Sequence variation in *DOCK9* and heterogeneity in bipolar disorder

Sevilla D. Detera-Wadleigh[a], Chun-yu Liu[c], Manjula Maheshwari[d], Imer Cardona[a], Winston Corona[a], Nirmala Akula[a], C.J.M. Steele[a], Judith A. Badner[c], Mukta Kundu[a], Layla Kassem[a], James B. Potash[b], NIMH Genetics Initiative for Bipolar Disorder Consortium[e,f,g,h,i,j,k], Richard Gibbs[d], Elliot S. Gershon[c] and Francis J. McMahon[a]

Background **Linkage of bipolar disorder to a broad region on chromosome 13q has been supported in several studies including a meta-analysis on genome scans. Subsequent reports have shown that variations in the *DAOA* (G72) locus on 13q33 display association with bipolar disorder but these may not account for all of the linkage evidence in the region.**

Objective **To identify additional susceptibility loci on 13q32-q33 by linkage disequilibrium mapping and explore the impact of phenotypic heterogeneity on association.**

Methods **In the initial phase, 98 single nucleotide polymorphism (SNPs) located on 13q32-q33 were genotyped on 285 probands with bipolar disorder and their parents were drawn from families in the NIMH Genetics Initiative consortium for bipolar disorder (NIMH1-4) and two other series. Fine scale mapping using one family series (NIMH1-2) as the test sample was targeted on a gene that displayed the highest evidence of association. A secondary analysis of familial component phenotypes of bipolar disorder was conducted.**

Results **Three of seven SNPs in *DOCK9,* a gene that encodes an activator of the Rho-GTPase Cdc42, showed significant excess allelic transmission ($P = 0.0477 - 0.00067$). Fine scale mapping on *DOCK9* yielded evidence of association at nine SNPs in the gene ($P = 0.02 - 0.006$). Follow-up tests detected excess transmission of the same allele of rs1340 in two out of three other sets of families. The association signals were largely attributable to maternally transmitted alleles (rs1927568: $P = 0.000083$; odds ratio = 3.778). A secondary analysis of familial component phenotypes of bipolar disorder detected significant association across multiple *DOCK9* markers for racing thoughts, psychosis, delusion during mania and course of illness indicators.**

Conclusion **These results suggest that *DOCK9* contributes to both risk and increased illness severity in bipolar disorder. We found evidence for the effect of phenotypic heterogeneity on association. To our knowledge this is the first report to implicate *DOCK9* or the Rho-GTPase pathway in the etiology of bipolar disorder.** ***Psychiatr Genet*** **17:274–286 © 2007 Lippincott Williams & Wilkins.**

Psychiatric Genetics 2007, **17:**274–286

Keywords: allele, association, Cdc42, phenotypes, transmission

[a]Genetic Basis of Mood and Anxiety Disorders, Mood and Anxiety Program, National Institute of Mental Health Intramural Research Program, National Institutes of Health, US Department of Health and Human Services, Bethesda, [b]Department of Psychiatry, Johns Hopkins University, Baltimore, Maryland, [c]Department of Psychiatry, University of Chicago, Chicago, Illinois, [d]Department of Molecular and Human Genetics, Human Genome Sequencing Center, Baylor College of Medicine, Houston, Texas, [e]Indiana University, Indianapolis, Indianapolis, [f]Washington University, St Louis, Missouri, [g]University of Pennsylvania, Philadelphia, Pennsylvania, [h]University of California at Irvine, California, [i]University of Iowa, Iowa, [j]University of California at San Diego, California and [k]Rush University, Illinois, USA

Correspondence to Sevilla D. Detera-Wadleigh, MAP, NIMH, NIH, Building 35 Room 1A205, 35 Convent Drive, Bethesda, MD 20892-3719, USA
Tel: +1 301 496 8089; fax: +1 301 402 9081;
e-mail: deteras@mail.nih.gov

Received 18 December 2006 **Revised** 31 January 2007
Accepted 5 February 2007

Introduction

Multiple genetic loci have been reported to predispose to bipolar disorder. As with other complex disorders, however, unclear convergence or frank divergence of independent studies has been a recurring problem. This is prominently illustrated by the largely divergent results from three meta-analyses on genome scans for linkage to bipolar disorder (Badner and Gershon, 2002; Segurado *et al.*, 2003; McQueen *et al.*, 2005). Key underlying causes of inconsistent results include genetic and phenotypic heterogeneity, risk-protective allele switches in different samples, gene–gene interaction, underpowered sample size, and contributions of as-yet undetermined environmental factors.

The diagnosis of bipolar disorder is based on a composite of diagnostic variables derived from a patient's history. Although reliable, and highly heritable, the diagnosis

0955-8829

could comprise many clinically similar conditions with differing genetic etiologies (Schulze and McMahon, 2004; MacQueen *et al.*, 2005) that could create 'noise' and conceal true findings. Thus, inconsistent genetic findings between independent samples could arise from uneven representations of cases with different, but clinically similar phenotypes.

The poorly understood phenomenon of risk-protective allele switching previously reported in G72 (reviewed in Detera-Wadleigh and McMahon, 2006) and COMT (Funke *et al.*, 2005) can mask association, as can gene–gene interaction. For example, epistatic effects generated through interaction of variations in serotonin transporter with variations in the dopamine transporter, 5-HT_{1B} and brain-derived neurotrophic factor have been documented in several studies in mice (Murphy *et al.*, 2003).

We have undertaken a search for susceptibility genes for bipolar disorder on chromosome 13q. Linkage of bipolar disorder and schizophrenia to this region has been strongly supported in several studies (Ginns *et al.*, 1996; Lin *et al.*, 1997; Stine *et al.*, 1997; Blouin *et al.*, 1998; Shaw *et al.*, 1998; Brzustowicz *et al.*, 1999; Detera-Wadleigh *et al.*, 1999; Badenhop *et al.*, 2001; Kelsoe *et al.*, 2001; Faraone *et al.*, 2002; Liu *et al.*, 2003; McInnis *et al.*, 2003; Potash *et al.*, 2003; Shaw *et al.*, 2003; Abecasis *et al.*, 2004; Park *et al.*, 2004). Single nucleotide polymorphism (SNP) screening in the 13q33 region detected association in schizophrenia at the G72/G30 locus (Chumakov *et al.*, 2002). Support for association in bipolar disorder was later found (Hattori *et al.*, 2003) and similar results from various studies in schizophrenia and bipolar disorder further underscored the relevance of G72/G30 variations in disease risk (Detera-Wadleigh and McMahon, 2006).

The broadness of the linkage peak suggested that other susceptibility loci may exist in 13q32-q33. To test this possibility we employed a mapping strategy that interrogated SNPs selected to sample common variation across a 7.6 Mb segment of 13q32.1-q33.1. We identified three SNPs associated with bipolar disorder that mapped to *DOCK9*, a gene that encodes an activator of the RhoGTPase, Cdc42. We further report that fine-scale mapping detected additional SNPs that showed association with various clinical features of the illness. These results implicate a novel pathway in the etiology of bipolar disorder and suggest that more than one gene may account for the genetic linkage of bipolar disorder to chromosome 13q.

Materials and methods

Patient samples and phenome file

Family samples that were collected under the auspices of the National Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder, comprising the NIMH Waves 1-2, 3 and 4 were used in this study. Families were ascertained on the basis of a sibling pair affected with bipolar I (BPI) or schizoaffective-bipolar disorder (SA-BP). The genotyped sample contains complete trios, probands with or without an affected sibling, and some families with only one parent and an affected offspring, with or without an affected sibling. Waves 1 and 2 (NIMH1-2) consist of 153 families and were ascertained the earliest by a consortium of four sites (Nurnberger *et al.*, 1997). Wave 3 (NIMH3) and Wave 4 (NIMH4) consist of 221 (Dick *et al.*, 2003) and 275 families, respectively, and were ascertained by a consortium of 10 sites. Additional bipolar disorder family samples included the 'CNG' which consists of 22 multiplex families ascertained by the Clinical Neurogenetics Branch in the 1980s (Berrettini *et al.*, 1991) and 73 multiplex families referred to as 'CHIP' collected by the Departments of Psychiatry at the University of Chicago and Johns Hopkins University and the Genetic Basis of Mood and Anxiety Disorders Unit at the NIMH Intramural Research Program.

Ascertainment and assessment of families were described previously (Berrettini *et al.*, 1991; Simpson *et al.*, 1992; Nurnberger *et al.*, 1997; Dick *et al.*, 2003). In the NIMH and the later portion of the CHIP samples, participants were assessed by use of the Diagnostic Instrument for Genetic Studies (Nurnberger *et al.*, 1994) and diagnosis was based on DSM-IIIR and DSM-IV criteria (American Psychiatric Association, 1987, 1994). In the CNG and earlier portions of the CHIP samples, participants were assessed with the Schedule for Affective Disorders and Schizophrenia-Lifetime version (Endicott and Spitzer, 1978) and diagnosed on the basis of Research Diagnostic Criteria (Spitzer *et al.*, 1978).

A file of phenotypic variables was generated by collating phenotype information on individual members of the NIMH Waves 1–4 families. This includes data on clinical features of mania and depression, course of illness indicators and comorbid psychiatric conditions. Familiality of variables was evaluated by use of a mixed effects regression model implemented in the MIXEDUP suite (Hedeker and Gibbons, 1996a, b; Schulze *et al.*, 2006). Continuous variables were log-transformed for use in association testing.

