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Combined Analysis from Eleven Linkage Studies of Bipolar Disorder Provides Strong Evidence of Susceptibility Loci on Chromosomes 6q and 8q

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Several independent studies and meta-analyses aimed at identifying genomic regions linked to bipolar disorder (BP) have failed to find clear and consistent evidence of linkage regions. Our hypothesis is that combining the original genotype data provides benefits of increased power and control over sources of heterogeneity that outweigh the difficulty and potential pitfalls of the implementation. We conducted a combined analysis using the original genotype data from 11 BP genomewide linkage scans comprising 5,179 individuals from 1,067 families. Heterogeneity among studies was minimized in our analyses by using uniform methods of analysis and a common, standardized marker map and was assessed using novel methods developed for meta-analysis of genome scans. To date, this collaboration is the largest and most comprehensive analysis of linkage samples involving a psychiatric disorder. We demonstrate that combining original genome-scan data is a powerful approach for the elucidation of linkage regions underlying complex disease. Our results establish genomewide significant linkage to BP on chromosomes 6q and 8q, which provides solid information to guide future gene-finding efforts that rely on fine-mapping and association approaches.

Introduction

Bipolar disorder (BP) is a common and often disabling mood disorder, from which individuals suffer from episodes of mania and depression. The symptoms of mania include an expansive, elevated, or irritable mood; inflated self-esteem; grandiosity; decreased need for sleep; increased talkativeness; racing thoughts; distractibility; increased goal-directed activity; and excessive involvement in pleasurable activities with a high potential for painful consequences (National Institute of Mental Health [NIMH] Genetics Workgroup 1999). The symptoms of depression include depressed mood, diminished interest or pleasure in activities, change in sleeping patterns, psychomotor agitation or retardation, fatigue or loss of energy, feelings of worthlessness or excessive guilt, inability to concentrate or act decisively, and recurrent thoughts of death or suicide (NIMH Genetics Workgroup 1999). Bipolar disorder I (BPI) is defined by the occurrence of one or more manic or mixed (manic/depressive) episodes and is often accompanied by at least one major depressive episode (American Psychiatric Association [APA] 1994). Bipolar disorder II (BPII) is characterized by milder manic episodes (hypomania) and recurrent major depressive episodes (APA 1994).

It is estimated that BPI has a lifetime prevalence of ~0.5%–1.5%, whereas BPII has a lifetime prevalence of ~0.5% (Kessler et al. 1994; Weissman et al. 1996). The burden of illness for BP is considerable. It is estimated that completed suicide occurs in 10%–15% of individuals who received a diagnosis of BP (APA 1994), as well as a higher rate of unemployment and marital

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dysfunction and an increased use of health services (Weissman et al. 1991). Further, treatment of BP is not curative and is not completely effective in mitigating symptoms. Extensive research efforts have been directed at uncovering the etiology of BP, with the hope that more-effective treatment and prevention strategies can be developed.

The importance of genetic factors in BP has been repeatedly confirmed using family, twin, and adoption studies. The recurrence risk ratio is ~7 for first-degree relatives and as high as 60 for MZ twins (NIMH Genetics Workgroup 1999). Twin studies have suggested heritability estimates of ~80%, providing evidence that genes contribute strongly to the familial aggregation of BP (Tsuang and Faraone 2000; Smoller and Finn 2003). The mode of inheritance for BP is complex and likely involves multiple genes. The specific number of susceptibility loci, the recurrence risk ratio attributable to each locus, and the degree of the interaction between loci are unknown. Nevertheless, it is clear that a single major locus does not explain any substantial proportion of the familial aggregation of BP (NIMH Genetics Workgroup 1999).

Genetic complexity underlying BP provides at least one plausible explanation for the many insignificant and/or inconsistent findings among gene-mapping efforts over the past 15 years. There have been numerous genome-wide scans of BP, and linkage signals from individual studies have been reported throughout the genome, including chromosomes 1, 4, 6, 10, 12, 13, 18, 21, 22, and X (reviewed by Baron [2002]). Consistent with other disorders of complex etiology, independent replication of linkage signals for BP has not been convincing.

The lack of reproducibility of linkage findings among different studies may reflect a variety of study-specific issues. For example, different studies have variable power to detect linkage, depending on the sample size and the number of affected individuals who meet ascertainment criteria for the study. There are often differences in the diagnosis and ascertainment of affected individuals, differences in the phenotypic model chosen for analysis (i.e., inclusion of recurrent unipolar depression [RUD]), or differences in the modeling parameters (i.e., penetrance, disease-allele frequency, etc.) specified for parametric linkage analysis. The genetic markers, marker densities, and the genetic maps used for each study can also vary considerably. Finally, in addition to false-positive results, there may be a considerable amount of genetic heterogeneity of BP among the populations studied.

Meta-analysis is one strategy that offers a systematic and quantitative approach to summarizing evidence from multiple genome scans. Two relevant meta-analyses of BP linkage studies have been published recently. Badner and Gershon (2002) developed the multiple scan probability (MSP) method to conduct a meta-analysis of reported *P* values in published linkage-analysis studies. Chromosomal regions that had a *P* value <.01 from 11 independently published BP linkage studies were included in their meta-analysis. The meta-analysis provided evidence of two susceptibility loci residing on chromosomes 13q and 22q. In a similar effort, Segurado et al. (2003) conducted a meta-analysis that included up to 18 independent linkage studies that used the genome scan meta-analysis (GSMA) method (Wise et al. 1999). In contrast to the meta-analysis by Badner and Gershon (2002), Segurado et al. (2003) reported no significant chromosomal regions linked to BP across the genome.

