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A Susceptibility Locus for Bipolar Affective Disorder on Chromosome 4q35

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Summary

Bipolar affective disorder (BAD) affects ~1% of the population and shows strong heritability. To identify potential BAD susceptibility loci, we undertook a 15-cM genome screen, using 214 microsatellite markers on the 35 most informative individuals of a large, statistically powerful pedigree. Data were analyzed by parametric two-point linkage methods under several diagnostic models. LOD scores >1.00 were obtained for 21 markers, with four of these >2.00 for at least one model. The remaining 52 individuals in the family were genotyped with these four markers, and LOD scores remained positive for three markers. A more intensive screen was undertaken in these regions, with the most positive results being obtained for chromosome 4q35. Using a dominant model of inheritance with 90% maximum age-specific penetrance and including bipolar I, II, schizoaffective/mania, and unipolar individuals as affected, we obtained a maximum two-point LOD score of 2.20 (θ = .15) at D4S1652 and a maximum three-point LOD score of 3.19 between D4S408 and D4S2924. Nonparametric analyses further supported the presence of a locus on chromosome 4q35. A maximum score of 2.62 (P = .01) was obtained between D4S1652 and D4S171 by use of the GENEHUNTER program, and a maximum score of 3.57 (P = .0002) was obtained at D4S2924 using the affected pedigree member method. Analysis of a further 10 pedigrees suggests the presence of this locus in at least one additional family, indicating a possible predisposing locus and not a pedigree-specific mutation. Our results suggest the presence of a novel BAD susceptibility locus on chromosome 4q35.

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Introduction

Bipolar affective disorder (BAD [MIM 125480]) is a major psychiatric condition characterized by severe disturbances of mood. The disorder has a variable age at onset, with the initial presentation of illness typically occurring during the 2d–4th decade, and is characterized by episodes of mania and depression. BAD affects 1%–2% of the population and occurs both in families and sporadically (MacKinnon et al. 1997). Twin, family, and adoption studies indicate that BAD has a strong genetic basis; however, the number of genes involved and the mode of transmission of the disorder are poorly understood (Kelsoe 1997). Craddock et al. (1995) suggest that a multiplicative model involving three or more loci is necessary, to explain the heritability of BAD. However, evidence indicating that a single major locus is responsible for BAD has also been reported (Spence et al. 1995). Many groups are currently investigating the genetic basis of BAD, with interest focused on both identification and confirmation of susceptibility loci on various chromosomes. However, to date, no specific mutations or genetic polymorphisms have been identified that predispose to the disorder.

Straub et al. (1994) reported a pedigree in which linkage to BAD and the PFKL locus, situated on chromosome 21q22.3, was observed. Subsequent reports have tended to support the view that a BAD susceptibility locus exists on chromosome 21q22.2 (Gurling et al. 1995; Detera-Wadleigh et al. 1996; Smyth et al. 1997; J. B. J. Kwok, L. J. Adams, J. A. Donald, P. B. Mitchell, and P. R. Schofield, unpublished data), although some groups have not found any evidence for this linkage (Byerley et al. 1995). Linkage to markers in the pericentromeric region of chromosome 18 was identified by Berrettini et al. (1994); this was supported by the results of Stine et al. (1995), but was not supported by all studies (Maier et al. 1995; Pauls et al. 1995; Claes et al. 1997). In addition, linkage to markers on the long arm of chromosome 18, but not the pericentromeric region, has been reported (Stine et al. 1995; Coon et al. 1996; De Bruyn et al. 1996; Freimer et al. 1996). The study