Genotyping

Of 199 validated SNPs, 98 were selected for genotyping with the Illumina Bead-Array assay (Illumina Inc., San Diego, California, USA). Follow-up analysis with selected *DOCK9* SNPs (minor allele frequency, MAF ≥ 0.1) included tagSNPs taken from HapMap determined by using Haploview, version 3.2 (Barrett *et al.*, 2005). Genotyping of *DOCK9* SNPs was done by utilizing one of the following methods: pyrosequencing

(Pyrosequencer PSQHS96, Pyrosequencing/Biotage), fluorescence polarization-TDI (Akula *et al.*, 2002) (Analyst HT, Molecular Devices) and TaqMan allele discrimination assay [Applied Biosystems, (ABI)]. PCR was performed using either HotMaster Taq polymerase (Brinkmann-Eppendorf, New York, USA), FastStart Taq (Roche, Valencia, California, USA) or HotStar Taq (Qiagen, Roche, Indianapolis, Indiana, USA) employing the manufacturer's recommended initial denaturation temperature followed by 40 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s and 72°C for 1 min. At the end of 40 cycles, an additional extension was done at 72°C for 7 min followed by a soak of 4°C. For some primer pairs, the annealing temperature was set at either 55 or 65°C. All genotypes were checked for consistency in duplicate samples and for Mendelian errors.

Association tests

Family-based association test (Laird *et al.*, 2000) was used to analyze genotype data from fine-scale mapping and to assess the effect of familial phenotype variables on association. TDTPHASE (UNPHASED package) (Dudbridge, 2003) was used for analysis of data from the initial screen with 98 SNPs, estimating odds ratios (ORs) and evaluation of transmission of parental alleles. A narrow affection status model (ASM I) that includes BPI and SA-BP as affected was used in the primary analysis. Analysis under a broader model (ASMIII) that includes BPI, SA-BP, BPII and recurrent major depression was also done on four SNPs (rs1340, rs9557134, rs9517575 and rs10492574).

Gene structure determination, polymorphism screening, haplotype block structure and resequencing

The predicted exons and splice junctions for KIAA1058 (AB028981) (Kikuno *et al.*, 1999) and NM_015296 ([Zizimin1] Meller *et al.*, 2002) [National Center for Biotechnology Information (NCBI)]; University of California Santa Cruz (UCSC) Genome Browser, May 2004), were sequenced on a panel of 22 cases drawn from the CNG families in an attempt to find new sequence variations. Sequencing was done in-house using the Big Dye terminator kit on an ABI 3100 sequencer. In addition, the entire gene, including all exons and introns of NM_015296 was resequenced on 12 unrelated BPI patients selected from the NIMH families at the Baylor Human Genome Sequencing Center. Additional resequencing of 16.5 kb of selected regions upstream of rs1927568 was done on 24 BPI unrelated probands from the NIMH families (Polymorphic DNA Technologies, Inc., Alameda, California, USA).

To generate the haplotype block structure we used HapMap-derived genotype data from trios of European (CEU) origin (release no. 19/phase II Oct05). Analysis was done using Haploview (version 3.2) (Barrett *et al.*, 2005) on SNPs that had MAFs ≥ 0.1 using the Gabriel Algorithm and default parameters (Gabriel *et al.*, 2002). Haplotype block structure was derived from the *DOCK9* SNP genotype data in NIMH1-2 in a similar manner.

Results

Linkage disequilibrium screening on 13q32-q33

In our previous study we have determined the region with a 95% confidence limit for the location of the susceptibility locus to be within the interval bounded by D13S122 and D13S280 (Liu *et al.*, 2001). Therefore, we targeted this region for our initial SNP screen covering a ~7.6 Mb segment from rs1012693 to rs1322713. A set of 98 validated SNPs were used to genotype a sample of 285 families composed of parent-offspring trios, one-parent-one-offspring pairs and affected sib-pairs. This sample included NIMH1-2, CHIP, CNG families and some drawn from the NIMH3 series. SNPs were selected from the Phase I HapMap and other public sources.

In this initial sparse screen, TDT analysis (TDTPHASE) (Dudbridge, 2003) yielded evidence of association with bipolar disorder at rs1927568 ($P = 0.00067$) (Table 1). The SNP mapped to the 5′ flanking region of the *DOCK9* gene (RefSeq NM_015296) (Meller *et al.*, 2002). In addition, two of the remaining six SNPs typed on *DOCK9* detected association with bipolar disorder: rs2000342 ($P = 0.04766$) and rs2390129 ($P = 0.01996$) (Table 1). No other clusters of significant results were detected in any of the genes sampled in this study (data not shown). On the basis of these results, we targeted *DOCK9* for further analysis.

DOCK9 transcripts and haplotype block structure

The *DOCK9* gene spans ~293 kb and encodes three major transcripts that differ in their amino terminal sequences (UCSC Genome Browser, May 2004; NCBI): KIAA1058 (AB028981) (Kikuno *et al.*, 1999; NCBI), NM_015296 (zizimin 1) (Meller *et al.*, 2002; NCBI) and AK127329 (UCSC Genome Browser, May 2004; NCBI) (Fig. 1). Our analysis indicated that the nucleotide sequence purportedly coding for the first 27 amino acids in KIAA1058 is part of the 5′ untranslated region therefore the initiation methionine immediately follows this sequence. AK127329 is an incomplete clone lacking the 3′-terminal segment that codes for the C-terminal portion of the protein. *DOCK9* has at least 50 exons and three alternative first exons: 1a, 1b and 1c (Fig. 1). Transcription is oriented opposite the genome-wise direction with exon 1a of NM_015296 as the most telomeric exon, ~71 kb downstream of exon 1b of AK127329, which in turn, is 37.5 kb distal to exon 1c of KIAA1058 (Fig. 1) (UCSC Genome Browser).

The haplotype block structure of *DOCK9* was calculated over a 320 kb interval delimited by rs1299066 and

Table 1 Transmission of *DOCK9* alleles to offspring with bipolar disorder

SNPs		Position, bp	ASMI (BPI/SABP)							
			TDT-PHASE	FBAT-e						
				NIMH1-2		NIMH3		NIMH4		CHIP-CNG	
No.	ID	UCSC genome browser, May 2004	Initial screen on 285 families (*P*)	No. of families	*P*	No. of families	*P*	No. of families	*P*	No. of families	*P*
1	rs1299066[a]	98 230 873		63	0.5837						
2	rs2899	98 243 865		57	0.3538	28	0.3402	45	0.1739		
3	rs772303	98 250 308	0.4886	NA							
4	rs2296983	98 255 162		62	0.3029	50	0.3971	69	0.5306		
5	rs772311[a]	98 266 480		62	0.1535						
6	rs1887856	98 277 348	0.1740	NA							
7	rs7333687	98 288 560		69	0.3520	36	0.8518	77	0.3033		
8	rs7986477	98 297 352		43	0.6260	22	0.9359	49	0.8875		
9	rs4772152	98 319 757		63	0.7407	41	0.2866	72	0.1181		
10	rs2296994	98 331 898		34	0.4778	16	0.2596	41	0.8132		
11	rs2026024	98 335 432	0.2969	NA							
12	rs1324982	98 342 134		57	0.3152	41	0.7513	41	0.2899		
13	rs7328282	98 352 956		72	0.6505	45	0.3679	53	0.1714		
14	rs12428661	98 373 569		54	1.0000	33	0.2214	48	0.7988		
15	rs1028910	98 377 729	0.4080	NA							
16	rs7331595[a]	98 395 733		58	0.4350						
17	rs8002389[a]	98 405 912		59	0.2421						
18	rs874199[a]	98 415 108		69	0.3142						
19	rs2390129[a]	98 426 081	**0.01996**	41	0.0558						
20	rs1359427[a]	98 436 409		61	0.1238						
21	rs1886553[a]	98 448 739		63	0.1064						
22	rs1041093[b]	98 453 658		46	0.0943	37	0.1103	59	0.3374		
23	rs1886554	98 460 081		44	**0.0156**	36	0.0872	59	0.3700		
24	docksnp81indel[c]	98 461 986		39	**0.0087**	35	0.1127	47	0.0817		
25	rs4772168	98 465 941		61	0.1401	46	0.4855	80	0.5924		
26	rs9517549	98 472 381		68	0.0904	47	0.1871	85	0.6600		
27	rs2000342	98 477 848	**0.04766**	71	0.1097	52	0.2670	84	0.4405		
28	rs6491476[b]	98 484 105		69	0.1569	52	0.3669	85	0.4953		
29	rs10492574	98 494 931		55	**0.0315**	43	0.0604	69	0.1525	40 (35)[d]	0.1202 (0.0595)
30	rs9517575	98 507 741		56	**0.0078**	43	0.0896	67	0.1673	39 (35)	**0.0412 (0.0156)**
31	rs9554547	98 514 478		66	0.0601	48	0.4814	84	0.7677		
32	rs2105425	98 524 566		62	0.0578	48	0.7339	78	0.1788		
33	rs9557134	98 526 453		53	**0.0064**	40	0.0626	67	0.2271	39 (36)	**0.0278 (0.0106)**
34	rs7334435	98 529 622		62	0.0751	47	0.6240	77	0.8361		
35	rs9557137	98 535 143		52	**0.0071**	45	0.0877	68	0.1038		
36	rs1927568	98 538 118	**0.00067**	56	**0.0119**	42	0.1037	67	0.1372		
37	rs1340	98 538 654		54	**0.0134**	40	**0.0396**	70	0.4437	36 (33)	0.0683
38	rs9513550[a]	98 550 511		51	**0.0222**						
39	rs3858781	98 555 678		62	0.0515	43	0.9758	87	0.4347		
40	rs1536657[a]	98 603 421		58	0.1799						