An advantage of the MSP and GSMA approaches is that they require only linkage statistics and/or *P* values from each study. However, many of the study-specific issues discussed above are also likely to affect these meta-analyses. One method to circumvent many of the issues encountered with the meta-analysis of linkage studies is through the use of the original genotype data rather than summary statistics (Levinson et al. 2003). Here, we explore whether combining the original genotype data provides benefits of increased power and control over sources of heterogeneity that outweigh the difficulty and potential pitfalls of the implementation. We report that, in this case, combining the original genotype data was clearly worth the effort, since we were able to demonstrate significant linkage to loci on chromosomes 6q and 8q while controlling for potential sources of heterogeneity.

Material and Methods

Source and Structure of the Original Data Sets

Our analysis included original genotype data from 11 independent linkage studies. Studies *excluded* from the present meta-analysis include genome scans that had (1) a unique population origin (i.e., founder populations) or (2) a unique ascertainment scheme (i.e., lithium-dependent BP); (3) studies containing largely multiplex complex pedigrees (these tended to be smaller studies with <20 pedigrees); or (4) no original data available. Table 1 summarizes the populations represented, as well as the numbers of families and individuals per data set. In sum, the 11 data sets include 1,067 families of 5,179 individuals from North America, Italy, Germany, Portugal, the United Kingdom, Ireland, and Israel. In addition, table 1 provides the number of markers that were genotyped from each study, as well as the number of markers that were used for the analysis (see the "Genotyping, Genetic Markers, and Map" section). Collection of blood and family history information for each study was done with informed consent and approval of the respective institutional review boards (or equivalent). We used raw genotype data that corresponded to the initial genome-wide scans from each data set (avoiding fine-mapping

Table 1

Overview of Data Sets

Data Set	Reference	Population	Race	No. of Pedigrees	No. of Genotyped Individuals	No. of Genetic Markers[a]	
						Genotyped	Mapped
Bonn	Cichon et al. 2001	Germany, Israel, Italy	White	75	387	389	386
Columbia	Liu et al. 2003	Israel, U.S.	White	40	358	334	333
Johns Hopkins 1	McInnis et al. 2003*b*	U.S.	Mixed	63	562	823	802
Johns Hopkins 2	Fallin et al. 2004	U.S.	White	40	175	381	380
NIMH Wave 1	NIMH Genetics Initiative Bipolar Group 1997	U.S.	Mixed	95	525	357	351
NIMH Wave 2	Dick et al. 2002; McInnis et al. 2003*a*; Willour et al. 2003; Zandi et al. 2003	U.S.	Mixed	55	348	465	458
NIMH Wave 3	Dick et al. 2003; NIMH Human Genetics Initiative Web Site	U.S.	Mixed	220	982	372	372
NIMH Wave 4	NIMH Human Genetics Initiative Web Site	U.S.	Mixed	274	1,053	384	384
Portuguese	Pato et al. 2004	Portugal	White	16	102	346	342
UCSD	Kelsoe et al. 2001	Canada, U.S.	White	20	163	331	324
Wellcome Trust	Bennett et al. 2002	Great Britain, Ireland	White	151	509	380	378
Total				1,067	5,179	4,562	4,510

[a] Genotyped = number of markers genotyped (autosomal only). Mapped = number of markers mapped to the common marker map (autosomal only).

[b] ARP = all possible ARPs; FS = all possible full-sibling pairs ; IF = families informative for nonparametric allele-sharing linkage analysis.

[c] Ascertainment scheme: 1SB = at least one affected sibling; 1FD = at least one affected first-degree relative; 1FD+ = at least one affected first- or greater-degree relative; 2FD+ = at least two affected first- or greater-degree relatives.

[d] Diagnosis of the relative(s) of proband (satisfying ascertainment scheme). BPNOS = bipolar disorder–not otherwise specified.

data, etc.), with the exception of the NIMH Wave 3, in which case we used the data available via the Web repository (NIMH Human Genetics Initiative Web Site).

Family Ascertainment and Assessment

Detailed descriptions of family ascertainment, clinical assessment, and diagnostic criteria are available in the respective primary references for each data set (table 1). With the exception of the Columbia and University of California at San Diego (UCSD) data sets, all studies included a proband with a Diagnostic and Statistical Manual of Mental Disorders, 3rd edition (DSM-IIIR), Research Diagnostic Criteria (RDC), or DSM-IV diagnosis of BPI but differed with respect to the number, type of relationship, and diagnosis of additional family members used for ascertainment conditions. A brief overview of pertinent information is provided in table 1.

Genotyping, Genetic Markers, and Map

Detailed information about specimen collection, DNA extraction, and genotyping methods for each data set can be found in the primary references for each data set (table 1). In all 11 data sets, variable-repeat microsatellite genetic markers were genotyped for family members with available DNA for each of the 22 autosomes. Genotypes from the X chromosome were not available from all data sets; therefore, they were excluded from the analysis. The number of markers genotyped for each data set was relatively consistent (~350–400), with the noted exception of the Johns Hopkins 1 data set ($n = 823$).

A unique feature available to meta-analyses of linkage studies that use original genotype data is the ability to construct a standardized genetic map. We mapped respective markers from each data set to one common sex-averaged map, using the Rutgers Combined Linkage–Physical Map of the Human Genome (Kong et al. 2004; Rutgers Combined Linkage–Physical Map of the Human Genome Web Site) as the backbone. If the genetic location (in cM) of a particular marker was found on the Rutgers map, then that location was used. Otherwise, if the genetic location from the Rutgers map was not available for a particular marker, we first determined the physical location (in bp) of that marker, using the National Center for Biotechnology Information (NCBI) Build 35.1 (University of California–Santa Cruz [UCSC] Genome Bioinformatics). We then identified the physical locations of two flanking markers that were found on the Rutgers map. The resultant genetic location of the unknown marker location was interpolated under the assumption that the ratio of the distances between markers on the physical map was the same as the ratio of the distances on the genetic map. If we could not identify a marker either on the Rutgers map or via the NCBI, that marker was discarded. We were able to map the vast majority of markers from each data set to the standardized, common map (table 1).

