Am. J. Hum. Genet. 77:1102–1111, 2005

Report

Genomewide Scan and Fine-Mapping Linkage Studies in Four European Samples with Bipolar Affective Disorder Suggest a New Susceptibility Locus on Chromosome 1p35-p36 and Provides Further Evidence of Loci on Chromosome 4q31 and 6q24

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We present the findings of a large linkage study of bipolar affective disorder (BPAD) that involved genomewide analysis of 52 families (448 genotyped individuals) of Spanish, Romany, and Bulgarian descent and further fine mapping of the 1p34-p36, 4q28-q31, and 6q15-q24 regions. An additional sample of 56 German families (280 individuals) was included for this fine-mapping step. The highest nonparametric linkage scores obtained in the fine mapping were 5.49 for 4q31 and 4.87 for 6q24 in the Romany families and 3.97 for 1p35-p36 in the Spanish sample. MOD-score (LOD scores maximized over genetic model parameters) analysis provided significant evidence of linkage to 4q31 and at least borderline significance for the 1p and 6q regions. On the basis of these results and previous positive research findings, 4q31 and 6q24 should now be considered confirmed BPAD susceptibility loci, and 1p35-p36 is proposed as a new putative locus that requires confirmation in replication studies.

Received June 6, 2005; accepted for publication September 23, 2005; electronically published November 2, 2005.

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0002-9297/2005/7706-0020$15.00

Bipolar affective disorder (BPAD [MIM 125480]) is a major psychiatric disorder characterized by episodes of mania and depression. It affects 0.5%–1.5% of the world population and constitutes a major public health problem, with significant morbidity and mortality (World Health Organization 2002). Family, twin, and adoption studies have provided strong evidence of a major genetic contribution to the etiology of BPAD (Craddock and Jones 1999). The pattern of inheritance is complex, sug-

Table 1

Overview of the Samples Studied in the Genomewide Scan and Fine-Mapping Linkage Analysis

Sample	No. of Families	No. of Family Members	No. of Affected/Mean No. Affected per Family		
			ASDI	ASDII	ASDIII
Spanish	42[a]	357	103/2.4	144/3.4	209/5
Bulgarian	7[a]	32	14/2	15/2.1	16/2.3
Romany	3[a]	59	13/4.3	17/5.7	35/11.7
German	56[b]	280	92/1.6	101/1.8	117/2.1
All:					
Genomewide scan	52	448	130/2.5	176/3.4	260/5
Fine-mapping linkage analysis	108	728	222/2	277/2.6	377/3.5

Note.—ASDI includes BPI; ASDII includes BPI, BPII, or SA/BP; ASDIII includes BPI, BPII, SA/BP, or UPR.

[a] Determined by genomewide scan and fine-mapping linkage analysis.

[b] Determined by fine-mapping linkage analysis.

gesting the involvement of both multiple genes and environmental factors. Although linkage analysis of BPAD has been an unsurprisingly challenging endeavor (Schulze and McMahon 2003), some genomic regions have gained consistent support from different studies and are therefore likely to contain BPAD susceptibility loci (Badner and Gershon 2002; Segurado et al. 2003).

Here, we present new data from a genomewide linkage analysis and subsequent fine-mapping analysis conducted in one of the largest collections of BPAD-affected families to date. The genome scan was performed on 52 families (Spanish, Bulgarian, and Romany, the latter recruited in Bulgaria) consisting of 448 subjects, of whom 260 were affected (according to the broad phenotype definition, ASDIII [described below]). An additional sample of 56 German families with 280 members (117 affected) for which a previously completed genome scan produced overlapping results (Cichon et al. 2001) was included for the fine-mapping stage. The characteristics of these families are summarized in table 1. Informed consent was obtained from all participating subjects. The study complies with all ethical guidelines of the institutions involved.

Phenotype evaluation (Fangerau et al. 2004) was based on DSM-IV criteria (American Psychiatric Association 1994). The inclusion criteria for BPAD-affected families were the presence of (1) a proband with bipolar I disorder (BPI) and (2) a secondary affected sibling with either BPI, bipolar II (BPII), schizoaffective disorder bipolar type (SA/BP), or unipolar recurrent depression (UPR). All individuals were interviewed by an experienced psychiatrist. The Schedule for Affective Disorders and Schizophrenia–Lifetime Version (SADS-L) (Endicott and Spitzer 1978) was used for the German and Spanish families. The Schedules for Clinical Assessment in Neuropsychiatry (Wing et al. 1990) was used for the Bulgarian and Romany families. The affected status definitions (ASD) used in the linkage analysis were as follows: ASDI (narrow) includes individuals with BPI only, with all other psychiatric diagnoses coded as "unknown"; ASDII (medium) includes all individuals who received a diagnosis of BPI, BPII, or SA/BP; and ASDIII (broad) also includes individuals with UPR.