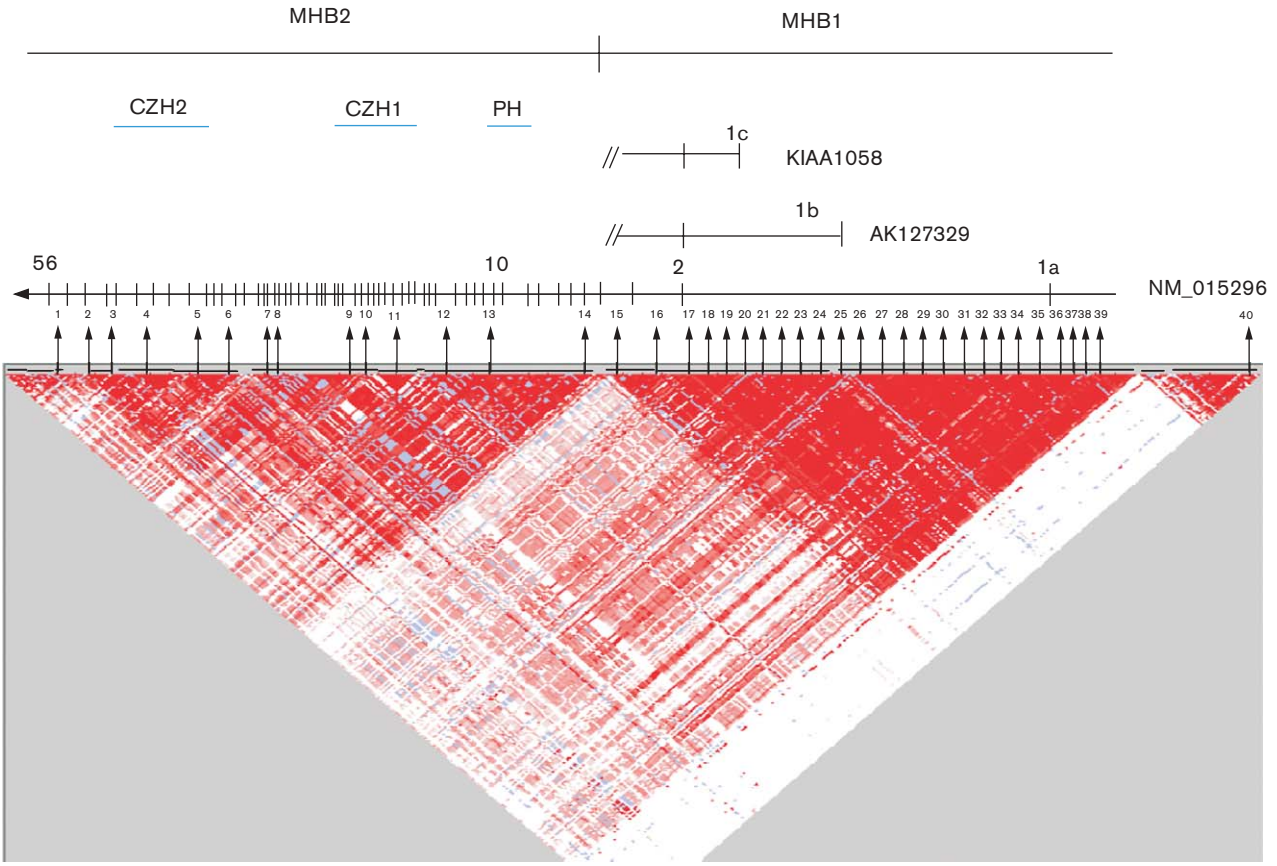
[a]Not typed in NIMH3 and NIMH4.
[b]In NIMH4, replaced by rs9554545 and rs7338227, respectively.
[c]-AAGTA-indel.
[d]Numbers in parenthesis indicate data under ASMIII.
NA, not analyzed.
P values <0.05 are shown in bold.

rs9513550. Genotype data generated by HapMap on 348 SNPs in the CEU including only those SNPs with MAF ≥ 0.1 was used. Haploview analysis produced 13 haplotype blocks within two major blocks of almost equal size (here designated MHB1 and MHB2), and interrupted by 525 bp of low linkage disequilibrium (LD) in intron 2 (Fig. 1). MHB1 spans about 164 kb, extending from the *DOCK9* 5′ flanking region up to exon 2. MHB2 covers $\sim$160 kb and incorporates the remaining exons that encode three functional motifs, PH, CZH1 and CZH2 (Meller *et al.*, 2002) (Fig. 1).

Fine-scale linkage disequilibrium mapping

To follow-up on the preliminary evidence of association, fine-scale mapping was done to interrogate the entire length of the *DOCK9* gene. Our approach was to individually genotype each of the four sets of bipolar disorder families. NIMH1-2 was used as the primary test sample upon which 40 SNPs were assayed. Follow-up genotyping using 31 of the 40 SNPs were done on NIMH3 and NIMH4. Association was also assessed in the CHIP and CNG family series (combined to achieve reasonable sample size), in which four of the 40 SNPs

Fig. 1



DOCK9 gene structure, haplotype structure in CEU and genotyped single nucleotide polymorphisms (SNPs). Diagrammatic representation of *DOCK9* isoforms and gene structure showing the exons (vertical lines), direction of transcription (thick arrow) and location of functional motifs. Haploview generated haplotype structure from HapMap-derived genotypes on 348 SNPs in CEU showing two major haplotype blocks (MHB1 and MHB2). Vertical arrows represent SNPs listed in Table 1. Distances are not drawn to scale.

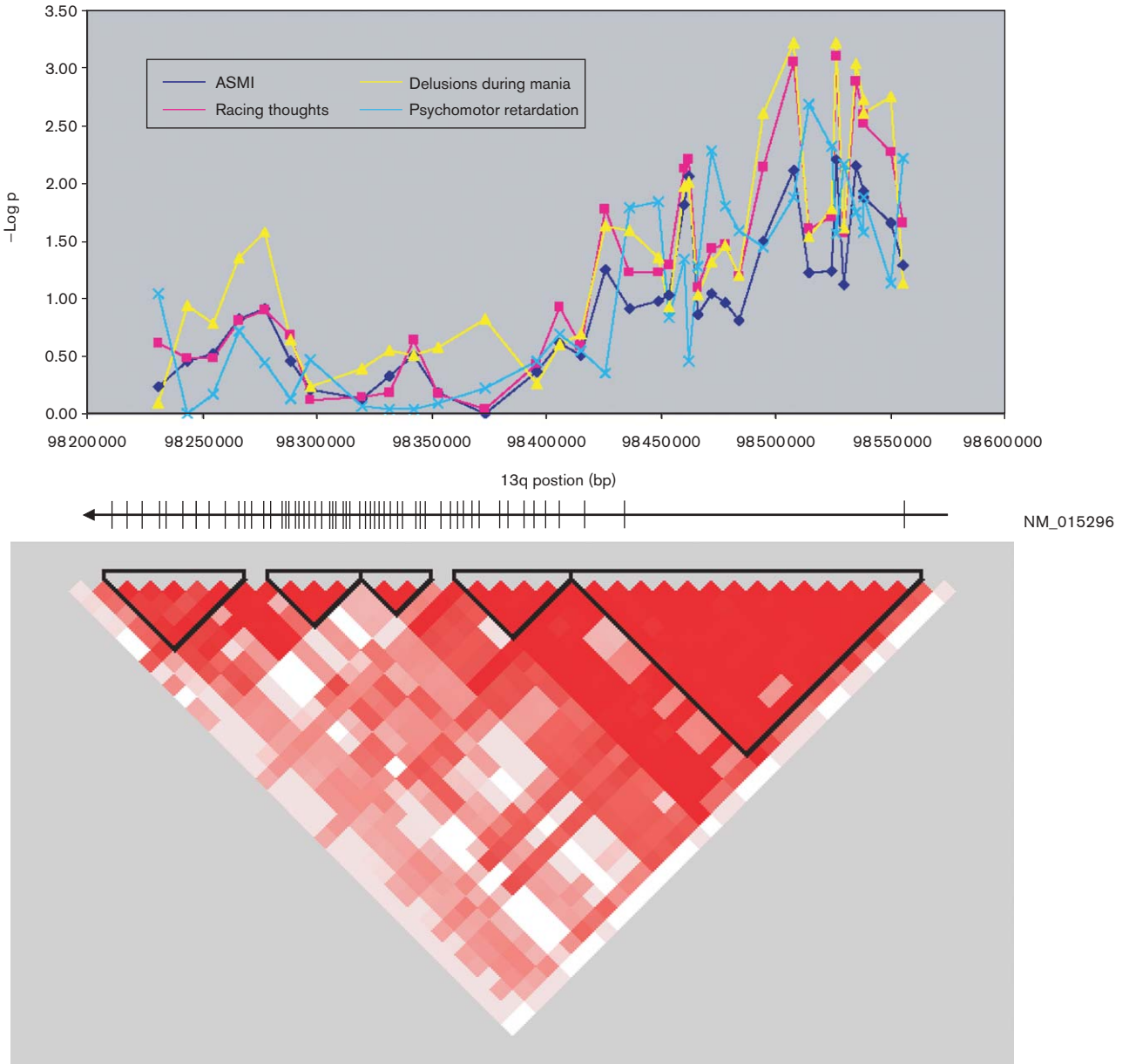
were analyzed. Haploview-predicted tag SNPs and other SNPs, some of which were detected through resequencing, were used. Six SNPs that deviated from Hardy–Weinberg equilibrium ($P < 0.05$) were excluded from the final analysis (Table 1, Fig. 1).