Pooling Procedure

We combined the raw genotype data from the 11 studies into one large pooled data set. To accommodate the variation in allele coding across different studies, we created unique marker names for each data set, such that

Diagnostic and Ascertainment Criteria				No. of Subjects with BP[b]							
				Narrow				Broad			
Diagnostic Criterion(a)	Diagnosis of Proband	Scheme[c]	Diagnosis of Affected Relative[d]	Total	ARP	FS	IF	Total	ARP	FS	IF
DSM-IIIR	BPI	1SB	BPI, SAB, BPNOS, RUD	103	62	45	27	124	98	69	36
RDC	BPI, SAB	2FD+	BPI, SAB	115	97	38	31	200	309	92	35
RDC	BPI	2FD+	BPI, BPII, SAB, RUD	125	73	65	32	216	243	169	55
DSM-IV	BPI	1FD+	BPI, BPII	73	33	19	22	95	53	38	40
DSM-IIIR, RDC	BPI	1FD	BPI, SAB	226	164	94	64	298	294	164	81
DSM-IIIR, RDC	BPI	1FD	BPI, SAB	135	85	54	39	174	170	110	46
DSM-IV	BPI	1SB	BPI, SAB	489	310	254	189	534	370	288	193
DSM-IV	BPI	1SB	BPI, SAB	620	416	338	234	665	482	380	243
DSM-IV	BPI	1FD	BPI, BPII, SAB	43	35	27	15	44	37	27	15
DSM-IIIR	BPI, BPII	2FD+	BPI, BPII, SAB, RUD	27	11	7	7	41	21	15	12
DSM-IV	BPI	1SB	BPI, BPII, BPNOS, RUD	288	159	150	109	313	195	186	122
				2,251	1,445	1,091	769	2,423	2,272	1,538	878

no two data sets shared an identical marker name (even if the same marker had been genotyped across more than one study). We then pooled the raw genotypes from each data set, generating missing genotypes for each occurrence of a marker from one of the other data sets. Pooling linkage samples in this way enabled us to estimate allele frequencies within study, which minimized any bias introduced through estimation of allele frequencies across the entire, potentially heterogeneous, pooled sample.

Error Detection

We used a variety of programs to check for Mendelian errors, including family-based association test (FBAT) (Laird et al. 2000) and PEDSTATS (Abecasis et al. 2002). Any incompatible genotypes were set to "unknown" for the entire family for that locus. In addition, we used the software package MERLIN (Abecasis et al. 2002) for its error-detection feature to identify unlikely genotypes ($P < .05$ from the r-statistic). Any unlikely genotypes detected by MERLIN were set to "unknown." Relationship errors were assessed using graphical relationship representation (Abecasis et al. 2001). Individuals with genotype data inconsistent with their pedigree relationships, including MZ twins, were excluded from the analysis. Finally, individuals with no genotype or phenotype information who were not needed to define relationships between other individuals in a pedigree were removed using MERLIN's *trim* option.

Affection-Status Models

Two hierarchical definitions for BP affection status were used: (1) a narrow model that included individuals who received a diagnosis of BPI only and (2) a broad model that included individuals who received a diagnosis of either BPI or BPII. Relatives who received a diagnosis of disorders other than BPI or BPII were coded as "unknown." The use of multiple affection models is common practice in linkage analysis of BP, primarily because diagnostic boundaries are ill defined and the underlying genetic model for BP is unknown. Table 1 provides an overview of the diagnostic criteria used for each data set, as well as the number of affected relative pairs (ARPs) that have complete phenotype and genotype information for both the narrow and broad BP definitions; we have the original genotype data for 1,445 ARPs for the narrow phenotype definition and 2,272 ARPs for the broad phenotype definition. Note that families ascertained through non-BPI–affected probands but that met criteria for the BP broad phenotype model were included in the analysis (i.e., BPII-affected probands from UCSD).

ARP LOD-Score Methods

The analytic objective of the project was to conduct nonparametric multipoint linkage analysis with use of the pooled sample for each of the two phenotype definitions. Marker-allele frequencies were estimated within study by use of founder genotypes. If there were no available founder genotypes for a particular family, a random family member with genotype information was used. Use of the default allele frequency calculation in MERLIN (all genotyped individuals) did not alter the results (data not shown). We used MERLIN's implementation of the Whittemore and Halpern (1994) algorithm to test for allele sharing among all affected individuals, and we generated the nonparametric LOD score via the Kong and Cox (1997) linear model. We also estimated the linkage information at each analysis position, using MERLIN's measure of entropy. At 1-cM intervals, across all 22 autosomal chromosomes, we analyzed each of the 11 data

sets individually, as well as the pooled data set comprising the 11 data sets combined.

Genomewide Significance Thresholds

We followed the guidelines of Lander and Kruglyak (1995) for genomewide significant and suggestive linkage thresholds. We estimated critical values for the LOD score from the pooled analysis, using the method described by Bacanu (2005); to compute genomewide significant and suggestive critical values, this method estimates the correlation between Gaussian statistics at adjacent map points. The threshold for genomewide significance was established at a LOD score of 3.03. Genomewide suggestive thresholds were established at a LOD score of 1.75. The thresholds were Bonferroni adjusted to account for the fact that two phenotypes were analyzed. In our study, the correlation between narrow and broad BP linkage statistics was 0.85. Our simulations (data not shown) suggest that, for this magnitude of correlation, Bonferroni-adjusted thresholds are very close to their empirical counterparts. Consequently, for the present analysis, we report only the Bonferroni adjustment for multiple testing. For comparison, we also calculated simulated thresholds (1,000 genomewide replicates) and confirmed that these were similar to the critical values obtained by the Bacanu (2005) method.

