Sequence variation in DOCK9 and heterogeneity in bipolar disorder

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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).

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...
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 gene interaction. For example, epistatic effects generated in dopamine transporter, 5-HT1B and brain-derived neurotrophic factor have been documented in several studies (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 breadth 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. Familiarity 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.
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. TDT analysis (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 (ASMMII) 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 (zizimin1) (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
rs9513550. Genotype data generated by HapMap on 348 SNPs in the CEU including only those SNPs with MAF ≥ 0.1 was used. Haplovie 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 ~160 kb and incorporates the remaining exons that encode three functional motifs, PH, CZH1 and CZH2 (Meller et al., 2002) (Fig. 1).

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

| SNPs | Position, bp | TDT-PHASE | ASMI (BPI/SABP) |
|------|--------------|-----------|-----------------|
|      |              | Initial screen on 285 families (P) | NIMH1-2 | NIMH3 | NIMH4 | CHIP-CNG |
|      |              | No. of families | P | No. of families | P | No. of families | P | No. of families | P | No. of families | P |
| 1    | rs1299066a   | 98 230 873 | 63 | 0.5837 | 28 | 0.3402 | 45 | 0.1739 |
| 2    | rs2899       | 98 243 865 | 57 | 0.3538 | 50 | 0.3971 | 69 | 0.5306 |
| 3    | rs772303     | 98 250 308 | 0.4886 | NA | 36 | 0.8518 | 77 | 0.3033 |
| 4    | rs2296983    | 98 255 162 | 62 | 0.3029 | 22 | 0.9359 | 49 | 0.8875 |
| 5    | rs772311     | 98 266 460 | 62 | 0.1535 | 16 | 0.2596 | 41 | 0.8132 |
| 6    | rs1887556    | 98 277 348 | 0.1740 | NA | 57 | 0.3679 | 72 | 0.1181 |
| 7    | rs733687     | 98 288 560 | 69 | 0.3520 | 43 | 0.9359 | 49 | 0.8875 |
| 8    | rs7986477    | 98 297 352 | 43 | 0.6260 | 22 | 0.2596 | 41 | 0.8132 |
| 9    | rs4772152    | 98 319 759 | 63 | 0.7407 | 16 | 0.2596 | 41 | 0.8132 |
| 10   | rs2296994    | 98 331 898 | 34 | 0.4778 | 16 | 0.2596 | 41 | 0.8132 |
| 11   | rs2026024    | 98 333 432 | 0.2969 | NA | 57 | 0.3679 | 72 | 0.1181 |
| 12   | rs124982     | 98 342 134 | 57 | 0.3152 | 41 | 0.7513 | 41 | 0.2899 |
| 13   | rs732822     | 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 | 69 | 0.3142 | 41 | 0.0558 |
| 16   | rs874199     | 98 386 108 | 69 | 0.3142 | 41 | 0.0558 | 41 | 0.0558 |
| 17   | rs2390129a   | 98 406 081 | 0.0196 | 41 | 0.0558 | 41 | 0.0558 |
| 18   | rs1359427a   | 98 436 409 | 61 | 0.1238 | 36 | 0.0872 | 59 | 0.3700 |
| 19   | rs1886653a   | 98 448 739 | 63 | 0.1064 | 36 | 0.0872 | 59 | 0.3700 |
| 20   | rs1041093a   | 98 453 658 | 46 | 0.0943 | 43 | 0.1103 | 59 | 0.3700 |
| 21   | rs1886654a   | 98 460 081 | 44 | 0.0156 | 36 | 0.0872 | 59 | 0.3700 |
| 22   | docksnbp81endf | 98 461 986 | 39 | 0.0007 | 36 | 0.0872 | 59 | 0.3700 |
| 23   | rs4772168    | 98 464 941 | 61 | 0.1401 | 46 | 0.4855 | 80 | 0.5924 |
| 24   | rs957549     | 98 472 381 | 68 | 0.0904 | 47 | 0.1871 | 85 | 0.6600 |
| 25   | rs2000342    | 98 477 488 | 0.04766 | 71 | 0.1097 | 52 | 0.2670 | 84 | 0.4405 |
| 26   | rs4691476b   | 98 484 105 | 69 | 0.1589 | 52 | 0.3699 | 85 | 0.4953 |
| 27   | rs1049257a   | 98 494 931 | 55 | 0.0315 | 43 | 0.0604 | 69 | 0.1525 |
| 28   | rs9517575    | 98 507 741 | 56 | 0.0078 | 43 | 0.0604 | 67 | 0.1673 |
| 29   | rs9554547    | 98 514 478 | 66 | 0.0601 | 48 | 0.4814 | 84 | 0.7877 |
| 30   | rs2105425    | 98 524 566 | 62 | 0.0578 | 48 | 0.7339 | 78 | 0.1788 |
| 31   | rs5557134    | 98 526 453 | 53 | 0.0064 | 40 | 0.0626 | 67 | 0.2271 |
| 32   | rs7334435    | 98 529 622 | 62 | 0.0751 | 47 | 0.6240 | 77 | 0.8361 |
| 33   | rs5957137    | 98 535 143 | 52 | 0.0071 | 45 | 0.0877 | 68 | 0.1038 |
| 34   | rs1927568    | 98 538 118 | 0.00067 | 56 | 0.0119 | 42 | 0.1037 | 67 | 0.1372 |
| 35   | rs1340       | 98 538 564 | 54 | 0.0134 | 40 | 0.0396 | 70 | 0.4437 |
| 36   | rs9513550a   | 98 550 511 | 51 | 0.0222 | 40 | 0.0396 | 70 | 0.4437 |
| 37   | rs3858781    | 98 555 678 | 62 | 0.0515 | 43 | 0.9578 | 87 | 0.4347 |
| 38   | rs1536657a   | 98 563 421 | 58 | 0.1799 | 36 | 0.0396 | 70 | 0.4437 |