At this phase of the study the primary association tests (see below) were conducted using Family-based association test-e (empirical variance option) to give more precise P values in the presence of linkage. In the NIMH1-2, analysis under ASMI detected evidence of association at the $P < 0.05$ level with nine MHB1 SNPs (smallest nominal $P = 0.006$) including rs1927568, one of three *DOCK9* markers that detected association in the initial screen (Table 1; Figs 1 and 2). These SNPs cluster in a ~100 kb block that comprises the 5′ flanking region, exon 1a, and intron 1 of NM_015296. Intron 1 of NM_015296 includes exon 1b of AK127329 and exon 1c of KIAA1058. This region is enclosed in a large haplotype block in the block structures generated using both the HapMap and NIMH1-2 genotype data (Figs 1 and 2).

Further analysis detected support for association in NIMH3 (rs1340) and CHIP-CNG (rs9517575 and rs9557134) (Table 1; Fig. 2). The same alleles of the same SNPs were transmitted in excess in NIMH1-2, NIMH3 and CHIP-CNG. In contrast, no significant over-transmission of any alleles was detected in NIMH4 (Table 1). The ORs for the over-transmitted allele C, T and C of rs9517575, rs9557134 and rs1927568 were 1.33, 1.80 and 1.93, respectively.

Additionally, we found evidence for a preferential transmission of maternal alleles to affected offspring suggesting that the observed association results were driven largely by maternal alleles. This was most strongly demonstrated by rs1927568 which displayed significant excess transmission of maternal alleles ($P = 0.000083$,

Fig. 2



Increased evidence of association of *DOCK9* variants with selected component phenotypes of bipolar disorder in NIMH1-2. Presented below is *DOCK9* gene structure and haplotype block structure generated from genotype data on 36 single nucleotide polymorphisms (SNPs).

OR = 3.778), and less over-transmission of paternal alleles ($P = 0.0532$, OR = 2.0).

Familial component phenotypes of bipolar disorder

To explore the impact of phenotype variability on the association results, we examined individual clinical features in the NIMH families as part of a secondary analysis. Potentially, some entities could impart 'noise' and various combinations of variables could conceal association that does exist. Many variables could be tested, so we evaluated only those that were significantly familial in these samples (Fisfalen *et al.*, 2005; Kassem *et al.*, 2006; Schulze *et al.*, 2006). Although familial variables are not necessarily genetic, those that are not familial are unlikely to have a strong genetic basis. By this criterion, we selected 25 phenotypic variables for analysis. These included symptoms of mania and depression, comorbid mental disorders such as psychosis and panic disorder, and course indicators such as age at onset.

Mania and depression variables

Secondary analyses on mania and depression variables in NIMH1-2 identified the same MHB1 SNPs that displayed association signals under the primary analysis. Interestingly, more SNPs showed significant association signals and *P* values were in general smaller than in the primary analyses, even though the effective sample sizes were smaller. For example, the variable 'racing thoughts during mania', which is known to be highly diagnostic of bipolar disorder (Goodwin and Jamieson, 1990) showed significant association with 16 SNPs ($P = 0.0366$–0.0008) (Fig. 2; Table 2), 'delusions during mania' with 18 SNPs (0.0483–0.0006) (Fig. 2), and 'grandiosity during mania' with 18 SNPs ($P = 0.048$–0.00072) (Table 2). In NIMH3, the greatest evidence of association was also detected for 'racing thoughts during mania' (nine SNPs, $P = 0.0323$–0.007) (Fig. 2; Table 2). For each associated SNP, the same allele was over-transmitted in both NIMH1-2 and NIMH3.

In familial depressive symptoms, psychomotor retardation detected excess transmission in the highest number of SNPs (16 SNPs, $P = 0.0458$–0.0049) in NIMH1-2 (Fig. 2) NIMH3 or NIMH4 did not display association signals with depression variables (data not shown).

Course of illness indicators: mania at onset, age at onset, episode frequency, age at first mania

In a recent study on the NIMH dataset, polarity of onset in bipolar disorder has been found to be heritable and this subset of families detected linkage on 16p (Kassem *et al.*, 2006). This analysis on NIMH1-2 showed excess allelic transmission with mania at onset in eight MHB1 and two MHB2 SNPs extending over a larger portion of the gene that includes several *DOCK9* exons (Table 3). Surprisingly, NIMH4 that has shown either no or scant evidence of association in prior analyses displayed signals for mania at onset in five MHB1 and four MHB2 SNPs (Table 3). NIMH4 over-transmitted, however, the alternative alleles indicating allele switching, that is, the potential susceptibility allele in NIMH1-2 is the protective allele in NIMH4. Association signals were detectable mostly in NIMH1-2 for variables such as age at first mania

Table 2 Transmission of *DOCK9* alleles with some familial component phenotypes of bipolar disorder in NIMH1-2 and NIMH3

	Racing thoughts during mania				Grandiosity during mania				Log no. manic episodes			
	NIMH1-2		NIMH3		NIMH1-2		NIMH3		NIMH1-2		NIMH3	
SNP	No. of families	*P*	No. of families	*P*	No. of families	*P*	No. of families	*P*	No. of families	*P*	No. of families	*P*
rs1299066	88	0.2479			81	0.3722			82	0.8816		
rs2899	72	0.3272	45	0.4904	66	0.1508	42	0.2752	58	0.7082	38	**0.0346**
rs2296983	80	0.3277	65	0.3991	75	0.2644	69	0.1062	76	0.8562	67	0.1981
rs772311	89	0.1562	NA		81	0.3208	NA		80	0.3568		
rs7333687	92	0.2104	60	0.9273	84	0.5341	59	0.8420	84	0.3513	66	0.5862
rs7986477	58	0.7733	35	0.2927	53	0.5355	39	0.6721	51	0.7074	41	0.5167
rs4772152	82	0.7255	60	0.1433	75	0.7609	59	0.1687	77	0.7375	63	0.7134
rs2296994	43	0.6495	26	0.5379	41	0.7214	31	0.5392	37	0.4430	30	0.4628
rs1324982	66	0.2314	54	0.2769	58	0.1521	52	0.4704	62	0.9734	54	0.9651
rs7328282	87	0.6707	65	0.1663	79	0.5711	64	0.2228	80	0.8890	63	0.6814
rs12428661	53	0.9190	32	0.6999	48	0.9135	39	0.3836	46	0.6280	37	0.1619
rs7331595	86	0.3676	NA		79	0.4077	NA		72	**0.0410**		
rs8002389	85	0.1201	NA		77	0.1023	NA		74	**0.0248**		
rs874199	91	0.2577	NA		88	0.1012	NA		80	0.2336		
rs2390129	54	**0.0171**	NA		48	0.0600	NA		47	0.1223		
rs1359427	91	0.0606	NA		83	**0.0124**	NA		83	**0.0106**		
rs1886553	93	0.0599	NA		87	**0.0179**	NA		85	**0.0217**		
rs1041093	64	0.0509	53	**0.0224**	57	0.1005	56	0.0761	55	0.0595	50	0.1414
rs1886554	64	**0.0077**	52	**0.0154**	57	**0.0181**	55	0.0553	55	**0.0041**	50	0.1331
docksnp81indel	51	**0.0063**	46	**0.0323**	47	0.0548	47	0.1021	47	0.0758	43	**0.0417**
rs4772168	88	0.0807	73	0.8213	83	**0.0485**	72	0.9266	79	**0.0328**	66	0.8985
rs9517549	97	**0.0366**	69	0.5678	90	**0.0154**	70	0.4331	90	**0.0180**	67	0.9398
rs2000342	96	**0.0339**	67	0.5018	90	**0.0101**	70	0.4067	90	0.0576	68	0.9805
rs6491476	94	0.0647	70	0.6299	88	**0.0186**	74	0.4615	89	0.0780	70	0.9749
rs10492574	73	**0.0073**	59	**0.0240**	67	**0.0025**	59	0.1268	65	**0.0064**	57	0.0950
rs9517575	72	**0.0009**	56	**0.0159**	66	**0.0007**	56	0.0926	62	**0.0013**	57	0.0946
rs9554547	96	**0.0249**	71	0.8212	90	**0.0076**	71	0.7560	88	**0.0050**	68	0.9471
rs2105425	88	**0.0200**	73	0.9322	83	**0.0087**	74	0.8562	82	**0.0045**	67	0.7686
rs9557134	70	**0.0008**	52	**0.0094**	66	**0.0010**	53	0.0581	63	**0.0018**	55	0.1002
rs7334435	86	**0.0273**	73	0.8214	82	**0.0130**	74	1.0000	81	**0.0072**	66	0.8901
rs9557137	71	**0.0013**	58	**0.0196**	65	**0.0012**	58	0.1078	62	**0.0034**	57	0.1007
rs1927568	73	**0.0026**	55	**0.0248**	67	**0.0025**	56	0.1484	64	**0.0049**	54	0.1206
rs1340	77	**0.0031**	56	**0.0070**	71	**0.0034**	57	**0.0341**	68	**0.0009**	56	0.0982
rs9513550	69	**0.0055**			64	**0.0036**			59	**0.0062**		
rs3858781	92	**0.0222**	66	0.8596	88	**0.0392**	65	0.7222	84	**0.0149**	59	0.3317
rs1536657	76	0.5563			68	0.7503			70	0.8016		

NA, not analyzed.
P values <0.05 are in bold.

