Article

Genome-Wide Association Study of Major Recurrent Depression in the U.K. Population

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Objective: Studies of major depression in twins and families have shown moderate to high heritability, but extensive molecular studies have failed to identify susceptibility genes convincingly. To detect genetic variants contributing to major depression, the authors performed a genome-wide association study using 1,636 cases of depression ascertained in the U.K. and 1,594 comparison subjects screened negative for psychiatric disorders.

Method: Cases were collected from 1) a case-control study of recurrent depression (the Depression Case Control [DeCC] study; N=1346), 2) an affected sibling pair linkage study of recurrent depression (probands from the Depression Network [DeNT] study; N=332), and 3) a pharmacogenetic study (the Genome-Based Therapeutic Drugs for Depression [GENDEP] study; N=88). Depression cases and comparison subjects were genotyped at Centre National de Génotypage on the Illumina Human610-Quad BeadChip. After applying stringent quality control criteria for missing genotypes, departure from Hardy-Weinberg equilibrium, and low minor allele frequency, the authors tested for association to depression using logistic regression, correcting for population ancestry.

Results: Single nucleotide polymorphisms (SNPs) in *BICC1* achieved suggestive evidence for association, which strengthened after imputation of ungenotyped markers, and in analysis of female depression cases. A meta-analysis of U.K. data with previously published results from studies in Munich and Lausanne showed some evidence for association near neuroligin 1 (*NLGN1*) on chromosome 3, but did not support findings at *BICC1*.

Conclusion: This study identifies several signals for association worthy of further investigation but, as in previous genome-wide studies, suggests that individual gene contributions to depression are likely to have only minor effects, and very large pooled analyses will be required to identify them.

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Depression is a common and disabling condition that is a leading cause of disability, responsible for a substantial proportion of absence from work, impaired quality of life, poor physical health, and deaths by suicide (1–3). Increasing our understanding of the etiology of depression is therefore an important priority for medical research.

Family and twin studies indicate a substantial role of genetic factors in the etiology of depression. However, attempts to quantify the genetic contribution to depression have led to estimates of heritability ranging from 17% to 75%, with an average of 37% (2, 4, 5).These discrepant estimates point to a heterogeneity in depression and can be explained by several features associated with heritability. First, cases of depression ascertained through clinical contact tend to have stronger genetic contribution than cases diagnosed by lay interviewers in the general population (4, 5). Second, recurrent depression is more heritable than a single episode (4, 6). Third, more reliable assessment of depression is associated with high heritability, suggesting that heritability is often underestimated due to measurement error (7–9). These lines of evidence all indicate a strong genetic contribution to severe and recurrent depression when it is reliably diagnosed in the context of clinical practice.

While the existence of genetic vulnerability to depression is well-established, progress in the identification of its molecular basis has been slow. Functional candidate gene studies have identified few replicable associations (10), and genome-wide linkage studies have yielded suggestive rather than conclusive results (11). Two genome-wide association studies (GWAS) have been published to date. A population-ascertained study of unipolar depression from the Netherlands, using healthy subjects selected for low liability to depression, detected suggestive evidence for a role of genetic variants in the *PCLO* gene, but replication studies were inconsistent (12). A second genome-wide study analyzed two cohorts of recurrent depression cases from Munich and Lausanne, along with healthy comparison subjects screened to exclude depression and anxiety, but found no evidence for genetic variants associated with depression (13). These studies indicate that the genetic liability to depression is likely to involve multiple genetic variants of weak effects.

Further studies are needed to identify genetic variants contributing to depression. We performed a genome-wide association study for depression in an extensive U.K. case-control collection. To optimize the power to detect associated variants, we focused on depression cases with the strongest genetic contribution: those with reliably assessed, clinically ascertained recurrent unipolar depression. To improve further power, these depression cases were compared with subjects screened for absence of psychiatric disorders. We also performed a meta-analysis of results from this study and from the Munich and Lausanne depression GWAS (13) to assess evidence for association to depression in over 3,000 cases.

Method

Samples

In the U.K. study, depression cases and comparison subjects were drawn from two studies of recurrent depression and a pharmacogenetic study of antidepressant response using similar methods of case definition and collected by the same clinical team. The studies were approved by the local ethical committees and informed written consent was obtained from all participants.

The Depression Case Control (DeCC) study consists of 1,346 cases (69.3% women) of recurrent depression fulfilling DSM-IV or ICD-10 criteria of at least moderate severity, ascertained from three U.K. centers (London, Cardiff, Birmingham) (14). The mean age at onset was 22.9 years (SD=10.8 years). Probands from the Depression Network (DeNT) affected sibling pair linkage study (15, 16) are cases of recurrent depression of at least moderate severity. The 332 U.K.-ascertained cases included in this analysis were primarily women (75.3%), with mean age at illness onset of 23.0 years (SD=10.2 years). The pharmacogenetic study, the Genome-Based Therapeutic Drugs for Depression (GENDEP) study, comprises subjects who have been investigated while in an episode of depression of at least moderate severity (17, 18). The majority of the 88 U.K. cases included in this analysis were female (63.6%), with mean age at onset of 26.4 years (SD=11.3). In contrast to the DeCC and DeNT studies, GENDEP subjects were not required to have had two or more episodes of depression, although 68 cases (77.3%) were recurrent.

All cases were interviewed with the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (19), focusing on their worst and second-worst episodes of depression in the DeCC and DeNT studies and on their current episode in GENDEP (see Farmer et al. [15] for full details), with study coordinators for all three studies (and the Munich study described below) trained by one investigator (A.E.F.), giving a homogenous cohort of depression cases. Subjects were excluded if there was a history or family history of schizophrenia or bipolar disorder or if mood symptoms were related to alcohol or substance misuse.