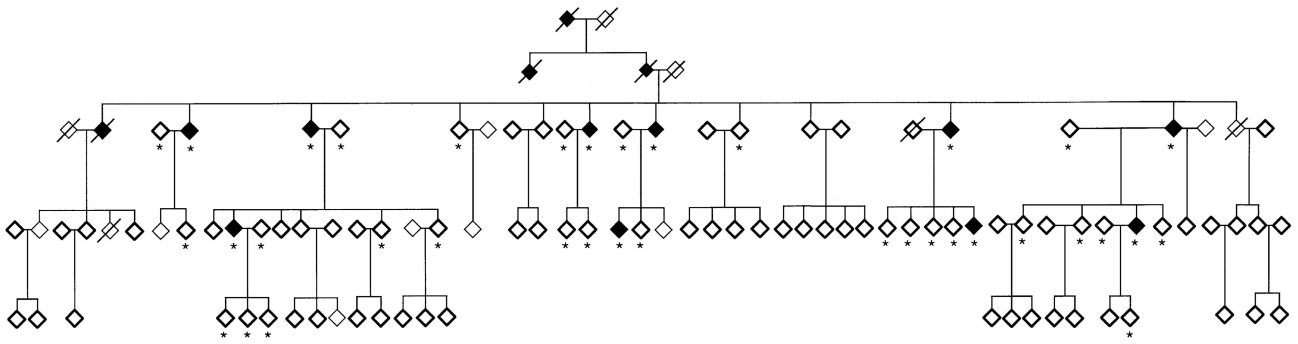

Figure 1 Family 01 structure. Individuals marked with an asterisk (*) were genotyped in the initial genome screen. Blackened symbols represent affected individuals, symbols with thick borders represent individuals typed in the follow-up screen, and symbols with fine borders represent individuals not typed in the study. Sex of the individuals has been disguised to provide anonymity.

by Stine et al. (1995) also suggested the possibility of a parent-of-origin effect in BAD. The results of several whole-genome screens have added new dimensions to the search for BAD susceptibility loci, with putative loci reported on chromosomes 4p (Blackwood et al. 1996), 18q (Freimer et al. 1996), and 6p, 13q, and 15q (Ginns et al. 1996). With predisposing loci having now been described on chromosomes 4p, 6p, 13q, 15q, 18p, 18q, 21q, as well as on Xq (Baron et al. 1993; Pekkarinen et al. 1996 [MIM 309200]), the evidence in favor of the existence of multiple BAD susceptibility loci is becoming increasingly strong.

We sought to identify BAD susceptibility loci by undertaking a genome screen of a large, informative pedigree, by use of microsatellite markers. Initially, we genotyped the 35 most informative members of the pedigree. Results of two-point linkage analysis on the data directed us to regions that were then investigated in the entire family (a further 52 individuals). Analysis of the most positive markers for all 87 individuals of this family resulted in one region (chromosome 4q35) that showed significant LOD scores. Analyses of the chromosome 4q35 region in this family, and in other pedigrees in our cohort, suggest that we have identified a novel susceptibility locus for BAD.

Material and Methods

Families

The families that participated in this study came from most states of Australia and were identified through a survey of the Mood Disorders Unit, Prince Henry Hospital, Sydney; the New South Wales Depression and Mood Disorders Association; and publicity through articles in popular magazines. These families were assessed by means of the Diagnostic Interview for Genetic Studies (Nurnberger et al. 1994), and best-estimate Research Diagnostic Criteria diagnoses were made after independent evaluation of all interviews and records. Individuals whose DNA was used in this study provided appropriate informed consent. Medium- to large-sized multigenerational pedigrees that contained a minimum of three affected individuals, at least two of whom were diagnosed with bipolar I, were recruited. The family used in the initial genetic screen (fig. 1) contains 87 members with available DNA samples, including 11 affected individuals: 5 bipolar I (BPI), 1 schizoaffective/mania (SZ/MA), and 5 recurrent unipolar (UP; Mitchell et al. 1991). The maximum and average expected LOD scores for this family, as determined by SLINK (Ott 1989; Weeks et al. 1990), are 8.64 and 3.95. Ten more pedigrees ascertained in the same manner were typed for markers in the most positive region identified from the genome screen. These pedigrees consisted of 137 individuals for genotyping, including 47 affecteds (22 BPI; 2 SZ/MA; 4 bipolar II [BPII]; and 19 UP). The pedigrees have combined maximum and average expected LOD scores under the assumption of homogeneity of 19.79 and 8.44, respectively, and maximum and average lod scores under the assumption of heterogeneity of 19.68 and 4.99, respectively, as determined by SLINK.