The genome scan was conducted at the Gene Mapping Center in Berlin, with use of procedures described by Lee et al. (2000). The 448 DNA samples were genotyped for 435 STRs, with an average intermarker distance of 8.3 cM (range 0–25.4 cM) (deCODE Genetics). An average heterozygosity of 0.77 was observed.

Nonparametric linkage (NPL) analysis of the genome scan data was performed using the current version of GENEHUNTER, version 2.1 release 5 (Kruglyak et al. 1996; Kruglyak and Lander 1998; Markianos et al. 2001). Marker-allele frequencies were estimated for all samples by a maximum-likelihood procedure with use of the program MENDEL (version 5.5). Identical-by-descent allele sharing among all affected family members was calculated for the narrow, medium, and broad affection–status definition with use of the GENEHUNTER score function S_{ALL}.

The genomewide linkage analysis (see detailed results in table 2 and figs. 1–4) produced nominal evidence of linkage ($P < .05$) to 17 chromosomal regions: 1p34-p36, 2p16-p25, 3p25, 4p16, 4q25-q31, 4q35, 6q21-q24, 10p13, 11p15, 11p13-q13, 12p11-p13, 13q12-q13, 15q14, 15q26, 17p12, 19p12-p13, and Xq23-q25. Strongest support was obtained for the 1p34-p36, 4q25-q31, and 6q21-q24 regions.

Table 2

Genomewide Scan: Multipoint NPL Analysis (Nominal $P < .05$) of the Entire Linkage Sample and Each Subsample

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics.*

Figure 1 Genomewide scan: multipoint NPL analysis of ASDI, ASDII, and ASDIII, across all chromosomes in the entire linkage sample.

On chromosome 1p34-p36, the maximum NPL score for the combined sample was 3.34 at 38.9 cM under the broad phenotype (ASDIII) definition. Nominally significant results were obtained in the Spanish (ASDIII: NPL = 3.36 at 37.78 cM) and the Romany (ASDI: NPL = 2.28 at 27.6 cM) samples. For 4q25-q31, for the entire sample, an NPL score of 2.89 was found at 138.7 cM for ASDII. Nominally significant results were obtained for the Spanish (ASDII: NPL = 2.75 at 136.5 cM) and the Romany (ASDII: NPL = 1.94 at 150.8 cM) families. In the 6q21-q24 region, the maximum NPL score for the combined sample was 2.66 at 134.2 cM for ASDIII. The breakdown showed nominally significant results in all subsamples: Romany (ASDIII: NPL = 3.65 at 145.0 cM), Spanish (ASDII: NPL = 2.25 at 125.4 cM), and Bulgarian (ASDIII: NPL = 1.89 at 134.2 cM).

Fine-mapping analysis of these three regions was performed for those family subsets demonstrating the strongest evidence of linkage: Spanish, Romany, and Bulgarian for chromosome 1p; Romany, Bulgarian, and German (Cichon et al. 2001) for chromosome 4q; and Spanish, Romany, Bulgarian, and German for chromosome 6q. The genotyping was performed by deCODE Genetics, with use of procedures described elsewhere (Björnsson et al. 2003). A total of 91 additional STR markers were analyzed, distributed by regions as follows: chromosome 1p34-p36, interval *D1S2672–D1S2741* (27.8–86.1 cM), 27 STRs, average intermarker distance 2.1 cM; chromosome 4q28-q31, interval *D4S1615–D4S2982* (129.7 cM–157.5 cM), 13 STRs, average intermarker distance 2.1 cM; chromosome 6q15-q24, interval *D6S1570–D6S1633* (101.5–170.2 cM), 51 STRs, average intermarker distance 1.3 cM. To determine the inferential validity of the maximum NPL scores that were obtained using the fine-mapping data and the program GENEHUNTER, we performed systematic simulations under the null hypothesis of no linkage. Because of insufficient computation time, we generated either 500 or 1,000 replicates, depending on the magnitude of the observed NPL score. The replicates were generated using MERLIN, version 0.10.2 (Abecasis et al. 2002). Each replicate was generated under the assumption of random segregation, with use of identical pedigree structure, affection status, marker spacing, allele frequencies, and patterns of the missing data in the real data set. Each replicate was analyzed, using MERLIN, in the same way as was the original data set, with computation of the Z_{mean} score, which is equivalent to the NPL score in GENEHUNTER. The empirical *P* value was calculated as the portion of all replicates showing an NPL score equal to or higher than the one observed in the real data set.

