observed. Of the strongest signals reported in the GenRED sample, the meta-analysis in the companion article<sup>17</sup> provides the greatest support for association to SNPs in *SP4*. Much larger samples may be needed to determine whether there are true associations between MDD and common SNPs.

### Conflict of interest

The authors declare no conflict of interest.

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