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**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
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 ($P = 0.000083$,
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 (Fisalen 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.

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).
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 (\( P = 0.048–0.000072 \)) (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 allelic 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 |

| SNP No. of families | Rounding thoughts during mania | Grandiosity during mania | Log. manic episodes |
|---------------------|-------------------------------|--------------------------|-------------------|
|                     | NIMH1-2 | NIMH3 | P | NIMH1-2 | NIMH3 | P | NIMH1-2 | NIMH3 | P |
| rs1299066           | 88      | 0.2479 | 81 | 0.3722 |
| rs2869              | 72      | 0.3277 | 45 | 0.4904 | 66 | 0.1508 | 42 | 0.2752 | 75 | 0.2644 | 65 | 0.1962 |
| rs2049683           | 80      | 0.3277 | 65 | 0.3991 | 75 | 0.2644 | 65 | 0.1962 | 76 | 0.3852 |
| rs772311            | 89      | 0.1562 | 81 | 0.3208 |
| rs7333867           | 92      | 0.2104 | 60 | 0.9273 | 84 | 0.5341 | 59 | 0.8420 |
| rs7986477           | 58      | 0.7733 | 35 | 0.2927 | 53 | 0.5355 | 39 | 0.6721 |
| rs4772152           | 82      | 0.7595 | 60 | 0.1433 | 75 | 0.7809 | 59 | 0.1687 |
| rs2096994           | 43      | 0.8498 | 26 | 0.9579 | 41 | 0.7214 | 31 | 0.5392 |
| rs1324982           | 66      | 0.2314 | 54 | 0.7269 | 58 | 0.1521 | 52 | 0.4704 |
| rs7328282           | 87      | 0.6707 | 65 | 0.1663 | 79 | 0.5711 | 64 | 0.2228 |
| rs1242661           | 53      | 0.9190 | 32 | 0.6999 | 48 | 0.9135 | 39 | 0.3836 |
| rs7331563           | 86      | 0.3676 | 79 | 0.4077 |
| rs7780239           | 85      | 0.1201 | 77 | 0.1021 | 74 | 0.0248 |
| rs841994            | 89      | 0.2577 | 88 | 0.1012 |
| rs2390129           | 54      | 0.0171 | 48 | 0.0060 | 91 | 0.0124 |
| rs1359427           | 93      | 0.0599 | 87 | 0.0179 |
| rs1886553           | 93      | 0.0599 | 87 | 0.0179 |
| rs1041093           | 64      | 0.0509 | 53 | 0.0224 | 57 | 0.1009 | 56 | 0.0761 |
| rs1886554           | 64      | 0.0707 | 52 | 0.0154 | 57 | 0.0181 | 55 | 0.0553 |
| dockspn81indel      | 51      | 0.0063 | 46 | 0.0323 | 47 | 0.0548 | 47 | 0.1021 | 47 | 0.0758 |
| rs2772168           | 88      | 0.8007 | 73 | 0.8213 | 83 | 0.4845 | 72 | 0.9266 | 79 | 0.0328 |
| rs9517549           | 97      | 0.0366 | 69 | 0.5679 | 90 | 0.0154 | 70 | 0.4331 |
| rs2000342           | 96      | 0.0339 | 67 | 0.5018 | 90 | 0.0101 | 70 | 0.4057 |
| rs6491476           | 94      | 0.0647 | 70 | 0.6299 | 88 | 0.0186 | 74 | 0.4615 |
| rs10439257          | 73      | 0.0073 | 59 | 0.0240 | 67 | 0.0025 | 59 | 0.1268 | 65 | 0.0064 |
| rs9517575           | 72      | 0.0009 | 56 | 0.0159 | 66 | 0.0007 | 56 | 0.0926 |
| rs9554547           | 96      | 0.0249 | 71 | 0.8212 | 90 | 0.0070 | 71 | 0.7560 |
| rs2105425           | 88      | 0.0200 | 73 | 0.9222 | 85 | 0.0087 | 74 | 0.8662 |
| rs9557134           | 70      | 0.0008 | 52 | 0.0094 | 66 | 0.0010 | 53 | 0.5081 |
| rs7334435           | 86      | 0.0273 | 73 | 0.8212 | 82 | 0.0130 | 74 | 1.0000 |
| rs9557137           | 71      | 0.0013 | 56 | 0.1916 | 65 | 0.0012 | 58 | 0.1078 |
| rs1927668           | 73      | 0.0026 | 55 | 0.0248 | 67 | 0.0025 | 56 | 0.1484 |
| rs1340              | 77      | 0.0031 | 66 | 0.0070 | 71 | 0.0034 | 57 | 0.0341 |
| rs9513550           | 69      | 0.0055 | 64 | 0.0036 | 64 | 0.0036 |
| rs3858781           | 92      | 0.0222 | 66 | 0.8596 | 88 | 0.0392 | 65 | 0.7222 |
| rs1536657           | 76      | 0.5563 | 68 | 0.7503 | 68 | 0.7503 | 68 | 0.7503 | 68 | 0.7503 | 68 | 0.7503 |