Genotyping

DNA was extracted from whole blood by standard techniques. Microsatellite markers analyzed were from the Cooperative Human Linkage Centre (CHLC) database and the Genome Database (GDB). Genotyping of CHLC markers was performed by Research Genetics. PCR of GDB markers was carried out in a 15-μl volume containing 60 ng DNA; 375 μM dATP, dCTP, dGTP, and dTTP; 5 μM each primer (the forward primer was labeled with either 6-Fam or Tet fluorescent dye); 2.5 mM $MgCl_2$, 1/10 volume of 10 × PCR buffer II (PE Applied Biosystems) and 0.6 units AmpliTaq Gold polymerase (PE Applied Biosystems). Samples were amplified on a Hybaid OmniGene thermal cycler, with the heated

lid. The following profile was used for DNA amplification: 95°C for 12 min; 10 cycles of 15 s at 94°C, 15 s at 55°C, and 30 s at 72°C; 20 cycles of 15 s at 89°C, 15 s at 55°C, and 30 s at 72°C; 30 min at 72°C; and 30 min at 35°C. Samples were diluted 1/10 in dH_2O, and 1.5 μl was added to the dye-loading mix (2.5 μl formamide, 0.5 μl blue dextran [50 mM EDTA, 50 mg/ml blue dextran], 0.5 μl GS500 Tamra size standard [PE Applied Biosystems]). One and five-tenths microliters of each sample was loaded onto a 4% denaturing polyacrylamide gel and electrophoresed on ABI377 sequencers. Products were detected by means of the Genescan program, version 2.0.2 (PE Applied Biosystems), and alleles were assigned by the Genotyper program, version 1.1.1 (PE Applied Biosystems). All genotyping was carried out by individuals blind to clinical diagnosis.

Chromosome 4q35 markers used and the distance between them as obtained from http://cedar.genetics.soton.ac.uk/pub/chrom4/gmap in the direction from centromere to q terminus were: D4S2417–9.2 cM–D4S2951–4.3 cM–D4S3041–3.1 cM–D4S1554–0.4 cM-D4S2920–4.4 cM–D4S2954–3.4 cM–D4S1535–1.0 cM–D4S408–4.7 cM–D4S2924–2.5 cM–D4S171–0.04 cM–D4S3051–0.4 cM–D4S3032–0.04 cM–D4S426–1.7 cM–D4S2930–0.003 cM–D4S1652. D4S1652 is the most distally reported polymorphic microsatellite marker on 4q.

Statistical Analysis

Two-point LOD scores were calculated between each marker and the disease by use of the MLINK program of the LINKAGE package, version 5.2 (Lathrop et al. 1984). Two disease thresholds were analyzed: Model I considered all BPI, BPII, and SZ/MA individuals as affected, and all other family members were considered unaffected. Model II considered all affected individuals from model I and all UP individuals as affected and all other family members as unaffected.

Two penetrance groups with maximum age-specific penetrance levels of either 60% or 90% and each containing four liability classes (class 1, <20 years; class 2, 20–29 years; class 3, 30–39 years; and class 4, ≥40 years) were used. Liability classes in the 90% penetrance group were defined with penetrances of 0.18, 0.45, 0.68, and 0.90; those in the 60% group were defined with penetrances of 0.12, 0.30, 0.45, and 0.60. Both dominant and recessive models were analyzed, with a disease allele frequency of .035 for the dominant model and .20 for the recessive model. Data were also analyzed with the dominant model and disease allele frequencies of .10 and .20. Multi-point analysis was undertaken for the 15 markers studied on chromosome 4q35 by use of the LINKMAP program of the LINKAGE package. The diagnostic models and penetrance groups used were the same as for two-point analysis. The HOMOG program (Ott 1991) was used to test for evidence of heterogeneity.

We have also used three nonparametric methods of analysis that are more suited to the larger-sized pedigrees found in our cohort. These nonparametric analyses included the GENEHUNTER (Kruglyak et al. 1995), MFLINK (Curtis and Sham 1995), and APM (Weeks and Lange 1988) programs. We have not presented nonparametric sib pair analyses (as has been typically done by other groups with smaller or nuclear pedigrees), since the small number of independent sib pairs in our pedigree cohort do not provide sufficient statistical power to draw significant conclusions.