Table 3 Transmission of alternative alleles with mania at onset in NIMH1-2 and NIMH4

		Mania at onset					
		NIMH1-2			NIMH4		
SNP	SNP code	No. of families	*P*	Overtransmitted allele	No. of families	*P*	Overtransmitted allele
rs1299066	M	51	NS		NA		
rs2899	W	45	NS		24	**0.0196**	A
rs2296983	S	55	NS		47	**0.0023**	G
rs772311	Y	55	NS		NA		
rs7333687	R	58	**0.0307**	G	53	**0.0198**	A
rs7986477	R	37	NS	NA	34	0.1060	A
rs4772152	W	52	NS	C	52	**0.0417**	T
rs2296994	R	26	0.0555	G	27	NS	
rs1324982	Y	46	0.1050	C	39	0.0551	T
rs7328282	R	53	NS		49	0.0565	A
rs12428661	R	34	0.0835	G	30	NS	
rs7331595	M	53	**0.0425**	A	NA		
rs8002389	R	54	0.0536	G	NA		
rs874199	Y	58	NS		NA		
rs2390129	R	39	0.0547	G	NA		
rs1359427	M	54	0.0538	A	NA		
rs1886553	S	56	0.0910	C	NA		
rs1041093[a]	S	44	**0.0429**	C	42	NS	
rs1886554	Y	44	**0.0084**	T	43	NS	
docksnp81indel	INDEL	37	**0.0341**	DEL	34	0.0667	INS
rs4772168	Y	54	0.0726	C	49	NS	
rs9517549	W	60	0.0710	A	57	NS	
rs2000342	Y	63	NS		56	NS	
rs6491476[a]	Y	62	NS		58	0.1293	
rs10492574	K	53	0.1146	T	50	**0.0181**	G
rs9517575	Y	53	0.0503	C	52	**0.0259**	T
rs9554547	Y	59	0.0541	C	57	NS	
rs2105425	R	57	**0.0380**	A	49	NS	
rs9557134	Y	53	**0.0317**	T	51	**0.0266**	C
rs7334435	R	58	**0.0380**	G	51	NS	
rs9557137	Y	53	0.0554	T	51	**0.0181**	C
rs1927568	Y	55	0.0587	C	50	**0.0123**	T
rs1340	W	54	**0.0204**	T	52	0.1298	A
rs9513550	R	50	0.0566	A	NA		
rs3858781	Y	59	**0.0318**	T	55	NS	
rs1536657	Y	48	NS		NA		

[a]Replaced with rs9554545 and rs7338227, respectively, in NIMH4.
NA, not analyzed; NS, not significant; SNP, single nucleotide polymorphism.
P values <0.05 are in bold.

(13 MHB1 SNPs, $P = 0.037$–0.0059), episode frequency (12 MHB1 SNPs, $P = 0.0434$–0.0034) and age at onset (13 MHB1 SNPs, $P = 0.0242$–0.0026) (Fig. 3), as well number of manic episodes (Table 2).

Bipolar disorder comorbid psychiatric phenotypes

Psychosis

Possible correlation of psychosis with the transmission pattern of *DOCK9* SNPs was of particular interest because both bipolar disorder and schizophrenia have been linked to 13q and prior studies have detected suggestive linkage of psychotic bipolar disorder to 13q (reviewed in Detera-Wadleigh and McMahon, 2006). In NIMH1-2, nine of the 10 MHB1 SNPs that detected excess transmission with psychosis at the $P < 0.05$ level were identical to those that detected association with bipolar disorder (Fig. 4). Similarly, allelic transmission at seven of these SNPs was associated with psychosis in NIMH3. Similar patterns were detected for the more narrowly defined delusions during mania (Fig. 2). It is important to note here that of the total number of ASMI offspring in NIMH1-4 only $\sim$6% had SA-BP diagnosis.

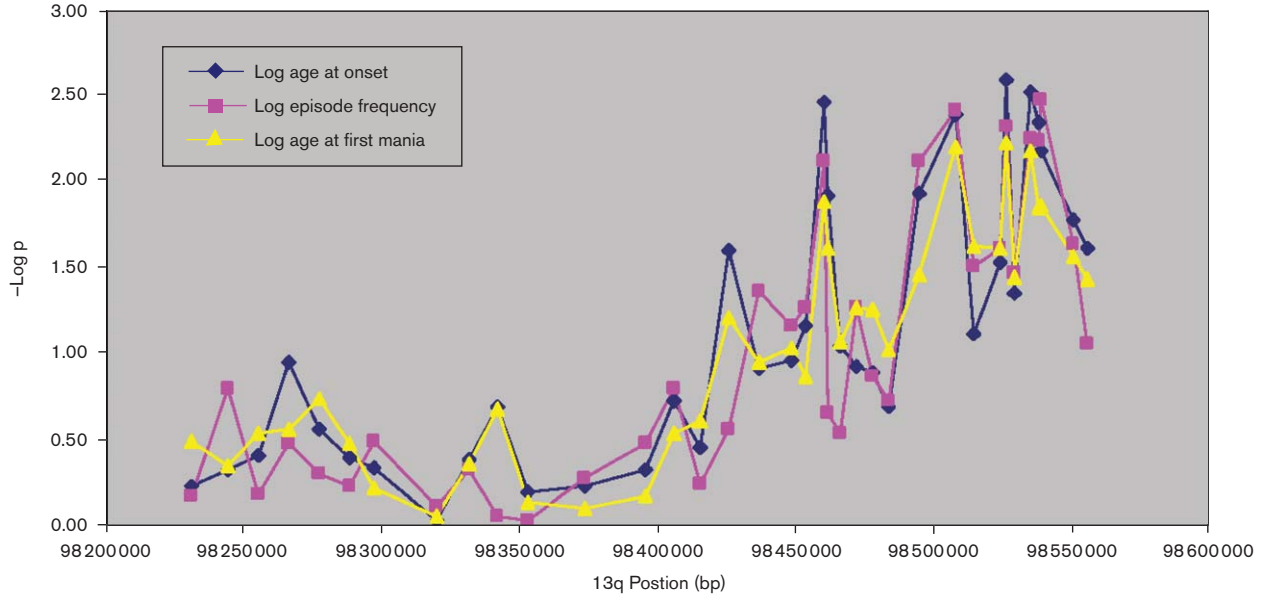
Suicide attempts

Suicidal behavior among patients with mood disorders has been well-documented (Angst *et al.*, 2005). In NIMH1-2, a history of suicide attempt(s) was associated only with a single marker, rs1340. This is also the only SNP that showed significant overall association with bipolar disorder in NIMH3 in the primary analysis. By contrast, there was greater evidence of association in NIMH3 involving five MHB1 SNPs ($P < 0.05$). Covariate analysis (suicidal attempt and bipolar disorder) detected signals in three MHB1 SNPs in NIMH1-2 (Fig. 4) and NIMH3. In 25–75% of NIMH4 families, excess transmission was displayed by the alternative alleles in three MHB1 and one MHB2 SNPs. Two-trait analysis retained signal in only one of the MHB1 SNPs (data not shown).

Panic disorder, alcoholism and substance abuse

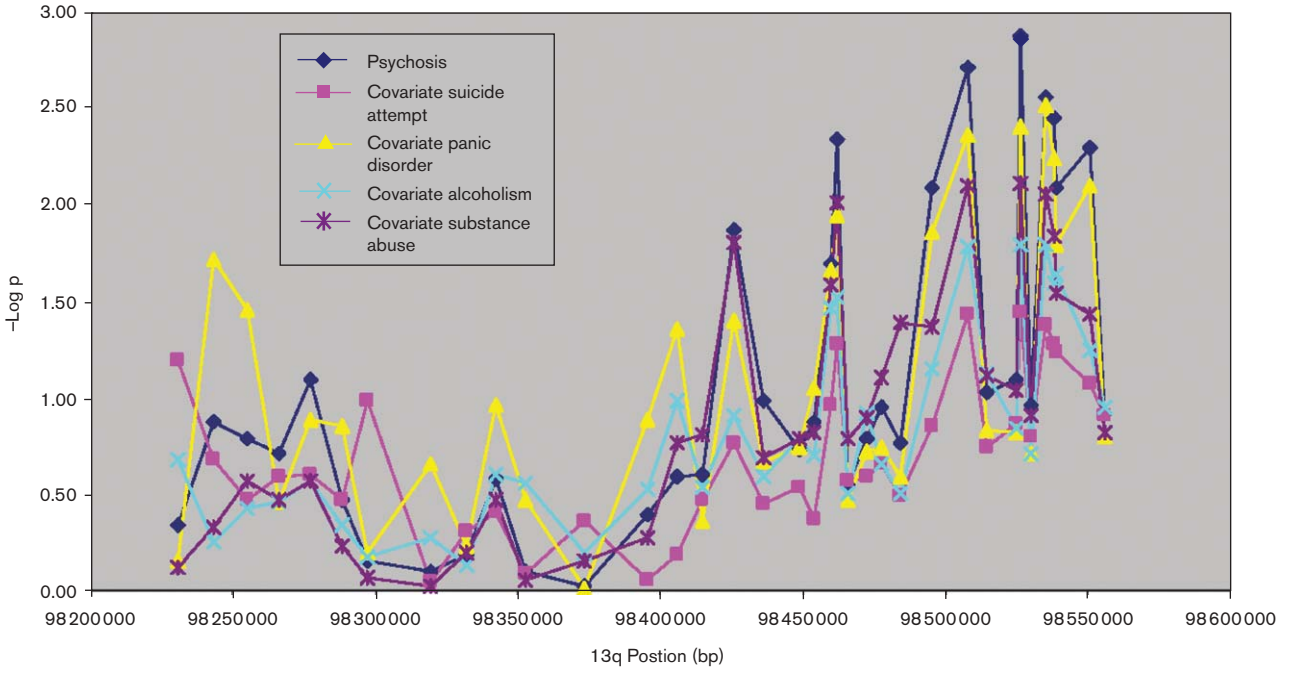
The co-occurrence of panic disorder, alcoholism and substance abuse with bipolar disorder has been well-documented in epidemiologic and family studies (Regier *et al.*, 1990; Winokur *et al.*, 1996; Freeman *et al.*, 2002; MacKinnon *et al.*, 2002; Doughty *et al.*, 2004). We explored

Fig. 3



Transmission of *DOCK9* variants in NIMH1-2 with course of illness indicators.