In parametric linkage analysis, the trait model must be specified prior to analysis. This is a disadvantage when the true disease model parameters are unknown, since the power to detect linkage decreases when the specified model is not sufficiently close to the true mode of inheritance (Clerget-Darpoux et al. 1986). Therefore, in addition to the NPL analyses, we performed multipoint LOD scores maximized over genetic model parameters (MOD)–score analyses in the fine-mapping linkage study, and we maximized parametric LOD scores with respect to the disease-model parameters. We used the new program GENEHUNTER-MODSCORE (Strauch 2003), which calculates MOD scores by automatically varying the disease-allele frequency and three penetrances. The program is a further development of GENEHUNTER-IMPRINTING (Strauch et al. 2000), which is based on the original GENEHUNTER, version 2.1 (Kruglyak et al. 1996; Kruglyak and Lander 1998; Markianos et al. 2001). We used the "modcalc single" option, under which GENEHUNTER-MODSCORE performs a separate maximization for each genetic position assumed for the putative disease locus. This procedure yields the MOD score in conjunction with the best-fitting penetrance and disease-allele frequency at each genetic position of a prespecified grid. The parameters can be considered an effect estimate for the particular locus. Given the nature of our large data set and its many extended pedigrees, a further reduction in the number of chromosomal regions to be analyzed was necessary for reasons of acceptable computation time. The intervals analyzed in this manner were *D1S2672–D1S1598* (27.82–65.0 cM) on 1p; *D4S1527–D4S2982* (134.67–157.54 cM) on 4q, and *D6S270–D6S1633* (138.78–170.24 cM) on chromosome 6q. For all regions and disease definitions, the analyses were run for the pooled set of populations.

Detailed results of fine mapping for both the combined sample and for the subsamples (Spanish, Romany, Bulgarian, and German) are presented in table 3.

On chromosome 1p34-p36, we genotyped 27 additional STR markers, which covered a region of 58.3 cM. In the combined sample, an NPL score of 3.69 (empirical $P = .003$) was obtained at 45.9 cM for ASDIII. The best

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Figure 2 Genomewide scan: multipoint NPL analysis of ASDI, ASDII, and ASDIII, across all chromosomes in the Spanish linkage sample.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics.*

Figure 3 Genomewide scan: multipoint NPL analysis of ASDI, ASDII, and ASDIII, across all chromosomes in the Romany linkage sample.

subsample score was observed in the Spanish families (ASDIII: NPL = 3.97 at 45.9 cM; empirical $P = .005$) (see fig. 5). The highest MOD score value, 3.40, was obtained in the combined sample at 45.9 cM for ASDIII and a near-dominant mode of inheritance (fig. 6). The data suggest that the susceptibility allele at this locus has a high frequency (16%) and incomplete penetrance (53%). The results for ASDI and ASDII were weaker, with MOD scores of 2.02 and of 1.36, respectively (fig. 6). The linkage region defined by the NPL and MOD-score analyses is well circumscribed and covers <5 cM between markers *D1S478* and *D1S493* on chromosome 1p35-p36. This region has not been proposed previously as a BPAD locus. However, review of published data shows that Curtis et al. (2003) observed a LOD score of 3.10 at *D1S432* in one large pedigree with BPAD. This marker is not, however, listed by the deCODE Genetics map. According to National Center for Biotechnology Information (NCBI) Build 35, *D1S432* is located <9 Mb centromeric of the linkage region implicated by our study. In addition, ~23 cM more telomeric, at marker *D1S1597* (23.2 cM), Zubenko et al. (2003) obtained a multipoint LOD score of 3.60 in a sample of 81 families with UPR. The fact that the most significant *P* value in our study was obtained with use of a phenotype definition that includes UPR may suggest the existence of a susceptibility locus on chromosome 1p35-p36 that contributes to a broad spectrum of affective disorders. According to the University of California–Santa Cruz (UCSC) RefSeq Genes track (UCSC Genome Bioinformatics), which assembles all known protein-coding genes taken from the NCBI mRNA reference sequences collection (RefSeq), the genomic interval on chromosome 1p identified by our fine-mapping analysis contains 129 genes.