Assessment of Heterogeneity

The identical-by-descent (IBD) sharing probabilities of each affected sibling pair (ASP) generated in MERLIN was used to calculate the maximum-likelihood estimates of sharing 0, 1, and 2 alleles IBD via the expectation-maximization algorithm. All possible ASPs for each family were used and weighted equally. The bootstrap variance estimation procedure (described below) automatically adjusts for correlation between sib pairs among families. Using a custom-written program, we estimated the IBD proportions (treating each sibling pair as independent) from MERLIN's IBD output by allowing the maximum-likelihood IBD estimates to converge *without* triangle constraints (Holmans 1993). We chose to use the unconstrained estimates, since imposing the triangle constraints in the presence of heterogeneity may be less powerful (Dizier et al. 2000). In addition, combination of estimates over studies is more meaningful with the unconstrained estimates. Note that our estimation procedure is identical to that of GENEHUNTER (Kruglyak et al. 1996), with the exception of the triangle-constraint restriction. Following a more traditional meta-analytic approach, we used estimates of mean IBD (IBD_m) sharing and its variance to quantify heterogeneity among the 11 data sets at selected regions along the genome. Using the unconstrained estimates of sharing 0, 1, or 2 alleles IBD (ibd_0, ibd_1, ibd_2) for ASPs, we calculated the IBD_m ($0.5 \times ibd_1 + ibd_2$) separately for each study. Note that use of the unconstrained probabilities allows the estimated IBD_m for a study to be <0.5, whereas use of the constraints forces IBD_m to be at least 0.5. To derive the variance of the IBD_m, we used a bootstrap procedure developed for estimating the variance of IBD_m sharing among ASPs in linkage analysis samples (M.B.M. and N.M.L., unpublished data). The Q-statistic was utilized to provide a formal test of heterogeneity (Laird and Mosteller 1990). Then, continuing along the traditional meta-analytic path, we pooled the study-specific estimates of IBD_m, using a random-effects model (Laird and Mosteller 1990) that allows for the incorporation of between-study heterogeneity and therefore a more realistic summary measure of IBD_m, as well as more accurate CIs. Gu et al. (1998) provide a detailed description of this general approach in the context of ASP linkage samples.

Results

LOD-Score Results

Overall, the average information content across all chromosomes of the pooled analysis was ~0.58 (range 0.49–0.63), which is consistent with genomewide scans that used ~10-cM marker density. Only one individual genome scan yielded a LOD score >3; eight other LOD scores >2 were found in individual scans. Some of these will be discussed later, in the context of the pooled results; additional results are available on request. The number of families informative for narrow and broad BP per data set is listed in table 1.

Table 2 displays the results for the pooled analysis. The largest Kong and Cox (1997) LOD score observed was with use of the narrow phenotype, which achieved genomewide significance (LOD > 3.03) on chromosome 6, at 115 cM (LOD 4.19). With the use of this same phenotype definition, no other chromosome achieved genomewide significance, although we observed genomewide suggestive linkage (LOD > 1.75) signals on chromosomes 9 (LOD 2.04), 8 (LOD 1.99), and 20 (LOD 1.91). Table 2 also shows the corresponding results from analysis of the broad phenotype. The LOD score found on chromosome 8 at 151 cM (LOD 3.40) obtained using this phenotype definition exceeds the genomewide significance threshold. Chromosome 9 revealed genomewide suggestive thresholds (LOD 2.04) in the location identical or near to that found with the narrow BP phenotype analysis. Note that the LOD score for chromosome 6 (LOD 1.74) was substantially lower for broad BP compared with narrow BP. In summary, two regions on chromosomes 6 (narrow BP) and 8 (broad BP) achieved genomewide significance. These two regions were selected for more-detailed analysis and presentation.

Figures 1*A* and 1*B* display the LOD scores from chro-

Table 2

Results from the Pooled Analysis

	Narrow BP			Broad BP		
Chromosome	Genetic Location[a] (cM)	Physical Location[b] (Mb)	LOD	Genetic Location[a] (cM)	Physical Location[b] (Mb)	LOD
1	200	185.0	.41	79	44.9	.59
2	92	68.0	.97	92	68.0	1.10
3	1	.6	.19	69	44.5	.14
4	152	154.0	.39	154	154.5	.56
5	79	67.0	.31	78	66.0	.11
6	115	108.5	4.19[c]	115	108.5	1.74
7	187	157.1	.57	187	157.1	.70
8	152	135.4	1.99[d]	151	134.5	3.40[c]
9	46	24.5	2.04[d]	48	25.6	2.06[d]
10	85	70.2	.07	50	25.8	.20
11	72	60.0	.54	72	60.0	.57
12	155	126.5	.40	155	126.5	.13
13	44	42.4	.62	50	46.4	.46
14	79	86.5	.54	79	86.5	.19
15	21	29.4	.95	25	31.2	.73
16	30	12.1	.18	35	13.4	.85
17	98	64.3	1.36	98	64.3	.91
18	70	44.9	1.47	87	58.5	1.05
19	73	51.5	.33	37	14.6	.13
20	12	4.2	1.91[d]	12	4.2	1.71
21	60	43.0	.06	48	39.2	.03
22	2	15.0	.12	9	16.0	.03