The comparison subjects were 1,288 individuals contacted via the Medical Research Council general practice research framework and screened using a composite index of depressive and anxiety symptoms (20); they were interviewed by telephone using the Past History Schedule (21). Of these subjects, 58.4% were women, with mean age of 47.24 years (range=20–69). A further 457 healthy volunteers (61.4% female) were staff or students of King's College London, again screened for mental health using the Past History Schedule.

Meta-analysis was performed with the U.K. study, a clinical cohort of recurrent depression cases from Munich, and a population-based study in Lausanne with a psychiatric component, as analyzed previously (13). Cases from Munich (N=926) were assessed in a similar manner to the DeCC and DeNT study subjects, with healthy comparison subjects screened for absence of anxiety and mood disorders (N=866) (22). The Lausanne genome-wide association study comprised 492 subjects meeting DSM-IV criteria for recurrent major depression and 1,052 unaffected subjects; these were selected from the Caucasians of a community survey carried out in the city of Lausanne, Switzerland. The methods and the assessment of subjects (based on the Diagnostic Interview for Genetic Studies) have been extensively described (23).

Genotyping

Genotyping for the U.K. depression cases and comparison subjects was performed using the Illumina HumanHap610-Quad BeadChips by the Centre National de Génotypage (CNG), Evry, France. Barcoded DNA samples were received by the CNG DNA banking facility in standard tubes together with the sample information. All DNA samples were subjected to stringent quality control, and processing was carried out under full Laboratory Information Management System (LIMS) control. Confirmatory genotyping of single nucleotide polymorphisms (SNPs) showing suggestive evidence of association was performed to confirm allele-calling and to provide verification of genotypes for imputed SNPs; these genotypes were analyzed separately in testing for association but had higher levels of missing genotypes, limiting their power to detect association.

Quality Control

Stringent quality control procedures were applied to individual and SNP data. Individuals were excluded if their genotypic data showed a missing rate >1%, abnormal heterozygosity, a sex assignment that conflicted with phenotypic data, if they were related (up to second degree) with other study members, or were of non-European ancestry. SNPs with minor allele frequency (<1%) or showing departure from Hardy-Weinberg equilibrium ($p<1\times10^{-5}$) were excluded. Principal component analysis was performed using EIGENSTRAT (24) after quality control procedures, and the two principal components showing significant differences in ancestry between depression cases and comparison subjects were used as covariates in association testing. Further details of quality control are given in

TABLE 1. SNPs With Strongest Signals for Association in U.K. Genome-Wide Association Study of Major Recurrent Depression[a]

						Frequency			Meta-Analysis[c]		
Chromosome	SNP	Base Pair Position	Alleles	Significance (p)	Odds Ratio[b]	Depressed Cases	Healthy Subjects	Healthy Subjects (Munich)	Significance (p)	Position	Closest Gene[d]
10	rs9416742[e]	60212700	A/G	1.30E-07	0.719	0.1852	0.2346	0.1917	0.03849	16768	*BICC1*
10	rs999845	60205926	T/C	3.12E-07	0.7273	0.1877	0.2358	0.1917	0.05116	22172	*BICC1*
2	rs2698195	61144678	T/C	3.54E-06	2.063	0.0400	0.02039	0.0410	0.00061	316	*KIAA1841*
16	rs8050326	84661643	A/G	4.07E-06	1.265	0.4532	0.3986	0.4134	0.00017	90	*IRF8*
X	rs747181	70349433	A/G	6.57E-06	1.318	0.3219	0.2633	-	-	-	*GJB1*
3	rs13079811[e]	82697593	C/A	8.14E-06	0.7868	0.3159	0.3714	0.3274	0.01432	6339	*GBE1*
1	rs2273289	11940877	C/T	8.16E-06	1.377	0.1632	0.1245	0.1293	0.00014	81	*PLOD1*
5	rs3805652	127789317	G/A	1.00E-05	1.38	0.1644	0.1249	0.1269	0.00155	767	*FBN2*
1	rs606149	192188171	T/C	1.26E-05	1.248	0.4872	0.4307	0.4452	2.6E-06	2	*LOC647167*
8	rs2930553[e]	86918262	G/T	1.43E-05	0.8047	0.4719	0.5254	0.5069	0.08632	36811	*REXO1L1*
5	rs10512971	51897194	G/A	1.44E-05	0.771	0.2182	0.2629	0.2436	0.00028	138	*PELO*
1	rs6662524	192153242	T/C	1.47E-05	0.7345	0.129	0.1696	0.1842	1.1E-05	9	*LOC647167*
5	rs1585698	51896668	G/T	1.49E-05	0.7725	0.22	0.2649	0.2442	0.00031	159	*PELO*
17	rs9944407	40017486	C/T	1.54E-05	0.7986	0.3631	0.4128	0.3782	0.00347	1585	*FZD2*
3	rs7433760	187284322	G/A	1.65E-05	0.788	0.2766	0.3262	0.2921	9.2E-05	60	*ETV5*
5	rs4705005	155691848	G/A	1.68E-05	0.8027	0.3955	0.4472	0.4073	0.00484	2205	*SGCD*
17	rs8067196	49822630	C/A	1.69E-05	1.494	0.0969	0.06838	0.0947	0.0103	4526	*TOM1L1*
20	rs2423618	11757442	C/T	2.12E-05	1.262	0.3395	0.2889	0.3106	0.00288	1322	*LOC728450*
18	rs949355	20419693	A/G	2.16E-05	0.7354	0.1252	0.1634	-	-	-	*LOC390843*
17	rs8066010	49808584	A/G	2.52E-05	1.482	0.0966	0.06864	0.0943	0.01107	4861	*TOM1L1*

[a] SNPs listed by p value from logistic regression analysis of depression cases and healthy subjects.
[b] For the first listed allele.
[c] U.K., Munich, and Lausanne studies.
[d] Closest gene to genotyped SNP, from build 36.2, using Illumina 610Quad annotation file.
[e] Also achieves suggestive significance ($p < 5 \times 10^{-6}$) in analysis of 1,152 female cases (see data supplement).

the data supplement that accompanies the online version of this article.