Results

Genome Scan

The 35 most informative individuals from family 01 (fig. 1) were subjected to an ~15-cM genome screen by use of 202 markers from the Weber (CHLC) 6 and 6a panels of microsatellite markers. Chromosome 16 was not genotyped, since each of these individuals had previously been genotyped for 12 microsatellite markers (Adams et al. 1997). These 35 individuals were chosen from the entire pedigree for this initial screen, since they provided sufficient power to detect linkage, the maximum LOD score being 6.43, as determined by SLINK (Ott 1989; Weeks et al. 1990). All data were analyzed by means of the MLINK program of the LINKAGE package. Disease models I (narrow definition, in which only BPI, BPII, and SZ/MA were classified as affected) and II (broad definition, in which BPI, BPII, SZ/MA, and UP were classified as affected) were analyzed with both dominant and recessive inheritance models and both 60% and 90% maximum age-specific penetrance levels. Under SLINK simulations, no markers from a 214-marker genome screen for a given genetic and diagnostic model were expected to give a LOD score >3.00 by chance, and less than one marker was expected to give a LOD score >2.00 by chance. Two-point LOD scores >1.00 were obtained for 21 of the analyzed markers for at least one diagnostic model, and four of these gave scores >2.00. The four markers that gave LOD scores >2.00 were D1S518 (LOD = 2.42; model I, 90% dominant), D4S1652 (LOD = 2.39; model II, 90% dominant), D5S807 (LOD = 2.12; model II, 90% dominant), and D5S1470 (LOD = 2.92; model II, 90% dominant), and each was then analyzed in more detail.

The 52 additional individuals in family 01 for whom DNA samples are available were typed for the four markers that gave LOD scores >2.00, and two-point analysis of the entire family was undertaken. The LOD score for D1S518 was reduced from 2.42 to 0.20, thus eliminating chromosome 1 from further analysis. LOD

Table 1

LOD Scores for the Entire 87-Member Pedigree for Chromosome 5Q Markers

	Dominant		Recessive	
Marker	90%	60%	90%	60%
D5S392	−10.34	−1.28	−3.65	−.57
D5S1492	1.00	.80	.79	.72
D5S2505	.33	.61	.74	.60
D5S807	1.10	.73	.89	.70
D5S817	−2.25	−.19	−.11	−.05
D5S1473	.66	.17	.29	.16
D5S819	.47	.12	.47	.18
D5S1470	1.03	.41	.43	.42
D5S2494	−1.94	.12	.23	.17

Note.— Linkage analysis was performed under affection status model II.

scores for the two chromosome 5 markers, D5S807 and D5S1470, located ~31 cM apart, remained >1.00 (table 1). A further seven markers on chromosome 5p were analyzed, one of which, D5S1492, gave a LOD score of 1.00 (table 1).

A Potential Susceptibility Locus at 4q35

The chromosome 4q35 marker D4S1652 was the only one with a LOD score that remained >2.00 after typing of the entire family. A maximum LOD score of 2.20 was obtained at recombination fraction (θ) of 0.20 for model II, with a dominant mode of inheritance and 90% maximum age-specific penetrance. Genotypes of the 87 pedigree members were obtained for a further 14 microsatellite markers spanning ~35 cM of the distal arm of chromosome 4q. Two-point analysis of the data revealed seven markers in an ~10-cM interval to have LOD scores >1.00 (table 2), with D4S1652 still having the largest

Table 2

Two-Point LOD Scores for Family 1 for Chromosome 4Q35 Markers Under Model I (Narrow Disease Definition) and Model II (Broad Disease Definition) for Dominant Transmission with Maximum Age-Specific Penetrance Levels of 60% and 90%