[a] Genetic location from the unified marker map.
[b] Physical location (approximate) from the Rutgers Combined Linkage-Physical Map.
[c] Genomewide significant (LOD > 3.03).
[d] Genomewide suggestive (LOD > 1.75).

mosomes 6 (narrow BP) and 8 (broad BP), respectively. In particular, we plotted the LOD score from the pooled data set superimposed on the plot of the LOD scores from each of the 11 component data sets (figs. 1*A* and 1*B*). For chromosome 6, the NIMH Wave 3 data set is the only data set that achieves a LOD score of >2.0, despite the fact that the pooled signal surpassed a LOD score of 4.0 (fig. 1*A*). There were three signals in this region that appear to achieve the highest LOD scores under the pooled signal (NIMH Wave 3, Portuguese, and Columbia). For chromosome 8, we found that no individual data set reached a LOD score of 2.0, despite the fact that the pooled signal exceeded 3.0 (fig. 1*B*). There were also clear overlapping signals from component data sets (e.g., Johns Hopkins 1 and NIMH Wave 3). Removal of the nonwhite families (and re-estimation of allele frequencies) from the NIMH samples ($n = 38$) did not impact the pooled linkage signal for either chromosome 6 or 8.

Assessment of Heterogeneity Results

In the region that encompasses the significant linkage signals on chromosome 6 and 8, there was no evidence of heterogeneity among data sets as tested via the *Q*-statistic ($P > .10$; results not shown). Using the location from each chromosome that reveals the peak LOD score, we provide a forest plot of the IBD_m and 95% CIs for each of the component data sets, as well as the summary IBD_m estimate and 95% CI (figs. 2*A* and 2*B*). We selected the 115-cM position for chromosome 6 (fig. 2*A*). Here, we see that only two data sets (NIMH Wave 3 and Portuguese) display significant excess allele sharing, whereas all others have 95% CIs that include 0.50. As seen in figure 2*A*, the combined estimate of IBD_m, which is the random-effects summary estimate that uses each component data set's IBD_m estimate and variance, suggests an excess in allele sharing at the position for the studies combined ($IBD_m = 0.55$; 95% CI 0.51–0.59). Figure 2*B* displays chromosome 8 at the 151-cM position. Here, two data sets display significant excess sharing (NIMH Wave 3 and Johns Hopkins 1) and another (Columbia) displays borderline excess sharing. The summary estimate of IBD_m also demonstrates excess sharing at this position, despite nine of the component data sets' 95% CIs including 0.50 ($IBD_m = 0.54$; 95% CI 0.52–0.56). Note that the summary IBD_m CIs are not adjusted for genomewide comparison.