Statistical Analysis

The primary test for association between SNPs and depression was logistic regression, including ancestry principal components, assuming a log-additive genetic model. Additional analyses were performed using the Cochran Armitage trend test and logistic regression assuming a full genotype model (2 df) and dominant and recessive models. Analyses were carried out for all depression cases and separately for male and female cases. The genomic control parameter λ was calculated for each analysis to assess inflation due to population stratification (25). Analysis was implemented using PLINK v1.05 (26) and R (www.r-project.org).

Two stringent thresholds of significance were used: genome-wide significance ($p<5\times10^{-8}$ [27, 28]) and suggestive significance ($p<5\times10^{-6}$). This study has a power of 86% at suggestive level of significance (and power of 58% at genome-wide significance) to detect an association explaining 0.75% of variance, assuming that cases account for the upper 10% of the depression liability and screened comparison subjects the lower 70%. For genetic regions where any SNP achieved suggestive evidence for association, follow-up analyses were performed. Genotypes at HapMap SNPs in 200-kb regions flanking the most highly associated SNP were imputed and analyzed using MACH version 1.0 (29) to determine whether stronger associations could be identified in the region. Haplotype analysis of genotyped and imputed SNPs was performed using UNPHASED (30), with two ancestry principal components as covariates and including only genotypes imputed with probability >0.9 in the analysis.

We tested specifically for association with a set of 84 candidate genes previously implicated in susceptibility to depression through their function or previous genetic association studies (see Supplementary Table 2 for list of genes, which was selected prior to genome-wide analysis). Results from the logistic regression analysis for 2,654 SNPs lying within the genes and in 20-kb flanking regions were extracted.

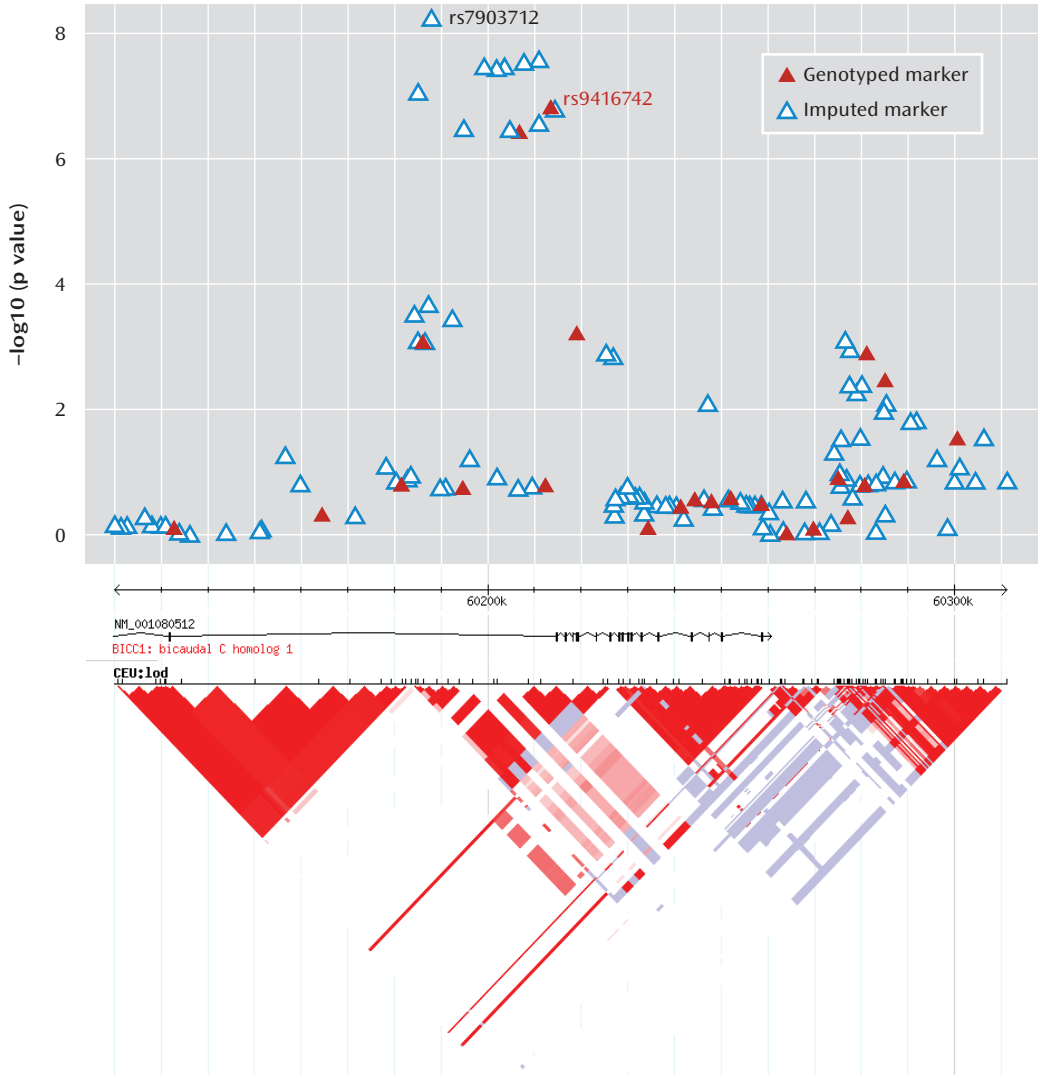
Meta-analysis was then performed on the U.K., Munich, and Lausanne studies. For the Munich and Lausanne studies, genome-wide summary statistics on the autosomal chromosomes were available (13). The Munich study had been genotyped on the Illumina 550K array, which overlaps substantially with the Illumina 610K array used in the U.K. study. For the Lausanne study, results from the same set of SNPs were available, which had been imputed where necessary, from genotypes on Affymetrix 500K chip. Meta-analysis was performed using the software METAL (www.sph.umich.edu/csg/abecasis/metal/).