NA, not analyzed.

\( P \) 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 as 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

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| SNP     | SNP code | No. of families | \( P \) | Overtransmitted allele | No. of families | \( P \) | Overtransmitted allele |
|---------|----------|----------------|--------|------------------------|----------------|--------|------------------------|
| rs1299066 | M        | 51             | NS     |                        | 24             | 0.0196 | A                      |
| rs2899   | W        | 45             | NS     |                        | 47             | 0.0023 | G                      |
| rs2996983 | S        | 55             | NS     |                        |                |        |                        |
| rs772311 | Y        | 55             | NS     |                        | 24             | 0.0196 | A                      |
| rs7333687 | R        | 58             | 0.0307 | G                      | 33             | 0.0198 | A                      |
| rs7986477 | R        | 37             | NS     | NA                     | 34             | 0.1060 | A                      |
| rs4772152 | W        | 52             | NS     | C                      | 52             | 0.0417 | T                      |
| rs2996994 | R        | 26             | 0.0555 | G                      | 27             | NS     | 39                     | 0.0561 | T                      |
| rs1324982 | Y        | 46             | 0.1050 | C                      | 49             | 0.0585 | A                      |
| rs7328282 | R        | 53             | NS     |                        | 49             | 0.0585 | A                      |
| rs1242661 | R        | 34             | 0.0835 | G                      | 30             | NS     |                        |
| rs7331595 | M        | 53             | 0.0425 | A                      | NA             |        |                        |
| rs8002389 | R        | 54             | 0.0536 | G                      | NA             |        |                        |
| rs6941999 | Y        | 58             | NS     |                        | NA             |        |                        |
| rs2990129 | R        | 39             | 0.0547 | G                      | NA             |        |                        |
| rs1359427 | M        | 54             | 0.0538 | A                      | NA             |        |                        |
| rs1886553 | S        | 56             | 0.0910 | A                      | NA             |        |                        |
| rs1041093a | S       | 44             | 0.0429 | C                      | 42             | NS     |                        |
| rs1886554 | Y        | 44             | 0.0084 | T                      | 43             | NS     |                        |
| docksnp81indel | INDEL | 37             | 0.0041 | DEL                    | 34             | 0.0687 | INS                    |
| rs4772168 | Y        | 54             | 0.0272 | C                      | 49             | NS     | 37                     | 0.0211 | G                      |
| rs9571549 | W        | 60             | 0.0710 | A                      | 57             | NS     | 58                     | 0.1293 | NS                     |
| rs2000342 | Y        | 63             | NS     |                        | 58             | NS     |                        |
| rs6491476a | Y       | 62             | NS     |                        | 58             | 0.1293 | NS                     |
| rs10492574 | K        | 53             | 0.1146 | T                      | 50             | 0.0181 | G                      |
| rs957575 | Y        | 53             | 0.0503 | C                      | 52             | 0.0259 | T                      |
| rs9554547 | Y        | 59             | 0.0838 | C                      | 57             | NS     | 59                     | 0.0838 | C                      |
| rs2105425 | R        | 57             | 0.0380 | A                      | 49             | NS     |                        |
| rs9557134 | Y        | 53             | 0.0317 | T                      | 51             | 0.0266 | C                      |
| rs7344435 | R        | 58             | 0.0380 | G                      | 51             | NS     |                        |
| rs9567137 | 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             | 0.0666 | A                      | NA             |        |                        |
| rs3858781 | Y        | 59             | 0.0218 | T                      | 55             | NS     |                        |
| rs1536527 | 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.