Although global tests for heterogeneity are often un-

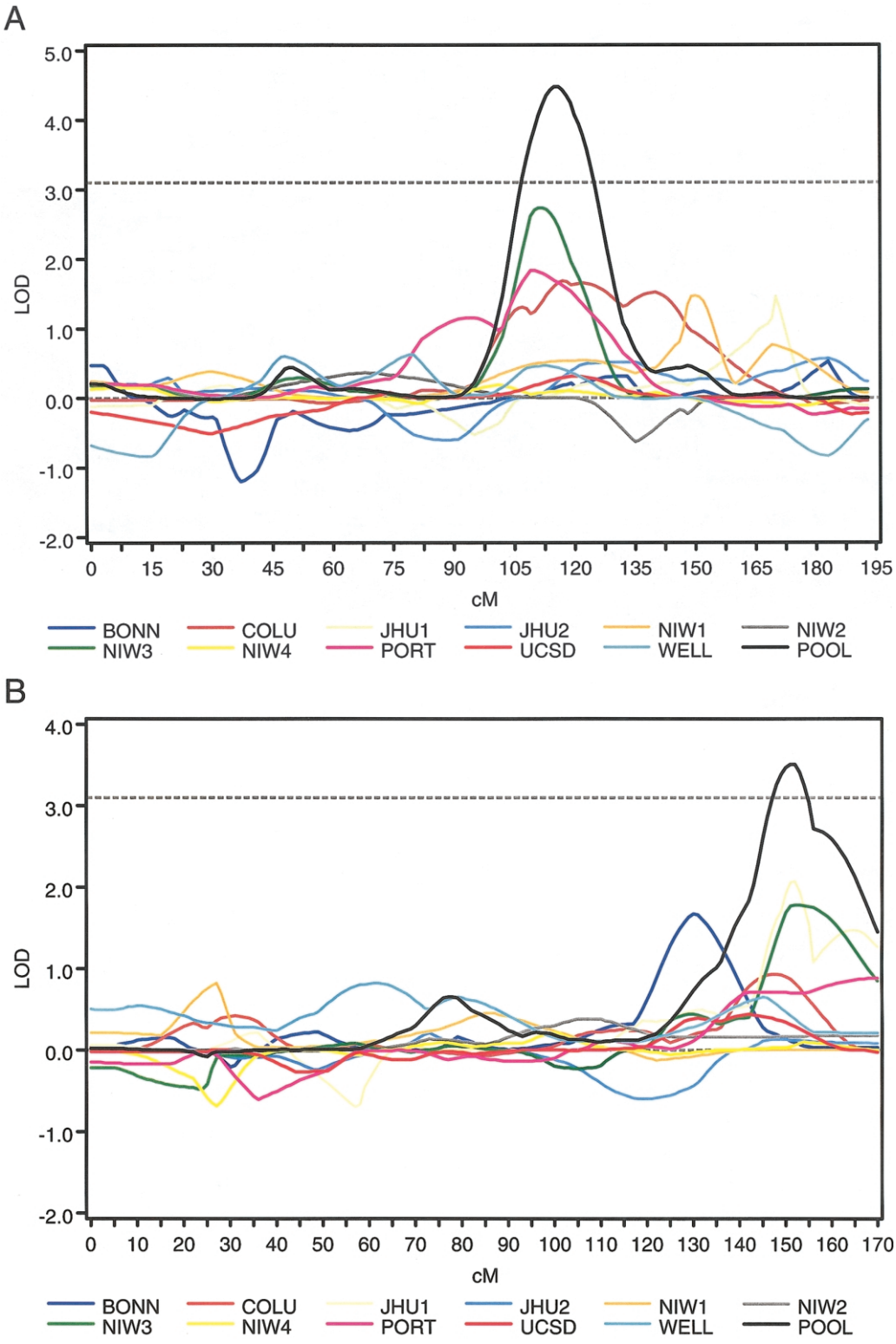

Figure 1 Relative contribution of component data sets to the pooled linkage signals. The LOD scores from the pooled analysis (*solid black line*) are overlaid with the LOD scores from the data set–specific analysis (the horizontal dotted line indicates the genomewide significance threshold). *A*, Narrow BP phenotype, chromosome 6. *B*, Broad BP phenotype, chromosome 8. BONN = Bonn; COLU = Columbia; JHU1 = Johns Hopkins 1; JHU2 = Johns Hopkins 2; NIW1 = NIMH Wave 1; NIW2 = NIMH Wave 2; NIW3 = NIMH Wave 3; NIW4 = NIMH Wave 4; PORT = Portuguese; UCSD = UCSD; WELL = Wellcome Trust; POOL = pooled sample.

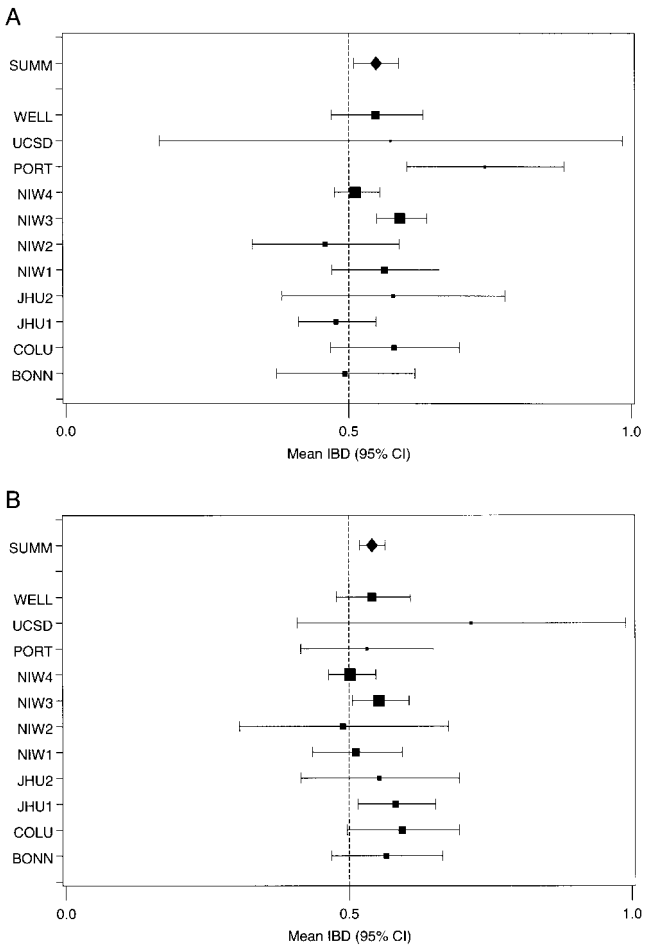

Figure 2 IBD_m and 95% CIs for the component data sets, as well as the random-effects summary point estimate. *A,* Narrow BP phenotype, chromosome 6 at 115 cM. *B,* Broad BP phenotype, chromosome 8 at 151 cM. BONN = Bonn; COLU = Columbia; JHU1 = Johns Hopkins 1; JHU2 = Johns Hopkins 2; NIW1 = NIMH Wave 1; NIW2 = NIMH Wave 2; NIW3 = NIMH Wave 3; NIW4 = NIMH Wave 4; PORT = Portuguese; UCSD = UCSD; WELL = Wellcome Trust; SUMM = random-effects summary IBD_m.

derpowered, the IBD_m procedure was able to detect statistically significant among-study heterogeneity at regions throughout the genome, including regions outside the linkage signals on chromosomes 6 and 8. In particular, the 6pter region and the 35–45-cM region on chromosome 6 have substantial heterogeneity (Q-statistic $P < .001$) as does the 40–50-cM region of chromosome 8 (Q-statistic $P < .001$).

There is mounting evidence that unselected sibling pairs tend to share more than half of their alleles IBD (Elston et al. 2005). To provide reassurance that the observed allele sharing among ASPs in the present study is attributable to affection status, we evaluated discordant sibling pairs as well. Estimation of IBD_m in unaffected siblings (excluding all psychiatric diagnoses, if available) paired with affected siblings resulted in either null sharing or slightly less than null sharing (not significant) at the locations of the reported linkage signals for each of the data sets that provided adequate diagnostic information (data not shown).

Discussion

To our knowledge, this is the largest and most comprehensive collaboration involving the original genotype data for BP linkage analysis. We identified loci on chromosomes 6q and 8q as meeting genomewide significance and loci on 9p and 20p as meeting suggestive linkage. Our results show that the extra expense and effort required to obtain original genotype data from genome scans has substantial benefits. Our observations from pooling data for the present analysis are consistent with an increase in power, since linkage signals from the pooled sample were generally higher than the linkage signal from any one component data set. Utilizing the more traditional meta-analytic approach, we constructed a summary measure of IBD_m to assess the among-study heterogeneity.