Results

Association Analysis

In the analysis testing for association with 1,636 depression cases and 1,594 screened comparison subjects at 471,747 SNPs that passed quality control procedures, no single SNP reached genome-wide significance. Four SNPs showed suggestive evidence for association: two SNPs in *BICC1* on chromosome 10q21.1 (rs9416742 [$p=1.3\times10^{-7}$] and rs999845 [$p=3.1\times10^{-7}$]), rs2698195 on chromosome 2p15, which is located 2 kb 5′ of *KIAA1841*, and rs8050326 (16q24.1, located over 100 kb from *IRF8*). The genomic control parameter (λ) decreased from 1.04 in the Cochran

FIGURE 1. Analysis of Imputed Genotypes in the *BICC1* Region of 10q21 Flanking rs9416742[a]



[a] Strongest evidence for association occurred at rs7903712 ($p=5.7\times10^{-9}$; imputation $r^2=0.9685$).

Armitage Trend test to 1.02 after accounting for two ancestry principal components, indicating little difference between depression cases and comparison subjects due to population ancestry or other systematic genomic effects. Details of the 20 SNPs with strongest signals for association are given in Table 1, with p values also provided from the meta-analysis with Munich and Lausanne cohorts.

Analysis of imputed genotypes in the *BICC1*-region of 10q21 strengthened the evidence for association. Genome-wide significant evidence for association was detected at six SNPs in strong linkage disequilibrium, with the strongest evidence for association occurring at rs7903712 (Figure 1). At the genotyped SNP rs9416742, the minor allele, which had a frequency of 0.235 in healthy subjects, conferred a protective effect against depression (odds ratio=0.719, 95% CI=0.6362–0.8127). The SNPs rs9416742 and rs7903712 were regenotyped in-house (see supplementary materials) and showed strong concordance with Illumina 610Quad BeadChip genotyping (concordance 99.95% for rs9416742) and with imputed genotypes (98.37% for rs7903712). For both SNPs, mismatch was uncorrelated with genotype. Association testing with regenotyped data showed a similar pattern of results, with rs7903712 having higher significance levels than rs9416742 ($p=9.65\times10^{-8}$ versus $p=3.07\times10^{-6}$). The decrease in significance reflects the higher missing rates for genotypes (10.5% and 8.6%); these genotypes were not used in subsequent analysis.

To explore the source of increased significance further, we analyzed haplotypes at these two markers. For rs7903712, imputed genotypes with estimated accuracy >90% were included, giving genotypes for 95.1% of the sample. Comparison with our regenotyped samples showed imputation missingness or genotype discordancy was not correlated with genotype. Only three haplotypes for rs9416742 and rs7903712 exist at frequency >1%. The increased evidence for association at rs7903712 was due to a haplotype of estimated frequency 1.7% in comparison

TABLE 2. *BICC1* Haplotype Analysis for rs9416742 (G/A) and rs7903712 (G/C)[a]

Haplotype	Case	%	Comparison	%	Odds Ratio	95% CI	Significance (p)
G-G	2567	0.805	2326	0.751	1		
A-C	556.7	0.175	706.8	0.228	0.692	0.610–0.785	9.04×10^{-8}
G-C	44.28	0.014	51.2	0.017	0.740	0.490–1.18	0.1525

[a] Allele A is protective at rs9416742, and allele C is protective at rs7903712. The p values are for each haplotype against the baseline haplotype G-G. Haplotype G-C carries the protective allele only at rs7903712, with an odds ratio of 0.74, similar to that of the A-C haplotype, but is too rare to show significance individually. The fourth haplotype A-G has a frequency of 0.004 in healthy subjects and was omitted from the analysis.

subjects, which carried the common allele at rs9416742 but conferred a protective effect (Table 2). In HapMap, the additional protective haplotype identified by rs7903712 has a frequency of 2.5%.

Results for top 20 SNPs for sex-specific analyses are given in Supplementary Tables 3 and 4. In women (1,152 cases), genome-wide association was observed with rs9416742 in *BICC1* ($p=1.8\times10^{-8}$; odds ratio=0.673, 95% CI=0.586–0.772), but men had a much weaker p value (p=0.039; odds ratio=0.830, 95% CI=0.695–0.991). A test for interaction between sex and genotype was not significant (p=0.149). In women, four further SNPs achieved suggestive significance. Three of these SNPs (rs8067196, rs2930553, and rs13079811) also showed strong association in the full study and are listed in Table 1. Suggestive evidence of association was also found at SNP rs987390, which lies 180 kb from rs2930553, near the *REXO1L1* gene, strengthening association in this genetic region. In men (N=477), only a single SNP, rs6989226, near the *TUSC3* gene reaches suggestive significance ($p=1.81\times10^{-6}$). No additional significant findings were identified using other genetic models (genotypic, dominant, and recessive).