		Recombination Fraction at θ =									
		.00		.05		.10		.15		.20	
Model and Locus	Penetrance:	90%	60%	90%	60%	90%	60%	90%	60%	90%	60%
Model 1:											
D4S2417		−3.89	−2.18	−2.08	−1.18	−1.33	−.76	−.86	−.49	−.55	−.31
D4S2951		−2.54	−1.35	−.91	−.24	−.28	.12	.07	.27	.25	.33
D4S3041		.22	.03	.19	.00	.15	.03	.11	.05	.07	.06
D4S1554		−3.45	−1.12	−1.45	−.45	−.77	−.20	−.41	−.07	−.20	−.01
D4S2920		.54	.48	.51	.42	.46	.36	.41	.29	.34	.23
D4S2954		−1.54	−1.26	−.72	−.74	−.46	−.53	−.32	−.39	−.23	−.29
D4S1535		−1.19	−.73	−.36	−.25	−.11	−.08	.01	−.01	.07	.02
D4S408		−3.66	−1.30	−1.50	−.57	−.81	−.28	−.44	−.14	−.22	−.06
D4S2924		−.31	−.30	−.30	−.26	−.29	−.22	−.26	−.17	−.21	−.12
D4S171		−6.12	−1.69	−2.75	−.91	−1.65	−.55	−1.02	−.34	−.61	−.21
D4S3051		−.26	−.18	−.26	−.15	−.25	−.12	−.21	−.08	−.15	−.05
D4S3032		.08	.13	.08	.11	.08	.08	.08	.06	.06	.04
D4S426		−.29	−.24	−.28	−.21	−.27	−.18	−.24	−.14	−.20	−.10
D4S2930		−6.51	−1.87	−3.00	−1.08	−1.78	−.68	−1.09	−.44	−.66	−.28
D4S1652		−6.50	−1.93	−2.60	−1.05	−1.43	−.63	−.80	−.37	−.42	−.22
Model 2:											
D4S2417		−6.30	−2.18	−3.06	−1.68	−1.96	−1.17	−1.29	−.80	−.83	−.53
D4S2951		−6.72	−3.97	−4.40	−2.60	−2.98	−1.93	−2.04	−1.42	−1.37	−.99
D4S3041		−1.15	−.65	−.93	−.57	−.76	−.49	−.60	−.40	−.47	−.31
D4S1554		−2.91	−2.04	−1.02	−.77	−.29	−.26	.11	.01	.32	.14
D4S2920		−1.64	−.97	−1.30	−.78	−1.03	−.64	−.81	−.51	−.63	−.41
D4S2954		−2.67	−1.89	−1.46	−1.32	−.99	−.92	−.69	−.63	−.46	−.41
D4S1535		−.19	−.40	.73	.18	.98	.39	1.06	.47	1.04	.47
D4S408		−.39	−.04	1.27	.87	1.66	1.13	1.75	1.17	1.67	1.10
D4S2924		.67	1.29	1.00	1.23	1.04	1.13	.99	1.01	.88	.86
D4S171		−.86	.74	1.34	1.38	1.87	1.55	2.03	1.56	1.98	1.45
D4S3051		.92	1.45	1.22	1.35	1.22	1.23	1.14	1.09	1.00	.92
D4S3032		−.43	−.49	−.22	−.33	−.10	−.22	−.02	−.14	.02	−.09
D4S426		.60	1.24	.93	1.18	.98	1.09	.94	.97	.84	.83
D4S2930		−1.57	.51	.63	1.15	1.28	1.34	1.53	1.37	1.57	1.28
D4S1652		−.02	1.68	1.76	2.02	2.15	2.02	2.20	1.89	2.07	1.69

Note.—LOD scores >1.00 are underlined.

Table 3

Variation in LOD Scores with Changes in Model Parameters

Variable	D4S1652	D4S171	D4S2924	D4S408
Normal[a]	2.20	2.03	1.04	1.75
Phenocopy 1%	2.16	2.07	1.00	1.77
Phenocopy 10%	1.15	1.48	.21	1.42
Disease frequency 10%	2.21	2.03	1.04	1.75
Disease frequency 20%	2.22	2.05	1.04	1.74
Maximum penetrance 30%	1.53	1.03	1.30	.56
Maximum penetrance 12%	1.00	.61	1.02	.25

[a] Normal = 90% maximum penetrance; 3.5% disease allele frequency; and 0.5% phenocopy rate.

two-point score. To ascertain the robustness of these linkage results, marked changes were made to a number of model parameters, including disease allele frequency, penetrance level, and phenocopy rate, to determine how they affected the maximum LOD scores. The LOD scores for these markers were found to be relatively robust to these changes (table 3) with all values remaining positive, supporting the presence of a susceptibility locus at chromosome 4q35.