Fig. 4



Transmission of *DOCK9* variants in NIMH1-2 with comorbid psychiatric disorders. Covariate analysis was performed to generate the majority of graphs.

the role of *DOCK9* in these comorbidities. In NIMH1-2 covariate analysis on panic disorder and ASMI, revealed nominally significant association in 10 MHB1 and four MHB2 SNPs ($P = 0.0457$–0.0033) (Fig. 4). Similar two-trait analysis on alcoholism and substance abuse highlighted seven MHB1 SNPs ($P = 0.0354$–0.0168) and 11

MHB1 SNPs ($P = 0.0378$–0.0081), respectively (Fig. 4). In NIMH3, covariate analysis detected no association in panic disorder. In alcoholism and substance abuse signals were displayed by one MHB1 SNP and three MHB2 SNPs, respectively.

Discussion

To our knowledge this is the first report to implicate DOCK9 or the Rho-GTPase pathway in the etiology of bipolar disorder. DOCK9 has not been well studied and its function in the brain, where it is highly expressed, remains to be established. DOCK9 has been shown to activate Cdc42, a RhoGTPase (Meller *et al.*, 2002), that has diverse roles including regulation of the actin cytoskeleton, cell migration, axonal guidance, neurite outgrowth and dendritic arbor growth (Threadgill *et al.*, 1997; Li *et al.*, 2000; Luo, 2002; Hall, 2005; Shen *et al.*, 2006). Other DOCK (dedicator of cytokinesis) genes exist that have been better characterized and these may provide clues to the function of DOCK9 (Meller *et al.*, 2005). *DOCK10*, on 2q36.2, also referred to as dopamine interacting protein 2 (NCBI), and *DOCK11*, on Xq24, are highly homologous to *DOCK9*. BLAST analysis also reveals an orthologous rat sequence that has been shown to be down-regulated by thyroid stimulating hormone (Pianese *et al.*, 1994).

Our study unveils associated variants located mostly in the 5′ flanking region and in intron 1 of NM_015296. The first exons, 1b and 1c, of AK127329 and KIAA1058, respectively, also lie within this intron therefore the associated SNPs might be located in the promoter region of these isoforms. Interestingly, several of the associated variants in intron 1 in the 5′ flanking region of NM_015296 are evolutionarily conserved suggesting a role in function. Studies have shown active regulatory sequences and enhancers in noncoding regions (Shin *et al.*, 2005; Woolfe *et al.*, 2005; Fisher *et al.*, 2006; Pennacchio *et al.*, 2006) but these remain to be identified in *DOCK9*. In addition, it is interesting that the ancestral allele in several of the MHB1-associated SNPs is significantly undertransmitted to affected offspring. Resequencing of NM_015296 in bipolar samples identified new polymorphisms (data not shown) but it has not disclosed any common nonsynonymous or splice junction mutations. DOCK9 might confer risk through altered levels of transcription and/or aberrant splicing patterns. Our findings set the stage for future work aimed at uncovering the key functional variation that accounts for the association results.

This study highlights evidence of association between bipolar disorder and several markers in a gene located within a bipolar disorder linkage peak on chromosome 13q that has been supported by several studies (reviewed in Detera-Wadleigh and McMahon, 2006). The sets of families tested display a heterogeneous pattern of association across individual component phenotypes of bipolar disorder. NIMH1-2 shows the highest evidence for association both in the primary and secondary analyses. For SNPs that detect association signals, the same alleles are over-transmitted in NIMH1-2, NIMH3 and CHIP-CNG. The largest single sample we examined, NIMH4, shows association only with a subset of component phenotypes and in this sample the alternative alleles are over-transmitted. This heterogeneity may reduce the power to replicate our findings in some samples.

Stronger association signals with racing thoughts, delusions during mania, course of illness indicators, and psychosis suggest that DOCK9 may contribute to increased illness severity and imply that future replication attempts should focus on severe cases. This also suggests that DOCK9 variations may have prognostic value.

Switching of over-transmitted (susceptibility) and under-transmitted (protective) alleles is a common phenomenon in complex disease. For example, different alleles of markers in DAOA (G72) are over-transmitted in different samples (reviewed in Detera-Wadleigh and McMahon, 2006), differing alleles of a functional COMT variant have been associated with various neurocognitive phenotypes (Funke *et al.*, 2005), and differing haplotypes of dysbindin have shown association with schizophrenia (Straub *et al.*, 2002; Schwab *et al.*, 2003). Although the possibility of a false positive signal in at least some samples is difficult to rule out, this phenomenon may also reflect true allelic heterogeneity, undetermined influences of other interacting risk loci, or differing allele frequencies and patterns of LD across samples.

The presence of comorbid psychiatric phenotypes possibly reflects the existence of genetic subtypes of bipolar disorder and shared genes for various phenotype presentations. Studies have documented the occurrence of psychotic features in a substantial proportion of bipolar disorder patients (Pope and Lipinski, 1978). The overlap of linkage peaks on 13q32-q33 for bipolar disorder and schizophrenia has led to the speculation of shared gene(s) for psychosis in both disorders (Blouin *et al.*, 1998; Detera-Wadleigh *et al.*, 1999). The association signals we observe with psychosis in both NIMH1-2 and NIMH3 suggest that DOCK9 may contribute to the linkage signals for psychotic disorders detected on chromosome 13q.

Other loci previously implicated in psychosis include G72/G30 in bipolar disorder with persecutory delusions (Schulze *et al.*, 2005) and in childhood psychosis and schizophrenia (Addington *et al.*, 2004). In contrast, G72/G30 variations that correlated with major mood episodes in schizophrenia and bipolar disorder did not show association with psychosis (Williams *et al.*, 2006). This

inconsistency in association findings possibly reflects differences in psychotic phenotypes across various samples. Dysbindin (*DTNBP1*) variants have been shown also to be nominally associated with psychotic bipolar disorder (Raybould *et al.*, 2005) and variations in neuregulin (*NRG1*) have shown correlation with bipolar disorder with mood incongruent psychotic features (Green *et al.*, 2005). Thus, it may be important to analyze *DOCK9* in schizophrenia samples as well as other samples of psychotic bipolar disorder.

This study has several limitations, and firm conclusions must await replication studies. The sample sizes were modest, and effective sample sizes were further reduced in the component phenotype analyses, potentially reducing our power to detect true associations. Although pooling all samples together from the start would have increased sample size, this would come at the cost of increased heterogeneity, which could decrease the true power to detect the modest association signals that one expects in complex disease. By treating each individual set of samples, we took into account real differences in the ways the samples were ascertained and evaluated. Our analysis of component phenotypes of bipolar disorder is an attempt to identify specific diagnostic entities that correlate with allelic over-transmission across sample panels, which, to our knowledge, is the first such exploration in bipolar disorder. Genetic analysis of multiple component phenotypes in a complex disease has been reported recently, for example, in migraine (Anttila *et al.*, 2006) and hypertension (Wallace *et al.*, 2006). This approach necessarily entails multiple tests whose mutual dependency is difficult to assess. Certainly, if we were to consider the individual component phenotypes to be independent, then the signals detected in the secondary analyses would not survive correction for multiple testing. Our approach examines variables that are constituent parts of a single overarching phenotype therefore tests on individual components may not be nonindependent, but additional replication studies are needed before firm conclusions can be reached.

The SNPs that consistently display association are clustered in MHB1, one of two CEU major haplotype blocks encompassing *DOCK9*. LD between SNPs in this region is clearly evident particularly in the analysis of some of the individual variables (Fig. 2; Table 2). It can be inferred that tests on the MHB1 SNPs are non-independent; hence may constitute a single test. Given that there are two major haplotype blocks in *DOCK9*, it may be reasonable to consider only two tests for gene-wise correction.

The results presented herein highlight some critical elements that contribute to the complexity of bipolar disorder and underscore the need for a refinement of phenotype classifications to improve our ability to uncover genetic and other risk factors. Multiple tests have been performed and therefore some results could have arisen from statistical fluctuations, but findings in the secondary analyses tended to agree with those from the primary tests. Future progress in collecting large, well-phenotyped samples and whole genome association studies should help address some of long-standing issues discussed here. Dissecting the patterns of allelic transmission in *DOCK9* may ultimately help untangle salient facets of heterogeneity in bipolar disorder.