In the analysis of 84 candidate genes, the strongest evidence for association was at an intronic SNP, rs13050655, in *PDE9A* ($p=3.58\times10^{-5}$, ranked 27 in the genome-wide analysis), a gene postulated to contribute to vulnerability to depression (31). Results by gene are shown in Supplementary Table 2.

Meta-Analysis

In the meta-analysis of U.K., Munich, and Lausanne studies, no SNP attained genome-wide significance and one SNP, rs606149, achieved suggestive significance ($p=2.57\times10^{-6}$). This SNP lies close to two other SNPs with p values of $<2\times10^{-5}$ in the analysis of U.K. cases and healthy comparison subjects (Table 1). The SNPs are located near *LOC647167* on chromosome 1, which is otherwise a gene desert. The top 20 significant findings are shown in Table 3. Notably, the third strongest finding is rs13074924, which is located 29.7 kb from the 3′ end of *NLGN1* (neuroligin 1) in 3q26.31, and >100 kb from the next gene. This SNP achieves modest p values in all three studies (p=0.001805 in the U.K. study, p=0.01554 in the Munich study, and p=0.03958 in the Lausanne study) with a consistent direction for allele effect, giving $p=8.54\times10^{-6}$ in the meta-analysis. In the meta-analysis, no replication was seen for the association with *BICC1* in the U.K. study. In the Munich, Germany, and Lausanne cohorts, rs9416742 had p values of 0.11 and 0.06, respectively, but in the opposite direction to the effect in the U.K. cohorts. Of the SNPs reported in the U.K. study (Table 1), only those near *LOC647167* have a p value of $<1\times10^{-4}$ in the meta-analysis.

Discussion

This genome-wide association study has found evidence for a role of genetic variants in the bicaudal C homologue 1 gene (*BICC1*) in recurrent unipolar major depression, however the finding that a minor allele of these variants has a protective effect against depression was not replicated in two independent samples of recurrent major depression collected and assessed with comparable procedures. The association of *BICC1* and depression is a novel finding, with no previous published evidence for a role of this gene in neuropsychiatric disease.

The *BICC1* gene product is an RNA-binding protein which has been found to form complex interactions with RNA and with other proteins. Several independent lines of evidence suggest a role for BICC1 in neurogenesis and plasticity, defects which may predispose to mood disorders. In *Drosophila*, it is required for dorsoventral patterning of the oocyte (32). The RNA binding activity of the protein is mediated by its KH domains (see Supplementary Figure 5) (33), which are characteristic of the heterogeneous nuclear ribonucleoprotein k family of transcription factors and bind both DNA and RNA (34). Other heterogeneous nuclear ribonucleoproteins are known to bind targets such as the serotonin transporter gene promoter (35). In humans, the C-terminus of the longer isoform-1 of the protein contains a sterile alpha motif (SAM) domain (Supplementary Figure 5), thought to be a highly specific postsynaptic targeting signal (36). This is interesting as our observed association is intronic to the long isoform, but is 15 kb 5′ from the first reported exon of a shorter isoform that does not contain a SAM domain and may highlight a haplotype which favors the expression of one or other of these isoforms. Both *BICC1* isoform RNAs are expressed widely in normal tissues and are expressed in all brain regions including cerebral cortex, hippocampus, and midbrain (see Supplementary Figures 6a-d), although *BICC1* isoform-1 RNA is more highly expressed than isoform-2, particularly in nerve tissue. *BICC1* can uncouple dishevelled-2 signaling from the canonical Wnt pathway in a SAM domain dependent manner, suggesting that the dif-

TABLE 3. Top 20 Significant Findings From a Meta-Analysis of U.K., Munich, and Lausanne Genome-Wide Association Studies of Major Recurrent Depression

Chromosome	SNP	Base Pair Position	Closest Gene	Odds ratio (U.K.)	Allele frequency (U.K.)	Significance Level (p)			Meta-Analysis Significance Result (p)
						U.K.	Munich	Lausanne	
1	rs606149	192188171	*LOC647167*	1.248	0.4593	1.26E-05	0.02255	0.4901	2.57E-06
5	rs310501	82925666	*VCAN*	1.158	0.3287	0.007339	0.04107	0.001001	6.98E-06
3	rs13074924	175511747	*NLGN1*	1.178	0.3558	0.001805	0.01554	0.03958	8.54E-06
1	rs550899	192149733	*LOC647167*	1.228	0.3919	7.80E-05	0.04067	0.2313	8.63E-06
6	rs4709845	164871009	*LOC728275*	0.8289	0.1526	0.008242	0.03299	0.001833	9.23E-06
11	rs7107938	27027694	*BBOX1*	1.141	0.4707	0.01009	0.002008	0.03854	9.77E-06
1	rs2884515	210796957	*ATF3*	0.8234	0.2881	0.0004611	0.005466	0.3759	9.99E-06
1	rs6662524	192153242	*LOC647167*	0.7345	0.149	1.47E-05	0.05889	0.4927	1.05E-05
14	rs1187738	95019742	*C14orf49*	1.16	0.455	0.003354	0.00704	0.07462	1.44E-05
1	rs6429449	242368653	*LOC440742*	0.7974	0.2666	9.34E-05	0.06101	0.2439	1.54E-05
12	rs2733449	56775580	*LOC338805*	1.195	0.3743	0.0007827	0.1052	0.0102	1.60E-05
2	rs7572559	54283769	*ACYP2*	1.25	0.1954	0.0005678	0.02558	0.1701	1.95E-05
15	rs13638	71968591	*TBC1D21*	0.8343	0.148	0.01041	0.007348	0.02759	2.08E-05
1	rs10921464	192156156	*LOC647167*	0.8137	0.4282	5.21E-05	0.03759	0.5709	2.13E-05
5	rs930076	9603642	*SEMA5A*	0.8257	0.2849	0.0005618	0.0313	0.2275	2.47E-05
12	rs1814317	106859234	*LOC728739*	0.8207	0.4433	0.0001301	0.04767	0.3533	2.63E-05
6	rs9456968	164890804	*LOC728275*	0.8368	0.1619	0.009379	0.07352	0.001449	2.77E-05
3	rs9290678	180499722	*LOC647244*	1.147	0.2639	0.01609	0.009342	0.01716	2.81E-05
5	rs4958272	150811835	*SLC36A1*	1.216	0.4864	0.000111	0.00811	0.7574	3.02E-05
12	rs10861843	106866844	*LOC728739*	0.8212	0.4427	0.0001407	0.04678	0.386	3.07E-05