Further Analysis of the 4q35 Locus

Three-point linkage analysis between sequential pairs of the fifteen 4q35 markers was undertaken with the LINKMAP program of the LINKAGE package. A maximal score of 3.19 was obtained between D4S408 and D4S2924 for model II with a dominant mode of inheritance and a maximum age-specific penetrance level of 90% (fig. 2). This score was also robust, since lowering the maximum age-specific penetrance level to 60% still resulted in a maximum score of 3.19 (fig. 2). Although the lack of a clear genetic or diagnostic model results in multiple hypothesis testing, the consistent positive results for both the two-point (table 3) and the three-point linkage analyses indicate a greater level of confidence in the data than if positive results were to be found in only some of the models tested.

Nonparametric methods of analysis were also used to examine the data. Further support was provided by the GENEHUNTER program (Kruglyak et al. 1996). Family 01 was too large for all 87 individuals to be analyzed simultaneously, so it had to be abridged for GENEHUNTER to run. A maximum score of 2.62 ($P = .01$) was obtained in the region spanning D4S1652 and D4S171. However, depending on the way in which the pedigree was subdivided, the GENEHUNTER scores ranged from a high score of 2.62 ($P = .01$) to a low of 0.59 ($P = .16$). The APM program (Weeks and Lange 1988) also provided limited support for a locus at 4q35, with scores of 3.57 ($P = .0002$) for $f(q) = 1$ and 1.97 ($P = .0245$) for $f(q) = 1\sqrt{q}$ for D4S2924. Significant APM scores were not obtained for the other chromosome 4 loci. The MFLINK program (Curtis and Sham 1995) was also used to analyze the data. A positive, but

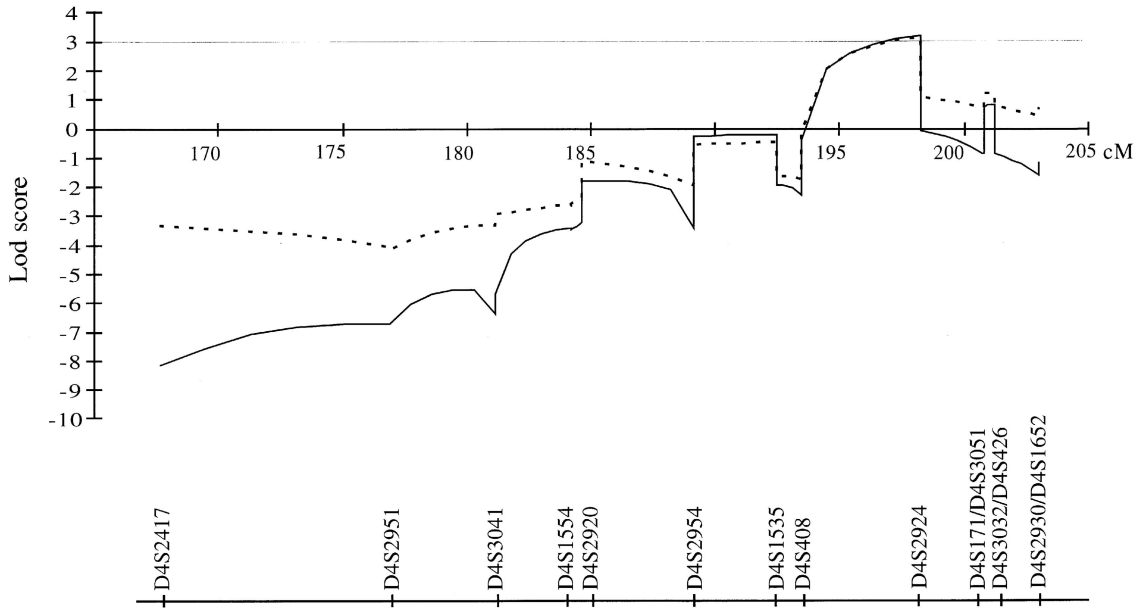

Figure 2 Three-point LOD scores of the 15 markers analyzed at 4q35 using model II with 90% (*solid line*) and 60% (*dotted line*) maximum age-specific penetrance levels.