On chromosome 4q28-q31, we analyzed 13 additional STR markers between *D4S1615* (129.7 cM) and *D4S2982* (157.5 cM). In the entire fine-mapping sample, we obtained evidence of linkage at 151.1 cM, with an NPL score of 3.18 (empirical $P = .004$) for ASDII (table 3 and fig. 7). The strongest evidence was produced by the Romany subsample (ASDIII: NPL = 5.49 at 148.4 cM; empirical $P = .015$). Also, the German subsample contributed to the chromosome 4 finding (ASDII: NPL = 2.58 at 151.1 cM; empirical $P = .012$). In the MOD-score analysis of the combined sample—including the Romany, German, and Bulgarian families—a MOD score of 4.24 was obtained at 147.2 cM for ASDII, with a disease-allele frequency of 4.5% and a near-additive mode of inheritance (fig. 8). Application of the two other phenotype definitions supported this region, with a MOD score of 3.22 at 147.6 cM for ASDI and of 2.76 for ASDIII (fig. 8). Our findings confirm the findings of genomewide analyses of other investigators. Using a dominant model of inheritance and a broad diagnostic definition, Ekholm et al. (2003) observed a 3-point LOD score of 3.60 at 152 cM (at marker *D4S1629*) in a sample of 41 BPAD-affected families from an isolated Finnish population. In another genomewide scan of 65 American families of European descent with BPAD, an NPL of 2.80 and a LOD score of 1.9 were reported, again at 152 cM (marker *D4S1629*) under a broad phenotype model (McInnis et al. 2003). A genomewide study of 40 pedigrees with BPAD from the United States and Israel obtained a 2-point LOD score of 3.16 under a dominant model and broad phenotype definition, with a maximum at 140 cM (marker *D4S1625*) (Liu et al. 2003). The peak identified in our fine-mapping NPL and MOD-score analysis spans ~10 cM and is situated precisely in the *D4S1625–D4S1629* interval supported by the above-mentioned genomewide analyses. The UCSC RefSeq Genes track (UCSC Genome Bioinformatics) lists 58 protein-coding genes as present in this genomic linkage interval on chromosome 4q.

Fine mapping of the 6q15-q24 region, flanked by *D6S1570* (101.5 cM) and *D6S1633* (170.2 cM), was performed with 51 additional STRs. This large region is of particular interest, since several previous studies have reported "suggestive" and "significant" evidence of linkage to BPAD. Rather than narrowing the region, the fine-mapping analysis led to the identification of three well-defined positive areas in each subsample, all of which have been reported elsewhere for BPAD—namely, 6q16, 6q23, and 6q24 (see fig. 9).

Evidence supporting 6q16 was obtained mainly from the German subsample, with an NPL score of 2.55 (empirical $P = .052$) at 112.0 cM for ASDII (table 3). Although, our results for 6q16 are not strong in terms of empirical *P* values, involvement of this region is supported by findings from independent linkage studies. The large National Institute of Mental Health study, which included a sample of 250 families, reported a LOD score

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Figure 4 Genomewide scan: multipoint NPL analysis of ASDI, ASDII, and ASDIII, across all chromosomes in the Bulgarian linkage sample.

of 2.26 at *D6S1021* (107 cM), with use of a broad disease definition (Dick et al. 2003; Schulze et al. 2004). At the same marker, Ewald et al. (2002) found a LOD score of 2.59 in two multiplex BPAD-affected families from Denmark with a narrow phenotype definition, and Pato et al. (2004) observed an NPL score of 2.59 in 16 Portuguese BPAD-affected families.

We also obtained evidence of linkage to 6q23 (full fine-mapping sample for ASDII: NPL = 2.85 at 135.1 cM; empirical $P = .022$ [table 3]), a region that was implicated in the Danish BPAD study (Ewald et al. 2002), with a 2-point LOD score of 2.49 at *D6S1009* (139 cM) with a narrow disease definition. At the same marker, Rice et al. (1997) found a 2-point LOD score of 2.08 in 97 American families of European descent. In the latter study, all markers on 6q between 112 cM and 155 cM yielded positive LOD scores for a broad affected-status model. Furthermore, this region was implicated by the genomewide study of Middleton et al. (2004), in which they investigated 25 multiplex BPAD-affected families of Portuguese origin, using the GeneChip Human Mapping 10K Array (HMA10K). They observed an NPL score of 4.20 at 125.8 Mb, which is located ~6 Mb centromeric of our strongest linkage finding within this chromosomal region.

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Figure 5 Fine-mapping linkage analysis: chromosomal 1p34-p36 multipoint NPL results in the fine-mapping sample and in the Spanish, Romany, and Bulgarian subsamples of ASDI, ASDII, and ASDIII. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

The third linkage region, 6q24, was supported by the combined sample, which produced an NPL of 3.18 (empirical $P = .008$) at 147.68 cM for ASDII. The Romany subsample produced the second-highest NPL score observed in our study (ASDIII: NPL = 4.87 at 152.0 cM; empirical $P = .044$). Given computational constraints and because the 6q24 results were the best finding in this chromosomal region, MOD-score analysis was restricted to this particular locus only. In the combined sample, this analysis identified two peaks, at 146.1 and 152.5 cM, with MOD scores 3.59 and 3.10 for ASDIII (fig. 10). For both peaks, the best genetic model was nearly additive, with low disease-allele frequencies and low penetrance. Using the medium and narrow phenotype definitions, we obtained MOD scores of 3.46 at

Table 3

Fine-Mapping Linkage Analysis: Multipoint NPL Analysis in the Fine-Mapping Linkage Sample (108 Families) and Each Subsample