ferent *BICC1* isoforms may play different biological roles (37). Gene expression studies (Array Express; http://www.ebi.ac.uk/microarray-as/ae/) suggest that BICC1 is also upregulated by sodium valproate (a mood stabilizer and Wnt signaling inhibitor used to treat bipolar disorder), with approximately twofold increase in *BICC1* expression after valproate treatment.

The other notable result is the meta-analysis finding of association with a SNP near neuroligin-1 (*NLGN1*) on 3q26.31. Echoing our BICC1 finding, it is known that all NLGNs are enriched at postsynaptic densities (38). *NLGN1* encodes a neuronal cell surface protein and a splice site-specific ligand for beta-neurexins. The Ca(2+)-dependent neurexin/neuroligin complex is required for efficient neurotransmission, and involved in the formation of synaptic contacts. Neuroligins are likely to be part of the machinery employed during the formation and remodeling of CNS synapses. The neuroligin/neurexin complex has also been implicated in schizophrenia (39), with deletions of *NLGN1* observed in autism (40); deletions of the closely related *NLGN3* and *NLGN4/5* result in a variety of psychiatric phenotypes (41, 42).

Several aspects of the finding in *BICC1* may be relevant to replication and follow-up studies. First, the strongest association was found with a SNP that was not genotyped on the Illumina panel but could be imputed with high accuracy based on genotyped markers in strong linkage disequilibrium. The high accuracy of imputation was confirmed by targeted regenotyping, and the association was statistically significant after accounting for uncertainty of imputation and for potential multiple testing of all common variants across the human genome (27, 28). However, this effect did not replicate in either the Munich or Lausanne samples. Such nonreplication occurs frequently; with the modest effect sizes expected for genetic contributions to depression, associations will remain elusive with large variation across studies. Even for a true association, the effect size may be inflated in the discovery sample (43, 44).

Replication of the *BICC1* finding will be necessary to determine whether this is a false positive result or a true effect which is also detectable in other studies. Such analyses are ongoing in the Psychiatric GWAS Consortium (45), of which this study is a part. The large, well-characterized samples that form the Psychiatric GWAS Consortium will be required before we can expect to see consistent signals of association through meta- and mega-analysis. The Psychiatric GWAS Consortium analysis should help resolve the role of *BICC1* and other putative associations in depression. However, replication is likely to remain a substantial challenge for all psychiatric disorders. While large sample sizes increase the power to detect genetic risk loci, variation between studies will be unavoidable, and potentially unquantifiable. Sources of diversity between studies include those related to phenotype (e.g., criteria for depression, assessment measures of depression for both cases and comparison subjects), those related to genotype (e.g., population ancestry, genotyping panel used), and more subtle effects such as differences in patient ascertainment methods by study or cultural differences in seeking help for depression, which may result in heterogeneous case samples even though all patients meet clinical criteria for recurrent depression.

In order to evaluate the present results and to make a qualified comparison with other studies, the strengths

and limitations of the study should be considered. The study used recurrent major depression, the type of depression that has been shown to be most heritable (4, 8), with collection of cases in the U.K. by the same clinical team giving a homogeneous clinical sample. As depression is common in the general population, excluding cases of lifetime depression from the comparison sample can substantially increase power of a study. We have therefore used a sample of comparison subjects screened to exclude those with higher liability to depression. The ability of the present study to detect true associations was limited by its sample size, which was comparable to the previously published genome-wide association studies (12, 13).

In conclusion, the present study provides evidence for a gene encoding a Wnt signaling component, *BICC1*, in the pathogenesis of depression. The meta-analysis implicated *NLGN1*, whose role in formation and remodeling of CNS synapses makes it a convincing functional candidate gene for depression. However, replication of these findings in other studies will be essential to confirm their role in depression. The results of this and other genome-wide association studies in depression suggest that effects of common genetic variants on depression are individually weak and multiple large samples will be needed to provide a more comprehensive picture of common genetic variants in depression.