Acknowledgements

The authors thank Tom I. Bonner for his valuable contribution and advice. They acknowledge the patients and their families for their generous participation in the study. They also thank the members of the NIMH Genetics Initiative for Bipolar Disorder consortium for their efforts in family ascertainment, collection and phenotyping. Data and biomaterials were collected as part often projects that participated in the National Institute of Mental Health (NIMH) Bipolar Disorder Genetics Initiative. From 1999–2003, the principal investigators and co-investigators were: Indiana University, Indianapolis, IN, R01 MH59545, John Nurnberger, MD, PhD, Marvin J. Miller, MD, Elizabeth S. Bowman, MD, N. Leela Rau, MD, P. Ryan Moe, MD, Nalini Samavedy, MD, Rif El-Mallakh, MD (University of Louisville). Husseini Manji, MD (Wayne State University). Debra A. Glitz, MD (Wayne State University). Eric T. Meyer, MS, Carrie Smiley. RN, Tatiana Foroud, PhD, Leah Flury, MS, Danielle M. Dick, PhD, Howard Edenberg, PhD: Washington University St Louis, MO, R01 MH059534. John Rice, PhD, Theodore Reich, MD, Allison Goate, PhD, Laura Bierut, MD: Johns Hopkins University, Baltimore, MD, R01 MH59533. Melvin McInnis MD, J. Raymond DePaulo Jr, MD, Dean F. MacKinnon, MD, Francis M. Mondimore, MD, James B. Potash, MD, Peter P. Zandi, PhD, Dimitrios Avramopoulos and Jennifer Payne: University of Pennsylvania, PA, R01 MH59553. Wade Berrettini, MD, PhD: University of California at Irvine, CA, R01 MH60068. William Byerley, MD and Mark Vawter, MD: University of Iowa, IA, R01 MH059548. William Coryell, MD and Raymond Crowe, MD: University of Chicago, IL. R01 MH59535, Elliot Gershon, MD, Judith Badner, PhD, Francis McMahon, MD, Chunyu Liu, PhD, Alan Sanders, MD, Maria Caserta, Steven Dinwiddie, MD, Tu Nguyen, Donna Harakal: University of California at San Diego, CA, R01 MH59567. John Kelsoe, MD, Rebecca McKinney, BA, Rush University, IL. R01 MH059556. William Scheftner, MD, Howard M. Kravitz, DO, MPH, Diana Marta, BS, Annette Vaughn-Brown, MSN, RN and Laurie Bederow, MA: NIMH Intramural Research Program, Bethesda, MD. 1Z01MH002810-01. Francis J. McMahon, MD, Layla Kassem, PsyD, Sevilla Detera-Wadleigh, PhD, Lisa

Austin, PhD, Dennis L. Murphy, MD. Collection of the CHIP families was supported by the NIMH Intramural Research Program (PI F.J.M.) and by collaborating R01 awards from the NIMH (E.S.G., J.P.). We wish to acknowledge the help of Sam Chen for providing us his protocol for scoring TaqMan-derived genotypes.

NCBI: *www.ncbi.nlm.nih.gov*
HapMap: *www.hapmap.org*
University of California Santa Cruz (UCSC) Genome Browser: *www.genome.ucsc.edu*.

References

Abecasis GR, Burt RA, Hall D, Bochum S, Doheny KF, Lundy SL, *et al.* (2004). Genomewide scan in families with schizophrenia from the founder population of Afrikaners reveals evidence for linkage and uniparental disomy on chromosome 1. *Am J Hum Genet* **74**:403–417.

Addington AM, Gornick M, Sporn AL, Gogtay N, Greenstein D, Lenane M, *et al.* (2004). Polymorphisms in the 13q33.2 gene *G72/G30* are associated with childhood-onset schizophrenia and psychosis not otherwise specified. *Biol Psychiatry* **55**:976–980.

Akula N, Chen YS, Hennessy K, Schulze TG, Singh G, McMahon FJ (2002). Utility and accuracy of template-directed dye-terminator incorporation with fluorescence-polarization detection for genotyping single nucleotide polymorphisms. *Biotechniques* **32**:1072–1076.

American Psychiatric Association (1987). *Diagnostic and Statistical Manual of Mental Disorders (DSM-III-R)*. 3rd ed. Washington, DC: APA.

American Psychiatric Association (1994). *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)*. Washington, DC: APA.

Angst J, Angst F, Gerber-Werder R, Gamma A (2005). Suicide in 400 mood-disorder patients with or without long-term medication: a 40 to 44 years' follow-up. *Arch Suicide Res* **9**:279–300.

Anttila V, Kallela M, Oswell G, Kaunisto MA, Nyholt DR, Hämäläinen E, *et al.* (2006). Trait components provide tools to dissect the genetic susceptibility of migraine. *Am J Hum Genet* **79**:85–99.

Badenhop RF, Moses MJ, Scimone A, Mitchell PB, Ewen KR, Rosso A, *et al.* (2001). A genome screen of a large bipolar affective disorder pedigree supports evidence for a susceptibility locus on chromosome 13q. *Mol Psychiatry* **6**:396–403.

Badner JA, Gershon ES (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. *Mol Psychiatry* **7**:405–411.

Barrett JC, Fry B, Maller J, Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**:263–265.

Berrettini WH, Goldin LR, Martinez MM, Maxwell ME, Smith AL, Guroff JJ, *et al.* (1991). A bipolar pedigree series for genomic mapping of disease genes: diagnostic and analytic considerations. *Psychiatr Genet* **2**:125–160.

Blouin JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, *et al.* (1998). Schizophrenia susceptibility loci on chromosomes 13q32 and 8p21. *Nat Genet* **20**:70–73.

Brzustowicz LM, Honer WG, Chow EW, Little D, Hogan J, Hodgkinson K, *et al.* (1999). Linkage of familial schizophrenia to chromosome 13q32. *Am J Hum Genet* **65**:1096–1103.

Chumakov I, Blumenfeld M, Guerassimenko O, Cavarec L, Palicio M, Abderrahim H *et al.* (2002). Genetic and physiological data implicating the new human gene G72 and the gene for D-amino acid oxidase in schizophrenia. *Proc Natl Acad Sci U S A* **99**:13675–13680.

Detera-Wadleigh SD, McMahon FJ (2006). G72/G30 in schizophrenia and bipolar disorder: review and meta-analysis. *Biol Psychiatry* **60**: 106–114.

Detera-Wadleigh SD, Badner JA, Berrettini WH, Yoshikawa T, Goldin LR, Turner G, *et al.* (1999). A high-density genome scan detects evidence for a bipolar-disorder susceptibility locus on 13q32 and other potential loci on 1q32 and 18p11.2. *Proc Natl Acad Sci U S A* **96**:5604–5609.

Dick DM, Foroud T, Flury L, Bowman ES, Miller MJ, Rau NL, *et al.* (2003). Genomewide linkage analyses of bipolar disorder: a new sample of 250 pedigrees from the National Institute of Mental Health Genetics Initiative. *Am J Hum Genet* **73**:107–114.

Doughty CJ, Wells JE, Joyce PR, Olds RJ, Walsh AES (2004). Bipolar-panic disorder comorbidity within bipolar disorder families: a study of siblings. *Bipolar Disord* **6**:245–252.

Dudbridge F (2003). Pedigree disequilibrium tests for multilocus haplotypes. *Genet Epidemiol* **25**:115–221.

Endicott J, Spitzer RL (1978). A diagnostic interview: the schedule for affective disorders and schizophrenia. *Arch Gen Psychiatry* **35**:837–844.

Faraone SV, Skol AD, Tsuang DW, Bingham S, Young KA, Prabhudesai S, *et al.* (2002). Linkage of chromosome 13q32 to schizophrenia in a large veterans affairs cooperative study sample. *Am J Med Genet* **114**:598–604.

Fisfalen ME, Schulze TG, DePaulo JR Jr, DeGroot LJ, Badner JA, McMahon FJ (2005). Familial variation in episode frequency in bipolar affective disorder. *Am J Psychiatry* **162**:1266–1272.

Fisher S, Grice Ea, Vinton RM, Bessling S, McCallion A (2006). Conservation of *RET* regulatory function from human to zebrafish without sequence similarity. *Science* **312**:276–279.

Freeman MP, Freeman SA, McElroy SL (2002). The comorbidity of bipolar and anxiety disorders: prevalence, psychobiology, and treatment issues. *J Affect Disord* **68**:1–23.

Funke B, Malhotra AK, Finn CT, Plocik AM, Lake SL, Lencz T *et al.* (2005). COMT genetic variation confers risk for psychotic and affective disorders: a case control study. *Behav Brain Funct* **1**:19–27.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, *et al.* (2002). The structure of haplotype blocks in the human genome. *Science* **296**:2225–2229.

Ginns EI, Ott J, Egeland JA, Allen CR, Fann CS, Pauls DL, *et al.* (1996). A genome-wide search for chromosomal loci linked to bipolar affective disorder in the Old Order Amish. *Nat Genet* **12**:431–435.

Goodwin FK, Jamieson KR (1990). *Manic-depressive illness*. New York: Oxford University Press.

Green EK, Raybould R, MacGregor S, Gordon-Smith K, Heron J, Hyde S, *et al.* (2005). Operation of the schizophrenia susceptibility gene, neuregulin 1, across traditional diagnostic boundaries to increase risk for bipolar disorder. *Arch Gen Psychiatry* **62**:642–648.

Hall A (2005). Rho GTPases and the control of cell behaviour. *Biochem Soc Trans* **33** (**Pt 5**):891–895.

Hattori E, Liu C, Badner JA, Bonner TI, Christian SL, Maheshwari M, *et al.* (2003). Polymorphisms at the G72/G30 gene locus, on 13q33, are associated with bipolar disorder in two independent pedigree series. *Am J Hum Genet* **72**:1131–1140.

Hedeker DR and Gibbons RD (1996a). MIXREG: a computer program for mixed-effects regression analysis with autocorrelated errors. *Comput Methods Programs Biomed* **49**:229–252.

Hedeker DR, Gibbons RD (1996b). MIXOR: a computer program for mixed-effects ordinal regression analysis. *Comput Methods Programs Biomed* **49**:157–176.

Kassem L, Lopez V, Hedeker D, Steele CJM, Zandi P, The NIMH Genetics Initiative Bipolar Disorder Consortium, McMahon FJ (2006). Polarity at onset is a familial feature of bipolar affective disorder. *Am J Psychiatry* **163**: 1754–1759.

Kelsoe JR, Spence MA, Loetscher E, Foguet M, Sadovnick AD, Remick RA, *et al.* (2001). A genome survey indicates a possible susceptibility locus for bipolar disorder on chromosome 22. *Proc Natl Acad Sci U S A* **98**: 585–590.

Kikuno R, Nagase T, Ishikawa K, Hirosawa M, Miyajima N, Tanaka A (1999). Prediction of the coding sequences of unidentified human genes. XIV. The complete sequences of 100 new cDNA clones from brain which code for large proteins in vitro. *DNA Res* **6**:197–205.