Chromosome Region and Sample	Position[a] (cM)	NPL (Empirical P[b])		
		ASDI	ASDII	ASDIII
1p35-p36:				
Fine-mapping sample	45.97	1.93 (.128)	2.65 (.020)	3.69 (.003)[c]
Spanish subsample	45.97	1.44 (.294)	2.06 (.116)	3.97 (.005)[c]
Romany subsample	45.97	3.21 (.034)	2.70 (.064)	1.78 (.184)
Bulgarian subsample	45.97	1.26 (.418)	1.26 (.416)	…
4q31:				
Fine-mapping sample	151.16	2.85 (.010)	3.18 (.004)[c]	2.36 (.016)
Romany subsample	148.44	3.52 (.014)	3.34 (.016)	5.49 (.015)[c]
German subsample	151.16	2.15 (.038)	2.58 (.012)	1.61 (.244)
Bulgarian subsample	151.16	1.29 (.258)	1.28 (.244)	…
6q16:				
Fine-mapping sample	112.06	…	1.42 (.358)	1.24 (.468)
German subsample	112.06	2.42 (.058)	2.55 (.052)	2.15 (.104)
6q23:				
Fine-mapping sample	135.15	1.75 (.246)	2.85 (.022)	2.59 (.054)
Bulgarian subsample	136.08	2.69 (.026)	2.69 (.026)	2.42 (.044)
Spanish subsample	135.15	…	2.08 (.150)	…
6q24:				
Fine-mapping sample	147.68	2.27 (.096)	3.18 (.008)	2.53 (.060)
Romany subsample: peak I	147.75	2.99 (.058)	3.21 (.040)	3.46 (.071)[c]
Romany subsample: peak II	152.00	2.52 (.096)	2.69 (.062)	4.87 (.044)[c]
German subsample	147.75	1.23 (.470)	1.76 (.230)	…
Bulgarian subsample	147.68	1.43 (.430)	1.43 (.444)	1.85 (.044)

Note.—ASDI includes BPI; ASDII includes BPI, BPII, or SA/BP; ASDIII includes BPI, BPII, SA/BP, or UPR.

[a] Determined from the deCODE Genetics sex-averaged map.

[b] Empirical P value based on 500 simulations unless otherwise noted.

[c] Empirical P values are based on 1,000 simulations.