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References

1. Cavanagh JT, Carson AJ, Sharpe M, Lawrie SM: Psychological autopsy studies of suicide: a systematic review. Psychol Med 2003; 33:395–405
2. ten Doesschate MC, Koeter MW, Bockting CL, Schene AH; DELTA Study Group: Health related quality of life in recurrent depression: a comparison with a general population sample. J Affect Disord 2010;120:126–132
3. Lopez AD, Murray CC: The global burden of disease, 1990–2020. Nat Med 1998; 4:1241–1243
4. McGuffin P, Katz R, Watkins S, Rutherford J: A hospital-based twin register of the heritability of DSM-IV unipolar depression. Arch Gen Psychiatry 1996; 53:129–136
5. Sullivan PF, Neale MC, Kendler KS: Genetic epidemiology of major depression: review and meta-analysis. Am J Psychiatry 2000; 157:1552–1562
6. McGuffin P, Rijsdijk F, Andrew M, Sham P, Katz R, Cardno A: The heritability of bipolar affective disorder and the genetic relationship to unipolar depression. Arch Gen Psychiatry 2003; 60:497–502
7. Peregrin-Alvarez I, Urbaneja-Lopez M, Quijada-To Ong D: Polycystic ovarian syndrome in a woman with polycystic kidney disease. Eur J Obstet Gynecol Reprod Biol 2008; 140:282–283
8. Foley DL, Neale MC, Kendler KS: Reliability of a lifetime history of major depression: implications for heritability and comorbidity. Psychol Med 1998; 28:857–870
9. Kendler KS, Neale MC, Kessler RC, Heath AC, Eaves LJ: The lifetime history of major depression in women: reliability of diagnosis and heritability. Arch Gen Psychiatry 1993; 50:863–870
10. Lopez-Leon S, Janssens AC, Gonzalez-Zuloeta Ladd AM, Del-Favero J, Claes SJ, Oostra BA, van Duijn CM: Meta-analyses of genetic studies on major depressive disorder. Mol Psychiatry 2008; 13:772–785
11. McGuffin P, Cohen S, Knight J: Homing in on depression genes. Am J Psychiatry 2007; 164:195–197
12. Sullivan PF, de Geus EJ, Willemsen G, James MR, Smit JH, Zandbelt T, Arolt V, Baune BT, Blackwood D, Cichon S, Coventry WL, Domschke K, Farmer A, Fava M, Gordon SD, He Q, Heath AC, Heutink P, Holsboer F, Hoogendijk WJ, Hottenga JJ, Hu Y, Kohli M, Lin D, Lucae S, Macintyre DJ, Maier W, McGhee KA, McGuffin P, Montgomery GW, Muir WJ, Nolen WA, Nöthen MM, Perlis RH, Pirlo K, Posthuma D, Rietschel M, Rizzu P, Schosser A, Smit AB, Smoller JW, Tzeng JY, van Dyck R, Verhage M, Zitman FG, Martin NG, Wray NR, Boomsma DI, Penninx BW: Genome-wide association for major depressive disorder: a possible role for the presynaptic protein piccolo. Mol Psychiatry 2009; 14:359–375
13. Muglia P, Tozzi F, Galwey NW, Francks C, Upmanyu R, Kong XQ, Antoniades A, Domenici E, Perry J, Rothen S, Vandeleur CL, Mooser V, Waeber G, Vollenweider P, Preisig M, Lucae S, Müller-Myhsok B, Holsboer F, Middleton LT, Roses AD: Genome-wide