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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
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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 genome-wide 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.

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References

Abecasis GR, Burt RA, Hall D, Bochoo S, Doheny KF, Lundy SL, et al. (2004). Genomewide scan in families with schizophrenia from the founder population of Afrikans 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. (2006).CHIP families was supported by the NIMH Intramural protocol for scoring TaqMan-derived genotypes.

Aguilera JL, Dombroski BA, Nath SK, Lasseter VK, Wolyniec PS, Nestadt G, et al. (2000). 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, Osigel WW, Kaunino MA, Nyhold HR, Hämäläinen E, et al. (2006). Trait components provide tools to disrupt the genetic susceptibility of migraine. Am J Hum Genet 78:85–99.

Badenhop RF, Moses MJ, Scimone A, Mitchell PB, Even KR, Rossa 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 6:398–403.

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, Goldin LR, 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.

Bruzewicz LM, Honer WG, Chow EW, Little D, Hogan J, Hodgkinson K, et al. (1999). Linkage of familial schizophrenia to chromosome 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 with bipolar disorder families: a study of siblings. Bipolar Disord 6:245–252.

Dudbridge F (2003). Pedigree disequilibrium tests for multifocus 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 AO, Tsuang DW, Bingham S, Young KA, Prabhudesai S, et al. (2002). Linkage of childhood-onset schizophrenia to chromosome 13q32 in a large veterans affairs cooperative study sample. Am J Med Genet 114:598–604.

Fusifan ME, Schulte 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, Besseling S, McCailllon 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 genotype variation contributes to affective and psychotic disorders: a case control study. Behav Brain Funct 1:1–9.

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.

Girino EI, Ott J, Egeland AJ, Allen CR, Fann CS, Pauls DL, et al. (1998). Operation of the schizophrenia susceptibility gene, neuroligin 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, Bonnor 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 Biol 49:229–252.

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

Kassern 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 163:1753–1759.

Kellsoe JR, Spence MA, Loetscher E, Foguet M, Sadowski 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, Hiroshawa 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, Goldin 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 105:375–380.

Luo L, Jiao HC, Dewan A, Grumm A, Tong X, Brito M, et al. (2003). Evidence for a putative bipolar disorder locus on 3p13–16 and another potential loci on 4q31, 7q54, 9q13, 9p91, 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, Aida M (2005). The phenotypes of bipolar disorder: relevance for genetic investigations. Mol Psychiatry 10:811–826.

NCBI: www.ncbi.nlm.nih.gov

HapMap: www.hapmap.org

University of California Santa Cruz (UCSC) Genome Browser: www.genome.ucsc.edu

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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 8:298–299.

McQueen MB, Devlin B, Faraoe 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). Murphy DL, Uhl GR, Holmes A, Ren-Patterson R, Hall FS, Sora I, Nurnberger JI Jr, Blehar MC, Kaufman CA, York-Coller C, Simpson SG, Nurnberger JI Jr, DePaulo JR, Gershon ES, Reich T, Blehar MC, Edenberg H, 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.

Shaw SH, McQueen MB, Devlin B, Shields G, Hopkins PJ, Loftus J, et al. (1998). A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 81:364–376.

Chow SH, Mroczkowski-Parker Z, Shekhtman T, Alexander M, Remick RA, 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, Joo 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.

Curran MA, Billett HD, Yen BM, et al. (1986). A molecular genetic classification of psychiatric phenotypes. Am J Psychiatry 849–859.

Pennacchio LA, Ahtuv 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, Ponzoni A, Avison V, Humke E, 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, Lipinska LJ Jr (1978). Diagnosis in schizophrenia and manic-depressive illness. Arch Gen Psychiatry 35:811–828.

Potash JB, Zandi PP, Willour VL, Lan TH, Hwu 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, Hyde S, et al. (2005). Bipolar disorder and polymorphisms in the dysbindin gene. Biol Psychiatry 57:696–701.

Reger 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, Hedeke 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 63:366–373.

Schwab SG, Knapp M, Mondabon S, Hallmayer J, Bornmann-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 Jr JI, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, 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, McQueen MB, Devlin B, Shields G, Hopkins PJ, Loftus J, et al. (1998). A genome-wide search for schizophrenia susceptibility genes. Am J Med Genet 81:364–376.