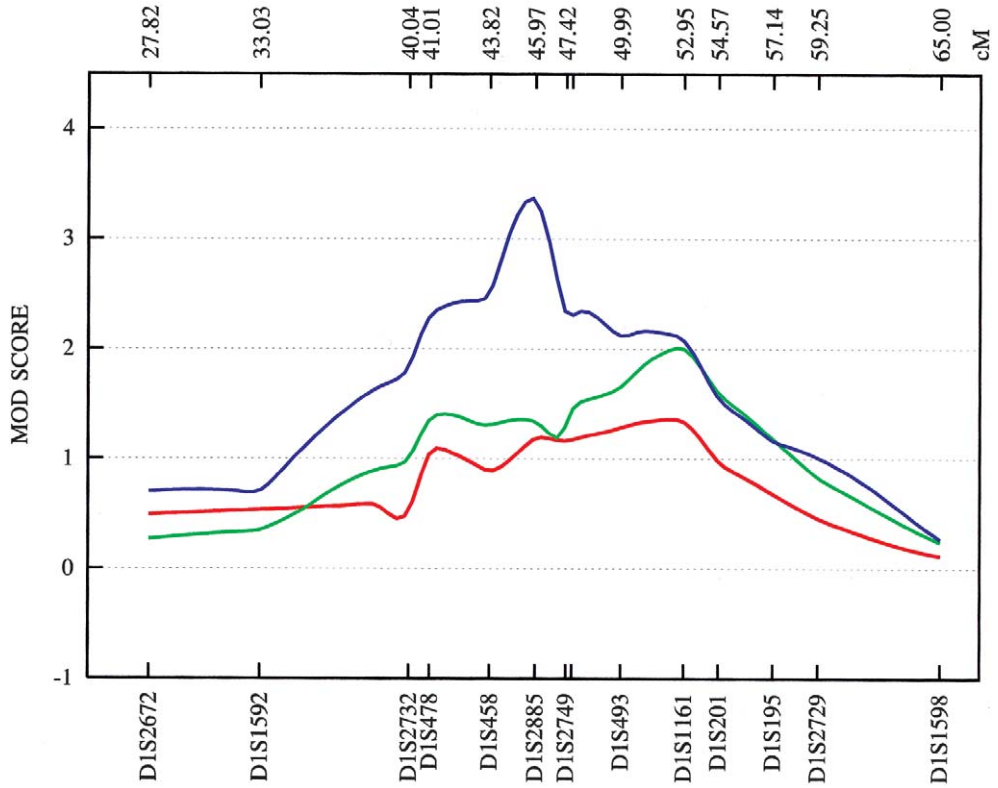

Figure 6 Plot of the MOD score for chromosome 1p35-p36, with a separate maximization over trait-model parameters for each genetic position assumed for the trait locus. ASDI (*red*) position of the maximum MOD score: 52.34 cM; penetrances {0.00; 0.49; 0.49}; disease-allele frequency 0.050. ASDII (*green*) position of the maximum MOD score: 52.63 cM; penetrances {0.00; 0.50; 0.50}; disease-allele frequency 0.040. ASDIII (*blue*) position of the maximum MOD score: 45.95 cM; penetrances {0.00; 0.53; 0.53}; disease-allele frequency 0.160. MOD scores are determined from the deCODE Genetics sex-averaged map. The penetrance of the disease models is obtained by MOD-score analysis and given in order $\{f_{+/+}; f_{m/+}; f_{m/m}\}$. A plus sign (+) indicates the wild-type allele; "m" indicates the mutant allele.

145.2 cM (for ASDII) and of 3.32 at 147.6 cM (for ASDI) (fig. 10). Although previous support for this region has been modest (Rice et al. 1997; see above), strong evidence of a 6q24 BPAD susceptibility locus was provided recently by a genomewide linkage study of an isolated population in northern Sweden (Venken et al. 2005). The study identified a 29-cM region on 6q (133–162 cM), with a multipoint LOD score of 2.48. Follow-up fine mapping analysis, under a recessive, affected-only model, led to a 3-cM candidate region (144–147 cM) and a multipoint LOD score of 3.25 at 146 cM, which matches the peak identified by our MOD-score analysis for the narrow and medium phenotype definitions (fig. 10). Collectively, the data provide strong support for the existence of a BPAD susceptibility locus on 6q24, a chromosomal region with relatively sparse gene entries. According to the RefSeq Genes track (UCSC Genome Bioinformatics), the genomic interval analyzed by our MOD-score analysis contains 32 genes.

The 108 affected families included in this study originate from four different European populations and represent one of the largest BPAD samples investigated to date. The fact that the extended Romany families produced the highest NPL scores, together with the findings of previous genome scans for BPAD in isolated populations, emphasizes the potential of such populations to facilitate gene identification in complex disorders. Although the number of susceptibility loci for BPAD in these isolated populations may not be substantially different from those of other populations, as suggested by our results and those of other studies (Fallin et al. 2004; Venken et al. 2005), isolated populations still offer advantages for genetic studies because of their limited diversity and presumed strong founder effects.

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 7 Fine-mapping linkage analysis: chromosomal 4q28-4q31 multipoint NPL results in the fine-mapping sample and in the German, Romany, and Bulgarian subsamples of ASDI, ASDII, and ASDIII. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