association study of recurrent major depressive disorder in two European case-control cohorts. Mol Psychiatry (advance online publication, December 23, 2008; doi:10.1038/mp.2008.131)
14. Cohen-Woods S, Gaysina D, Craddock N, Farmer A, Gray J, Gunasinghe C, Hoda F, Jones L, Knight J, Korszun A, Owen MJ, Sterne A, Craig IW, McGuffin P: Depression Case Control (DeCC) Study fails to support involvement of the muscarinic acetylcholine receptor M2 (CHRM2) gene in recurrent major depressive disorder. Hum Mol Genet 2009; 18:1504–1509
15. Farmer A, Breen G, Brewster S, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen M, Perry J, Preisig M, Rietschel M, Reich T, Jones L, Jones I, McGuffin P: The Depression Network (DeNT) Study: methodology and sociodemographic characteristics of the first 470 affected sibling pairs from a large multi-site linkage genetic study. BMC Psychiatry 2004; 4:42
16. McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen MJ, Perry J, Preisig M, Reich T, Rice J, Rietschel M, Jones L, Sham P, Farmer AE: Whole genome linkage scan of recurrent depressive disorder from the depression network study. Hum Mol Genet 2005; 14: 3337–3345
17. Uher R, Huezo-Diaz P, Perroud N, Smith R, Rietschel M, Mors O, Hauser J, Maier W, Kozel D, Henigsberg N, Barreto M, Placentino A, Dernovsek MZ, Schulze TG, Kalember P, Zobel A, Czerski PM, Larsen ER, Souery D, Giovannini C, Gray JM, Lewis CM, Farmer A, Aitchison KJ, McGuffin P, Craig I: Genetic predictors of response to antidepressants in the GENDEP project. Pharmacogenomics J 2009; 9:225–233
18. Uher R, Maier W, Hauser J, Marusic A, Schmael C, Mors O, Henigsberg N, Souery D, Placentino A, Rietschel M, Zobel A, Dmitrzak-Weglarz M, Petrovic A, Jorgensen L, Kalember P, Giovannini C, Barreto M, Elkin A, Landau S, Farmer A, Aitchison KJ, McGuffin P: Differential efficacy of escitalopram and nortriptyline on dimensional measures of depression. Br J Psychiatry 2009; 194:252–259
19. Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, Jablenski A, Regier D, Sartorius N: SCAN: Schedules for Clinical Assessment in Neuropsychiatry. Arch Gen Psychiatry 1990; 47:589–593
20. Sham P, Sterne A, Purcell S, Cherny SS, Webster M, Rijsdijk FV, Asherson P, Ball D, Craig I, Eley T, Goldberg D, Gray J, Mann A, Owen M, Plomin R: GENESiS: creating a composite index of the vulnerability to anxiety and depression in a community-based sample of siblings. Twin Res 2000; 3:316–322
21. McGuffin P, Katz R, Aldrich J: Past and present state examination: the assessment of "lifetime ever" psychopathology. Psychol Med 1986; 16:461–465
22. Tozzi F, Prokopenko I, Perry JD, Kennedy JL, McCarthy AD, Holsboer F, Berrettini W, Middleton LT, Chilcoat HD, Muglia P: Family history of depression is associated with younger age of onset in patients with recurrent depression. Psychol Med 2008; 38:641–649
23. Preisig M, Waeber G, Vollenweider P, Bovet P, Rothen S, Vandeleur C, Guex P, Middleton L, Waterworth D, Mooser V, Tozzi F, Muglia P: The PsyCoLaus study: methodology and characteristics of the sample of a population-based survey on psychiatric disorders and their association with genetic and cardiovascular risk factors. BMC Psychiatry 2009; 9:9
24. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D: Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006; 38:904–909
25. Devlin B, Roeder K: Genomic control for association studies. Biometrics 1999; 55:997–1004
26. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC: PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81:559–575
27. Dudbridge F, Gusnanto A: Estimation of significance thresholds for genomewide association scans. Genet Epidemiol 2008; 32:227–234
28. Pe'er I, Yelensky R, Altshuler D, Daly MJ: Estimation of the multiple testing burden for genomewide association studies of nearly all common variants. Genet Epidemiol 2008; 32:381–385
29. Li Y, Abecasis G: Mach 1.0: rapid haplotype reconstruction and missing genotype inference. Am J Hum Genet 2006; S79:2290
30. Dudbridge F: Likelihood-based association analysis for nuclear families and unrelated subjects with missing genotype data. Hum Hered 2008; 66:87–98
31. Wong ML, Whelan F, Deloukas P, Whittaker P, Delgado M, Cantor RM, McCann SM, Licinio J: Phosphodiesterase genes are associated with susceptibility to major depression and antidepressant treatment response. Proc Natl Acad Sci U S A 2006; 103:15124–15129
32. Snee MJ, Macdonald PM: Bicaudal C and trailer hitch have similar roles in gurken mRNA localization and cytoskeletal organization. Dev Biol 2009; 328:434–444
33. Bouvrette DJ, Price SJ, Bryda EC: K homology domains of the mouse polycystic kidney disease-related protein, bicaudal-C (Bicc1), mediate RNA binding in vitro. Nephron Exp Nephrol 2008; 108:e27–e34
34. Tomonaga T, Levens D: Heterogeneous nuclear ribonucleoprotein K is a DNA-binding transactivator. J Biol Chem 1995; 270:4875–4881
35. Klenova E, Scott AC, Roberts J, Shamsuddin S, Lovejoy EA, Bergmann S, Bubb VJ, Royer HD, Quinn JP: YB-1 and CTCF differentially regulate the 5-HTT polymorphic intron 2 enhancer which predisposes to a variety of neurological disorders. J Neurosci 2004; 24:5966–5973
36. Grabrucker AM, Vaida B, Bockmann J, Boeckers TM: Efficient targeting of proteins to post-synaptic densities of excitatory synapses using a novel pSDTarget vector system. J Neurosci Methods 2009; 181:227–234
37. Maisonneuve C, Guilleret I, Vick P, Weber T, Andre P, Beyer T, Blum M, Constam DB: Bicaudal C, a novel regulator of Dvl signaling abutting RNA-processing bodies, controls cilia orientation and leftward flow. Development 2009; 136:3019–3030
38. Sudhof TC: Neuroligins and neurexins link synaptic function to cognitive disease. Nature 2008; 455: 903–911
39. Bennett AOM: Dual constraints on synapse formation and regression in schizophrenia: neuregulin, neuroligin, dysbindin, DISC1, MuSK and agrin. Aust N Z J Psychiatry 2008; 42:662–677
40. Glessner JT, Wang K, Cai G, Korvatska O, Kim CE, Wood S, Zhang H, Estes A, Brune CW, Bradfield JP, Imielinski M, Frackelton EC, Reichert J, Crawford EL, Munson J, Sleiman PM, Chiavacci R, Annaiah K, Thomas K, Hou C, Glaberson W, Flory J, Otieno F, Garris M, Soorya L, Klei L, Piven J, Meyer KJ, Anagnostou E, Sakurai T, Game RM, Rudd DS, Zurawiecki D, McDougle CJ, Davis LK, Miller J, Posey DJ, Michaels S, Kolevzon A, Silverman JM, Bernier R, Levy SE, Schultz RT, Dawson G, Owley T, McMahon WM, Wassink TH, Sweeney JA, Nurnberger JI, Coon H, Sutcliffe JS, Minshew NJ, Grant SF, Bucan M, Cook EH, Buxbaum JD, Devlin B, Schellenberg GD, Hakonarson H: Autism genome-wide copy number variation reveals ubiquitin and neuronal genes. Nature 2009; 459:569–573
41. Tabuchi K, Blundell J, Etherton MR, Hammer RE, Liu X, Powell CM, Südhof TC: A neuroligin-3 mutation implicated in autism increases inhibitory synaptic transmission in mice. Science 2007; 318:71–76
42. Lawson-Yuen A, Saldivar JS, Sommer S, Picker J: Familial deletion within NLGN4 associated with autism and Tourette syndrome. Eur J Hum Genet 2008; 16:614–618

43. Goring HH, Terwilliger JD, Blangero J: Large upward bias in estimation of locus-specific effects from genomewide scans. Am J Hum Genet 2001; 69:1357–1369
44. Ioannidis JP, Thomas G, Daly MJ: Validating, augmenting and refining genome-wide association signals. Nat Rev Genet 2009; 10:318–329
45. Cichon S, Craddock N, Daly M, Faraone SV, Gejman PV, Kelsoe J, Lehner T, Levinson DF, Moran A, Sklar P, Sullivan PF: Genomewide association studies: history, rationale, and prospects for psychiatric disorders. Am J Psychiatry 2009; 166:540–556