Laird N, Horvath S, Xu X (2000). Implementing a unified approach to family based tests of association. *Genet Epidemiol* **19** (**Suppl 1**):S36–S42.

Li Z, Van Aelst L, Cline HT (2000). Rho GTPases regulate distinct aspects of dendritic arbor growth in Xenopus central neurons in vivo. *Nat Neurosci* **3**:217–225.

Lin MW, Sham P, Hwu HG, Collier D, Murray R, Powell JF (1997). Suggestive evidence for linkage of schizophrenia to markers on chromosome 13 in Caucasian but not Oriental populations. *Hum Genet* **99**:417–420.

Liu C, Badner JA, Christian SL, Guroff J, Detera-Wadleigh SD, Gershon ES (2001). Fine mapping supports previous linkage evidence for a bipolar disorder susceptibility locus on 13q32. *Am J Med Genet (Neuropsych Genet)* **105**:375–380.

Liu J, Juo SH, Dewan A, Grunn A, Tong X, Brito M, *et al.* (2003). Evidence for a putative bipolar disorder locus on 2p13-16 and other potential loci on 4q31, 7q34, 8q13, 9q31, 10q21-24, 13q32, 14q21 and 17q11-12. *Mol Psychiatry* **8**:333–342.

Luo L (2002). Actin cytoskeleton regulation in neuronal morphogenesis and structural plasticity. *Ann Rev Cell Dev Biol* **18**:601–635.

MacKinnon DF, Zandi P, Cooper J, Potash JB, Simpson SG, Gershon E, *et al.* (2002). Comorbid bipolar disorder and panic disorder in families with a high prevalence of bipolar disorder. *Am J Psychiatry* **159**:30–35.

MacQueen GM, Hajek T, Alda M (2005). The phenotypes of bipolar disorder: relevance for genetic investigations. *Mol Psychiatry* **10**:811–826.

McInnis MG, Lan TH, Willour VL, McMahon FJ, Simpson SG, Addington AM, *et al*. (2003). Genome-wide scan of bipolar disorder in 65 pedigrees: supportive evidence for linkage at 8q24, 18q22, 4q32, 2p12, and 13q12. *Mol Psychiatry* **8**:288–298.

McQueen MB, Devlin B, Faraone SV, Nimgaonkar VL, Sklar P, Smoller JW, *et al*. (2005). Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. *Am J Hum Genet* **77**:582–595.

Meller N, Irani-Tehrani M, Kiosses WB, Del Pozo MA, Schwartz MA (2002). Zizimin1, a novel Cdc42 activator, reveals a new GEF domain for Rho proteins. *Nat Cell Biol* **4**:639–647.

Meller N, Merlot S, Guda C (2005). CZH proteins: a new family of Rho-GEFs. *J Cell Sci* **118** (**Pt 21**):4937–4946.

Murphy DL, Uhl GR, Holmes A, Ren-Patterson R, Hall FS, Sora I, *et al*. (2003). Experimental gene interaction studies with SERT mutant mice as models for human polygenic and epistatic traits and disorders. *Genes Brain Behav* **2**:350–364.

Nurnberger JI Jr, Blehar MC, Kaufman CA, York-Coller C, Simpson SG, Harkavy-Friedman J, *et al*. (1994). Diagnostic interview for genetic studies: rationale, unique features, and training. *Arch Gen Psychiatry* **51**: 849–859.

Nurnberger JI Jr, DePaulo JR, Gershon ES, Reich T, Blehar MC, Edenberg H, *et al*. (1997). Genomic survey of bipolar illness in the NIMH genetics initiative pedigrees: a preliminary report. *Am J Med Genet* **74**:227–237.

Park N, Juo SH, Cheng R, Liu J, Loth JE, Lilliston B, *et al*. (2004). Linkage analysis of psychosis in bipolar pedigrees suggests novel putative loci for bipolar disorder and shared susceptibility with schizophrenia. *Mol Psychiatry* **9**:1091–1099.

Pennacchio LA, Ahituv N, Moses AM, Prabhakar S, Nobrega MA, Shoukry M, *et al*. (2006). In vivo enhancer analysis of human conserved non-coding sequences. *Nature* **444**:499–502.

Pianese I, Porcellini A, Avvidemento VE, D'Esposito F, Felliciello A, Monticelli A, *et al*. (1994). A novel thyroid transcript negatively regulated by TSH. *Mol Biol (Life Sci Adv)* **13**:75–83.

Pope HG Jr, Lipinski JF Jr (1978). Diagnosis in schizophrenia and manic-depressive illness. *Arch Gen Psychiatry* **35**:811–828.

Potash JB, Zandi PP, Willour VL, Lan TH, Huo Y, Avramopoulos D, *et al*. (2003). Suggestive linkage to chromosomal regions 13q31 and 22q12 in families with psychotic bipolar disorder. *Am J Psychiatry* **160**:680–686.

Raybould R, Green E, MacGregor S, Gordon-Smith K, Heron J, Hyde S, *et al*. (2005). Bipolar disorder and polymorphisms in the dysbindin gene. *Biol Psychiatry* **57**:696–701.

Regier DA, Farmer ME, Rae DS, Locke BZ, Keith SJ, Judd LL, Goodwin FK (1990). Comorbidity of mental disorders with alcohol and other drug abuse. Results from the epidemiologic catchment area (ECA) study. *JAMA* **264**:2511–2518.

Schulze TG, McMahon FJ (2004). Defining the phenotype in human genetic studies: forward genetics and reverse phenotyping. *Hum Hered* **58**: 131–138.

Schulze TG, Ohlraun S, Czerski PM, Schumacher J, Kassem L, Deschner M, *et al*. (2005). Genotype-phenotype studies in bipolar disorder showing association between the DAOA/G30 locus and persecutory delusions: a first step toward a molecular genetic classification of psychiatric phenotypes. *Am J Psychiatry* **162**:2101–2108.

Schulze TG, Hedeker D, Zandi P, Rietschel M, McMahon FJ (2006). What is familial about familial bipolar disorder? Resemblance among relatives across a broad spectrum of phenotypic characteristics. *Arch Gen Psychiatry* **63**:1368–1376.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Borrmann-Hassenbach M, Albus M, *et al*. (2003). Support for association of schizophrenia with genetic variation in the 6p22.3 gene, dysbindin, in sib-pair families with linkage and in an additional sample of triad families. *Am J Hum Genet* **72**:185–190.

Segurado R, Detera-Wadleigh SD, Levinson DF, Lewis CM, Gill M, Nurnberger JI Jr, *et al*. (2003). Genome scan meta-analysis of schizophrenia and bipolar disorder, Part III: Bipolar Disorder. *Am J Hum Genet* **73**: 49–62.

Shaw SH, Kelly M, Smith AB, Shields G, Hopkins PJ, Loftus J, *et al*. (1998). A genome-wide search for schizophrenia susceptibility genes. *Am J Med Genet* **81**:364–376.

Shaw SH, Mroczkowski-Parker Z, Shekhtman T, Alexander M, Remick RA, Sadovnick AD, *et al*. (2003). Linkage of a bipolar disorder susceptibility locus to human chromosome 13q32 in a new pedigree series. *Mol Psychiatry* **8**:558–564.

Shen W, Wu B, Zhang Z, Dou Y, Rao ZR, Chen YR, Duan S (2006). Activity-induced rapid synaptic maturation mediated by presynaptic cdc42 signaling. *Neuron* **50**:401–414.

Shin JT, Priest JR, Ovcharenko I, Ronco A, Moore RK, Burns CG, *et al*. (2005). Human-zebrafish non-coding conserved elements act in vivo to regulate transcription. *Nucl Acids Res* **33**:5437–5445.

Simpson SG, Folstein SE, Meyers DA, DePaulo JR (1992). Assessment of lineality in bipolar linkage studies. *Am J Psychiatry* **149**:1660–1665.

Spitzer RL, Endicott J, Robins E (1978). Research diagnostic criteria: rationale and reliability. *Arch Gen Psychiatry* **35**:773–782.

Stine OC, McMahon FJ, Chen L, Xu J, Meyers DA, MacKinnon DF, *et al*. (1997). Initial genome screen for bipolar disorder in the NIMH genetics initiative pedigrees: chromosomes 2, 11, 13, 14, and X. *Am J Med Genet* **74**: 263–269.

Straub RE, Jiang Y, MacLean CJ, Ma Y, Webb BT, Myakishev MV, *et al*. (2002). Genetic variation in the 6p22.3 gene *DTNBP1*, the human ortholog of the mouse dysbindin gene, is associated with schizophrenia. *Am J Hum Genet* **71**:337–348.

Threadgill R, Bobb K, Ghosh A (1997). Regulation of dendritic growth and remodeling by Rho, Rac, and Cdc42. *Neuron* **19**:625–634.

Wallace C, Xue MZ, Newhouse SJ, Marçano ACB, Onipinla AK, Burke B, *et al*. (2006). Linkage analysis using co-phenotypes in the BRIGHT study reveals novel potential susceptibility loci for hypertension. *Am J Hum Genet* **79**: 323–331.

Williams NM, Green EK, Macgregor S, Dwyer S, Norton N, Williams H, *et al*. (2006). Variation at the DAOA/G30 locus influence susceptibility to major mood episodes but not psychosis in schizophrenia and bipolar disorder. *Arch Gen Psychiatry* **63**:366–373.

Winokur G, Coryell W, Endicott J, Keller M, Akishkal H, Solomon D (1996). Familial alcoholism in manic-depressive (bipolar) disease. *Am J Med Genet* **67**:197–201.

Woolfe A, Goodson M, Goode DK, Snell P, McEwen GK, Vavouri T, *et al*. (2005). Highly conserved non-coding sequences are associated with vertebrate development. *PLoS Biology* **3**:116–130.