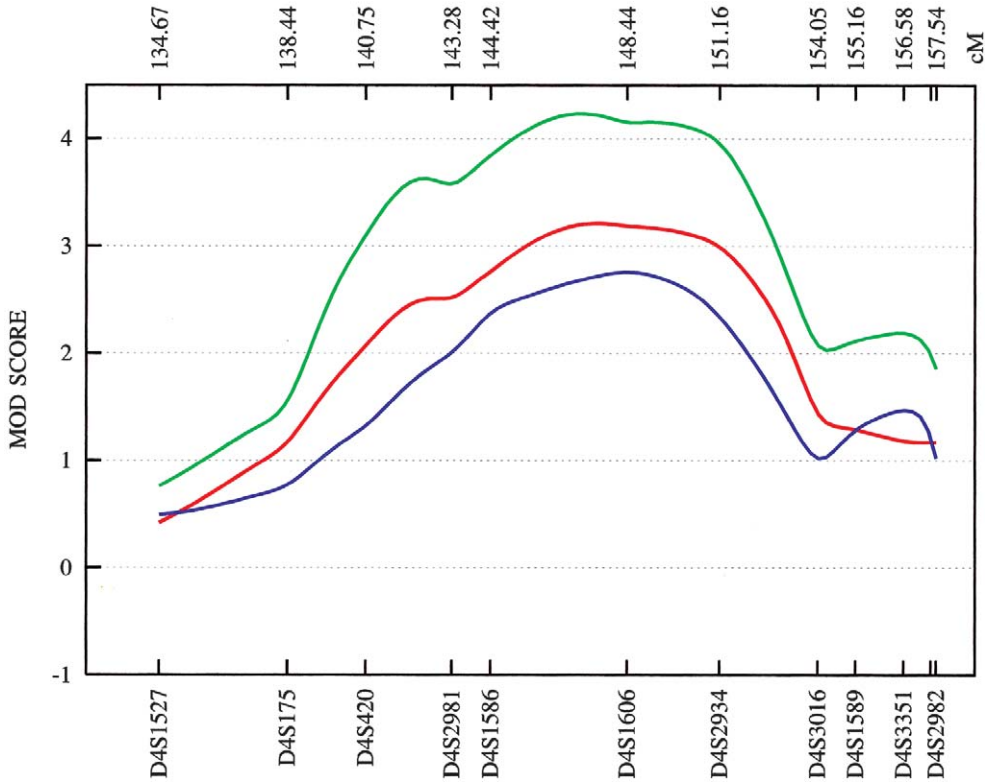

Figure 8 Plot of the MOD score for chromosome 4q31, with a separate maximization over trait-model parameters for each genetic position assumed for the trait locus. ASDI (*red*) position of the maximum MOD score: 147.64 cM; penetrances {0.00; 0.30; 0.92}; disease-allele frequency 0.025. ASDII (*green*) position of the maximum MOD score: 147.23 cM; penetrances {0.00; 0.33; 0.99}; disease-allele frequency 0.045. ASDIII (*blue*) position of the maximum MOD score: 148.44 cM; penetrances {0.00; 0.49; 0.96}; disease-allele frequency 0.040. MOD scores are determined from the deCODE Genetics sex-averaged map. The penetrance of the disease models is obtained by MOD-score analysis and given in order $\{f_{+/+}; f_{m/+}; f_{m/m}\}$. A plus sign (+) indicates the wild-type allele; "m" indicates the mutant allele.

Our genomewide scan identified several genomic regions likely to contain BPAD-susceptibility loci, some of which overlap with and provide further support for previous findings. Our subsequent fine-mapping linkage analysis led to substantially better results in all of the investigated regions and shaped the linkage peaks (e.g., in the Romany subsample, the NPL scores increased from 2.28 to 3.21 for chromosome 1p, from 1.65 to 5.49 for 4q, and from 3.65 to 4.87 for 6q, whereas, in the Spanish subsample, the NPL scores for 1p increased from 3.36 to 3.97). In addition, we applied parametric multipoint MOD-score analysis to the fine-mapping linkage data. Since the power to detect linkage is reduced when the trait model specified for a parametric (LOD score) analysis is not close to the true underlying model (Clerget-Darpoux et al. 1986), MOD-score analysis is a promising strategy for analysis of linkage data for complex traits. Although applied only to those chromosomal regions identified by the preceding NPL analysis, this approach is clearly explorative and thus does not allow stringent control of type I error. In principle, *P* values of MOD scores can be obtained by performing simulations for the investigated data set under the null hypothesis of no linkage. For our BPAD-affected family sample, a substantial amount for computation time is already required for the MOD-score analysis of the original data set. Analysis of many replicates for each of the loci identified here would therefore not be feasible; we relied instead on the criteria of Weeks et al. (1990) and Hodge et al. (1997). By performing simulations, they have found that, for MOD scores, a critical LOD score of 3 should be adjusted by a value in the range of 0.3–1.0 to maintain a comparable type I error, with a conservative upper boundary. When applied to the results

The figure is available in its entirety in the online edition of *The American Journal of Human Genetics*.

Figure 9 Fine-mapping linkage analysis: chromosomal 6q15-q24 multipoint NPL results in the fine-mapping sample and in the German, Romany, Spanish, and Bulgarian subsamples of ASDI, ASDII, and ASDIII. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