nonsignificant, score of 1.43 was obtained between D4S171 and D4S2924.

Support for the 4q35 Locus in Other Pedigrees

A further 10 pedigrees in our cohort were genotyped for four microsatellite markers at chromosome 4q35 (D4S1652, D4S171, D4S2924, and D4S408). Two-point linkage analysis of all 11 families, under the broad diagnostic model (model II) with dominant inheritance and 90% maximum age-specific penetrance, gave a maximum score of 2.03 ($\theta = .20$) at D4S1652, indicating that, as a whole, the additional 10 pedigrees do not provide additional support for—but also do not refute—the observation of linkage to chromosome 4q35. The LOD score for D4S171 was 1.46 ($\theta = .25$), whereas scores for the other two markers were <1.00.

Of the additional pedigrees genotyped, family 11 gave a LOD score of 1.07 for marker D4S1652, which was 100% of the maximum LOD score that was expected from SLINK analysis, thus indicating that linkage was possible in this family as well. LOD scores >0.80 were also obtained for markers D4S171 and D4S408. To enable a preliminary disease haplotype to be constructed, members of family 11 were genotyped for a further three markers at 4q35 (D4S2930, D4S426, and D4S3032), and positive LOD scores were obtained: D4S426, LOD score of 1.08 ($\theta = .00$); D4S2930, LOD score of 0.61 ($\theta = .00$); and D4S3032, LOD score of 0.14 ($\theta = .20$). Three-point analysis of family 11 with the seven markers resulted in a maximum LOD score of 1.16 between D4S2930 and D4S1652.

Small positive results were obtained in two additional families for the four markers (D4S1652, D4S171, D4S408, and D4S2924), but none of these were statistically significant or represented a major fraction of the maximum possible LOD score when compared with the results obtained from simulation analysis (data not shown). Heterogeneity analysis with the HOMOG program (Ott 1991) showed statistically significant evidence for linkage of D4S1652 ($P < .05$) and D4S171 ($P < .01$) to BAD but no statistically significant evidence for heterogeneity.

Discussion

A genome screen of 214 microsatellite markers in a large Australian BAD pedigree has led to the identification of a novel BAD susceptibility locus on chromosome 4q35. Importantly, the positive LOD scores obtained were robust to changes in the model, with strong support for linkage even when a maximum age-specific penetrance level of 60% was used in the analysis (maximum multipoint LOD score of 3.19 between D4S408 and D4S2924). Furthermore, increasing the disease allele frequency to as high as 0.20, or the occurrence of phenocopies to 0.10, still gave positive results at 4q35, thus substantiating the robustness of this finding. Results of the nonparametric analyses also provide support for this linkage result.

The lack of a clear genetic model has resulted in some degree of multiple hypothesis testing, which would tend to inflate the genomewide level of significance of the 3.19 LOD score. Until our results are independently replicated, one must also consider the possibility that they are due solely to chance. However, the robustness of the linkage results to variation in model parameters provides some degree of confidence in our conclusions. The fact that at least one other pedigree in our cohort may also be linked suggests that the locus may be a general BAD predisposition gene and not a family-specific mutation. In addition, the construction of a disease haplotype based on the results of those pedigrees that show evidence for linkage will be an important step in the further elucidation of the genetic etiology of BAD. Lander and Kruglyak (1995) have proposed that the term "suggestive" linkage be used to describe statistical evidence for linkage that is likely to occur one time at random in a genome screen. A LOD score of 2.20 is required to support such a conclusion, which has been obtained in the two-point analysis of our data. Although our three-point linkage analyses reveal LOD scores of 3.19, they fall just short of the criteria necessary to claim "significant" linkage. Definitive evidence in support of a bipolar susceptibility locus in the 4q35 region will require independent confirmation.