Heterogeneity

In general, replication of linkage signals from complex diseases such as BP is likely to be complicated by known sources of heterogeneity among linkage studies. In particular, sources of heterogeneity include different populations under investigation, varied number of affected individuals (with varying diagnoses) per family, varied family size, different ascertainment criteria, varied instruments of assessment and diagnostic criteria, different sets of marker genotypes and corresponding marker maps, as well as dissimilar analytic methods. We restricted our analyses to uniform disease classifications and the use of common methods, including a standardized common marker map. Both within- and between-study heterogeneity likely remains, since subjects were ascertained, were assessed, and received a diagnosis with considerable variation. The presence of genetic heterogeneity may also limit the utility of pooling original data geared toward the identification of candidate loci. Despite these issues, the regions of significant or suggestive linkage lacked evidence of substantial heterogeneity. However, tests of heterogeneity are often underpowered, and acceptance does not preclude heterogeneity in these regions. On the other hand, substantial heterogeneity would likely limit the extent to which signals could be detected; such regions may be worthy of further study.

Phenotype Definition

Our results provide some support for the view that genetic influences on BPI and BPII may be distinguish-

able. Our most significant result was seen for narrow BP (BPI-only phenotype) on chromosome 6q. When the analysis was expanded to include BPII, the linkage signal on chromosome 6q was attenuated, despite the increase in the number of ARPs. In contrast, removal of the individuals with BPII from the analysis reduced the evidence of linkage on chromosome 8q. It has been argued that the diagnostic reliability of BPII may be less robust than that of BPI, although this may not be true when careful diagnostic procedures are applied (Simpson et al. 2002). Genetic epidemiologic data suggest that BPII is probably a genetically heterogeneous entity in which some cases are genetically distinct from BPI, whereas others are part of a spectrum that includes BPI and/or unipolar depression (Smoller and Finn 2003).

It could be argued from our results that a locus on chromosome 6q is linked specifically to BPI, so that the addition of subjects with BPII resulted in "phenotypic noise," effectively pulling the estimates of allele sharing toward the null. On the other hand, inclusion of subjects with BPII enhanced the linkage signal on chromosome 8q, suggesting that a locus in this region may influence a broader bipolar spectrum phenotype. It should be noted that, because we did not analyze a BPII-only phenotype, we cannot address the question of whether the 8q locus or loci elsewhere in the genome have more-specific effects on BPII itself.

Prior Meta-Analyses in Context

Table 3 provides an overview of the variations of different data sets included in each of the two prior meta-analyses as well as the present combined analysis. Overall, the MSP meta-analysis included 8 data sets (Badner and Gershon 2002), the GSMA (Segurado et al. 2003) included 18 data sets, and the present combined analysis included 11 data sets (table 3). Only four data sets (Bonn, NIMH Wave 1, UCSD, and Wellcome Trust) were common across all three meta-analyses. There were additional data sets that were common to one or more analyses; however, different variations of the data were often included. For example, the Johns Hopkins 1 data set was included in all three analyses; however, only the GSMA and the present combined analysis used the full sample (65 pedigrees). Much of the variability in the data sets—as well as data-set versions used for each meta-analysis—is largely explained through what was available at the time of each of the meta-analyses. The Johns Hopkins 2; NIMH Waves 2, 3, and 4; and Portuguese data sets became available after the MSP and GSMA analyses. Therefore, as is true in any meta-analysis context, updating analyses to include new information as it becomes available is essential to the confirmation and identification of genomic regions that may harbor disease-susceptibility loci.

Chromosomes 6 and 8

The strongest support derived from this analysis under the narrow BP phenotype definition is for chromosome 6q and under the broad BP phenotype definition, chromosome 8q. Because of practical considerations, an exhaustive review of the BP linkage analysis literature will not be the focus of this discussion (for more detailed review, see Baron [2002]). Rather, a brief discussion of each region follows.

Linkage signals of varying degrees on chromosome 6 have been reported from data sets used in the present analysis as well as other data sets not included here. Figure 1*A* provides a graphical representation of the relative contribution from each of the component data sets to the signal on 6q. The NIMH Wave 3, Portuguese, and Columbia data sets appear to be the most influential. From the estimated IBD_m, all studies in this analysis—with the exception of Bonn, Johns Hopkins 1, and NIMH Wave 2—showed excess allele sharing in ASPs at the pooled linkage peak (fig. 2*A*). The NIMH Human Genetics Initiative also noted linkage at this region (at or near marker *D6S1021*) through a linkage analysis incorporating an interaction effect with 6p (Schulze et al. 2004). The study of the Portuguese sample reported a linkage signal to chromosome 6q22 in a follow-up marker scan with use of high-density genotyping (Middleton et al. 2004). In addition, the Wellcome Trust study reported a signal on 6q through a follow-up analysis that included larger sample size and additional marker genotypes (Lambert et al. 2005). Further, in a Danish study that was not a part of the present meta-analysis, Ewald et al. (2002) reported a maximum LOD score of 3.8 for the marker (*D6S1021*) closest to the maximum LOD score in our pooled sample analysis. Another study that used families from northern Sweden also reported a signal from parametric linkage analysis of chromosome 6q; however, that signal appears nearer to the smaller pooled linkage signal observed in the present meta-analysis (Venken et al. 2005). Moreover, although not statistically significant, Segurado et al. (2003) reported bin 6.4 as meeting a P_{AveRnk} of $<.10$ for their model 1 (BPI or schizoaffective disorder-bipolar type [SAB]). Badner and Gershon (2002) did not report linkage to BP on 6q.