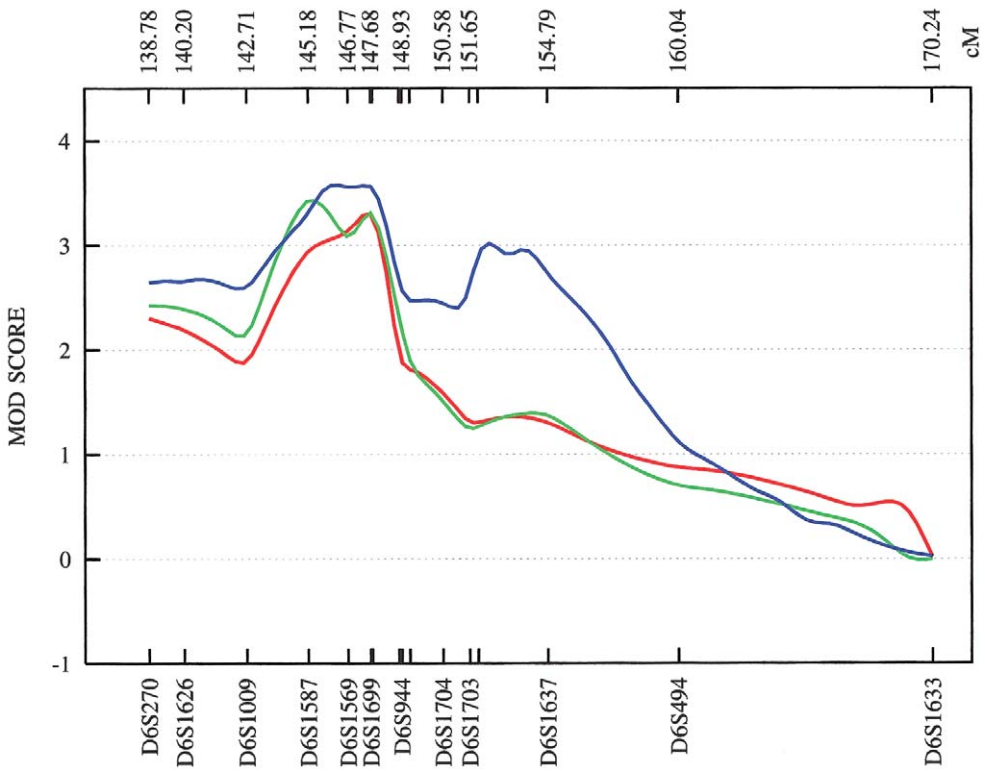

Figure 10 Plot of the MOD score for chromosome 6q24, with a separate maximization over trait-model parameters for each genetic position assumed for the trait locus. ASDI (*red*) position of the maximum MOD score: 147.68 cM; penetrances {0.00; 0.50; 0.50}; disease-allele frequency 0.005. ASDII (*green*) position of the maximum MOD score: 145.18 cM; penetrances {0.00; 0.52; 0.52}; disease-allele frequency 0.010. ASDIII (*blue*) position of the maximum MOD score: 146.13 cM; penetrances {0.00; 0.11; 0.26}; disease-allele frequency 0.005. MOD scores are determined from the deCODE Genetics sex-averaged map. The penetrance of the disease models is obtained by MOD-score analysis and given in order $\{f_{+/+}; f_{m/+}; f_{m/m}\}$. A plus sign (+) indicates the wild-type allele; "m" indicates the mutant allele.

of our study, that means that we have obtained "significant" evidence of a susceptibility locus in the 4q region and at least "borderline significance" for the regions on chromosomes 1p and 6q.

On the basis of our findings in two independent family samples, we propose the 1p35-p36 locus as a new susceptibility locus for BPAD. Chromosomal regions 4q31 and 6q24 have already been implicated in previous studies and, in the light of our results, should now be considered to be true-positive findings. The present study is the first application of the newly developed GENEHUNTER-MODSCORE program to BPAD, which allows a more precise definition of linkage peaks and estimation of disease inheritance parameters. The different pattern of linkage findings observed in the comparison of the individual subsamples in our study is not surprising. This phenomenon is to be expected with a genetically complex disorder (Suarez et al. 1994), since factors such as the structure and size of the family samples, incomplete penetrance, and locus heterogeneity influence the linkage results and may lead to interstudy differences (Terwilliger et al. 1997). Thus, our results reflect and support the opinion that BPAD is caused by multiple genes, with presumed genetic heterogeneity and with different combinations of predisposing genes segregating in different families and populations. Some of these were found within those regions identified by our extensive fine-mapping NPL and MOD-score analyses. The next step will be systematic linkage disequilibrium mapping in these identified regions, followed by the application of positional cloning methods.

Acknowledgments

The authors are grateful to the patients and their families for their cooperation and blood samples. This study was supported by Deutsche Forschungsgemeinschaft grants SFB 400 and GRK 246 and by the National Genomic Network of the Bundesministerium für Bildung und Forschung (to P.P., T.F.W., and M.R.). The collection of families from Bulgaria was supported by the National Science Fund. The contribution of Dr. T. Milenska and the Mental Health Services in Kjustendil, Bulgaria, to the collection of Romany families is gratefully acknowledged. We thank Prof. Wolfgang Maier, Dr. Dirk Lichtermann, Dr. Jürgen Minges, Prof. Margot Albus, Dr. Margitta Borrmann-Hassenbach, Dr. Mario Lanczik, Prof. Ernst Franzek, Prof. Jürgen Fritze, Dr. Ulrike Reuner, Dr. Bettina Weigelt,

and Dr. Roland Kreiner for their contribution to the collection of German families. M.M.N. received support for this work from the Alfried Krupp von Bohlen und Halbach-Stiftung. TGS was supported by a Young Investigator Award from the National Alliance for Research on Schizophrenia and Depression. R.K. was a research fellow of the Alexander von Humboldt Foundation. L.K. and A.J. acknowledge support from the Australian National Health and Medical Research Council. We thank Dr. Christine Schmael for help preparing the manuscript.

Web Resources

The URLs for data presented herein are as follows:

deCODE Genetics, http://www.decode.com/ (for information about the genetic map)
NCBI, http://www.ncbi.nih.gov/ (for BPAD)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for BPAD)
UCSC Genome Bioinformatics, http://genome.ucsc.edu/ (for marker positions and the RefSeq Genes track)

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