All of the most positive scores identified at 4q35 were for markers within an ~10-cM region, defining a relatively small region for other researchers to investigate in their pedigrees. Evidence for linkage to 4q35 was primarily identified under the broad diagnostic model (model II), which includes both BP and UP individuals as affected. Maximal LOD scores have also been obtained in other studies using this diagnostic classification (Berrettini et al. 1994; Straub et al. 1994). The finding of no statistically significant evidence for heterogeneity by the HOMOG program is probably due to the relatively small data set currently analyzed and the fact that, except for family 01, the pedigrees used in this study are too small to produce large LOD score values.

Many groups have now reported positive linkage findings in the study of BAD (reviewed in Risch and Botstein 1996; Baron 1997; Kelsoe 1997; MacKinnon et al. 1997), yet the most notable feature of the results of these genomewide scans is the lack of studies replicating previously reported susceptibility loci. One identifiable feature of these studies is that significant evidence for linkage resulting from either parametric or nonparametric analyses have frequently been obtained from one or a few large pedigrees. For example, the original identifi-

cation of loci on chromosomes 21q22 (Straub et al. 1994), 18p (Berrettini et al. 1994), 18q (Freimer et al. 1996), 4p16 (Blackwood et al. 1996), Xq26 (Pekkarinen et al. 1995), and three separate loci on chromosomes 6p, 13q, and 15q in the Old Order Amish (Ginns et al. 1996) were all made using large, statistically powerful pedigrees. Our identification of a susceptibility locus on chromosome 4q35 from the genome screen of the large Australian pedigree is consistent with this pattern of a specific linkage being identified through the use of a large, statistically powerful pedigree.

Assuming that our results, and those of other groups that have identified linkage, are real and not due to chance, how could one explain this observation? The occurrence of pedigree-specific mutations seems unlikely. Additionally, in our study, a second pedigree also showed evidence for a locus on chromosome 4q35. For other candidate susceptibility regions, there have been a number of attempted replication studies, and general support has been reported for chromosomes 21q22, 18p, 18q, and possibly the long arm of the X chromosome (reviewed in Baron 1997; Kelsoe 1997; MacKinnon et al. 1997). Clearly, each of these reports adds further support to the conclusion that BAD has a complex genetic etiology. One possible explanation is that, within a large pedigree where a single susceptibility locus of modest effect is segregating and several other susceptibility loci are substantially homozygous, one may observe significant, although modest, evidence for linkage for that single locus. If multiple independent susceptibility loci are segregating, or a number of smaller pedigrees are used, linkage would be difficult to identify. This interpretation could explain the difficulties that have been seen in undertaking replication studies and the negative results obtained from some genome screens of large pedigrees. It would also suggest that increasing cohort size, and thus the number of informative meioses, will not necessarily lead to an immediate refinement of the map position of the candidate locus. It may therefore be necessary to significantly increase pedigree cohorts above the typical current study size of several hundred individuals before one is able to precisely map and identify such loci.

Potential susceptibility genes within the region of interest identified in this study and specific susceptibility alleles remain to be identified. Although a number of transcripts expressed in the brain map to this region, the lack of an underlying biochemical or neuroanatomical basis to the disorder hampers the prioritization and examination of positional candidate genes.

The consistent lack of replication of linkage results reinforces the notion that BAD has a complex genetic etiology (Risch and Botstein 1996; Baron 1997; Kelsoe 1997; MacKinnon et al. 1997). In isolation, our results strongly suggest the presence of a novel BAD susceptibility gene at 4q35, with linkage in two of our pedigrees providing evidence for a possible general susceptibility locus rather than a rare pedigree-specific mutation. Replication of linkage to this locus by other groups and the eventual identification of the allelic variation that results in the presence of this susceptibility locus may provide a means by which the molecular basis of this severe psychiatric disorder can be defined and understood.

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