The pooled signal from chromosome 8 appears to be driven by the Bonn, Johns Hopkins 1, and NIMH Wave 3 data (fig. 1*B*). All studies in our analysis, aside from NIMH Waves 2 and 4, show excess allele sharing among ASPs at the linkage peak (fig. 2*B*). In addition, a genome-wide scan of psychosis in the Columbia data set provided evidence of linkage to 8q24 (Park et al. 2004). Aside from previous reports from data sets included in this collaboration, we are unaware of any linkage signals that have been reported for 8q from studies outside the collaboration. Segurado et al. (2003) reported that bin 8.6

Table 3

Data Sets Included in the Three Recent Meta-Analyses of BP Linkage Scans

Study	Comment	Meta-Analysis[a]		
		MSP	GSMA	Combined
Antwerp 1 (Segurado et al. 2003)	Unpublished data		Antwerp 1	
Antwerp 2 (Segurado et al. 2003)	Unpublished data		Antwerp 2	
Bonn (Cichon et al. 2001)		Cichon	Bonn	Bonn
Columbia (Liu et al. 2003)			Columbia	Columbia
Costa Rica (McInnes et al. 1996)	Two-point analysis		Costa Rica	
Costa Rica (Garner et al. 2001)	Markov chain Monte Carlo analysis	Garner		
Edinburgh (Blackwood et al. 1996)	1 pedigree	Blackwood		
Edinburgh (Blackwood et al. 1996; Segurado et al. 2003)	7 pedigrees (includes unpublished data)		Edinburgh	
Finland (Ekholm et al. 2003)			Finland	
Johns Hopkins 1 (Friddle et al. 2000)	50 pedigrees (initial sample)	Friddle		
Johns Hopkins 1 (McInnis et al. 2003*b*)	65 pedigrees (expanded sample)		Hopkins/Dana	Johns Hopkins 1
Johns Hopkins 2 (Fallin et al. 2004)	Ashkenazi population			Johns Hopkins 2
NIMH Intramural (Detera-Wadleigh et al. 1999)	Includes 1 Old Order Amish pedigree	Detera-Wadleigh	NIMH-IM	
NIMH Wave 1 (NIMH Genetics Initiative Bipolar Group 1997)		NIMH	NIMH	NIMH Wave 1
NIMH Wave 2 (Dick et al. 2002; McInnis et al. 2003*a*; Willour et al. 2003; Zandi et al. 2003)				NIMH Wave 2
NIMH Wave 3 (Dick et al. 2003; NIMH Human Genetics Initiative Web site)				NIMH Wave 3
NIMH Wave 4 (NIMH Human Genetics Initiative Web site)				NIMH Wave 4
Ottawa (Turecki et al. 2001)			Ottawa	
Portugal (Pato et al. 2004)	16 pedigrees (microsatellite scan)			Portuguese
Quebec (Morissette et al. 1999)		Morissette	Quebec	
Sydney (Badenhop et al. 2002)	13 pedigrees		Sydney 1	
Sydney (Badenhop et al. 2002; Segurado et al. 2003)	15 pedigrees (includes unpublished data)		Sydney 2	
Turkey (Radhakrishna et al. 2001)		Radhakrishna		
University College (UC) London (Curtis et al. 2003)			UC London	
UCSD (Kelsoe et al. 2001)		Kelsoe	UCSD	UCSD
Utah (Coon et al. 1993)		Coon	Utah	
Wellcome Trust (Bennett et al. 2002)		Bennett	U.K./Irish	Wellcome Trust

[a] When a study was included in a meta-analysis, the variable entry for that meta-analysis is listed as it was referenced in each meta-analysis.

failed to reach genomewide significance; however, they report that 8q might contain a locus that is "weakly linked" to BP (P_{AveRnk} <.05) for their model 2 (BPI, BPII, and SAB). Badner and Gershon (2002) did not report linkage to BP on 8q.

Conclusions

The present analysis is the first comprehensive collaboration of BP linkage studies to use original genotype data that is aimed at identifying potential candidate regions for future investigation. Using the pooled data, we demonstrated genomewide significant linkage to chromosomes 6q and 8q and genomewide suggestive linkage to chromosomes 9p and 20p. The finding on chromosome 6q, which is based on the BPI phenotype, is supported by some of the individual studies analyzed herein, as well as by other studies that we could not include in our analysis. When subjects with BPII are included in the analysis, the results for chromosome 6q are diminished, and a locus on 8q becomes significant. Evidence for the 8q locus appears limited to studies included in this analysis. Effect size estimates from the ASP analysis suggest relative risks (λ_s) of 1.34 and 1.26 attributable to loci on chromosomes 6q and 8q, respectively. Thus, the majority of the genetic constituents underlying BP remain unidentified, which suggests that their discovery may require more-elaborate and -focused investigations of bipolar-spectrum phenotypes, as well as more-refined methods for gene mapping.

We have demonstrated that combining data from disparate genome scans provides an effective mechanism for summarizing quantitative information and that lack of consistent findings in individual scans does not preclude finding a significant region in the combined data. There are particular strengths for use of the original data, in that it allows control of many potential sources of variability in the original studies. Fortunately, original genome-scan data are becoming increasingly available, such as those from the NIMH Human Genetics Initiative, and our results suggest these efforts should be supported. Our approach offers a powerful methodology for the identification of linkage regions underlying complex diseases, such as BP, for which there are likely multiple disease-susceptibility loci.

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Web Resources

The URLs for data presented herein are as follows:

NIMH Human Genetics Initiative, http://zork.wustl.edu/nimh/NIMH_initiative/NIMH_initiative_link.html

Rutgers Combined Linkage–Physical Map of the Human Genome, http://compgen.rutgers.edu/maps/

UCSC Genome Bioinformatics, http://genome.ucsc.edu/

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