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The First Genomewide Interaction and Locus-Heterogeneity Linkage Scan in Bipolar Affective Disorder: Strong Evidence of Epistatic Effects between Loci on Chromosomes 2q and 6q

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We present the first genomewide interaction and locus-heterogeneity linkage scan in bipolar affective disorder (BPAD), using a large linkage data set (52 families of European descent; 448 participants and 259 affected individuals). Our results provide the strongest interaction evidence between BPAD genes on chromosomes 2q22-q24 and 6q23-q24, which was observed symmetrically in both directions (nonparametric LOD [NPL] scores of 7.55 on 2q and 7.63 on 6q; $P < .0001$ and $P = .0001$, respectively, after a genomewide permutation procedure). The second-best BPAD interaction evidence was observed between chromosomes 2q22-q24 and 15q26. Here, we also observed a symmetrical interaction (NPL scores of 6.26 on 2q and 4.59 on 15q; $P = .0057$ and .0022, respectively). We covered the implicated regions by genotyping additional marker sets and performed a detailed interaction linkage analysis, which narrowed the susceptibility intervals. Although the heterogeneity analysis produced less impressive results (highest NPL score of 3.32) and a less consistent picture, we achieved evidence of locus heterogeneity at chromosomes 2q, 6p, 11p, 13q, and 22q, which was supported by adjacent markers within each region and by previously reported BPAD linkage findings. Our results provide systematic insights in the framework of BPAD epistasis and locus heterogeneity, which should facilitate gene identification by the use of more-comprehensive cloning strategies.

Bipolar affective disorder (BPAD [MIM 125480]) is characterized by severe episodes of mania and depression and represents a common disorder affecting ~1% of the world's population. Therefore, BPAD is considered to be one of the top public health problems associated with a significant morbidity (World Health Organization, World Health Report 2002). Although formal genetic studies consistently provide strong evidence of a major genetic contribution to BPAD,[1] the underlying genetic architecture is poorly understood. The pattern of inheritance is complex, reflecting the actions and interactions of multiple genetic and environmental factors, which has led to difficulties in mapping individual risk genes by conventional linkage studies. Although some promising loci have been identified in BPAD by genomewide linkage studies (reviewed in the work of Craddock and Forty[2]), the overall linkage picture is characterized by failures to replicate even the most interesting loci indicated by individual studies, and levels of statistical linkage significance point to more-modest effects for each single locus. Even in the most extensive and detailed linkage meta-analysis performed by Segurado et al.,[3] no genomewide significant linkage evidence was observed.

Here, we present a systematic approach that allows for a genomewide consideration of susceptibility from multiple loci and may therefore improve the ability to map genes for BPAD. We used a large BPAD linkage data set and performed a genomewide interaction linkage scan. The families represent the same data set with which we previously performed a one-dimensional linkage analysis.[4] To estimate the overall significance of the genomewide interaction findings, we performed a permutation pro-

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cedure, analyzing 10,000 replicates. The BPAD regions showing the strongest interaction evidence were subsequently covered by genotyping additional sets of linkage markers. This step was performed to narrow the susceptibility intervals. In addition, we performed a genomewide locus-heterogeneity analysis to identify BPAD loci in families that show negative linkage evidence at a conditional marker. In particular, we were interested in the pattern of locus heterogeneity within the identified BPAD-interaction regions.

Material and Methods

Subjects

The genomewide interaction scan was performed with 52 families with Spanish, Bulgarian, and Romany descent, consisting of 448 subjects, of whom 259 were affected. Informed consent was obtained from all participants. The study complied with all ethical guidelines of the institutions involved. A description of the family structure is presented in table 1. The ascertainment scheme is given in detail in the work of Schumacher et al.[4] In brief, the phenotype evaluation was based on DSM-IV criteria.[5] The inclusion criteria for families with BPAD were the presence of a proband with bipolar I (BP I) disorder and a secondary affected sibling with either BP I, bipolar II (BP II), schizoaffective disorder bipolar type (SA/BP), or unipolar recurrent depression (UPR). Given computational constraints, and to reduce the number of statistical tests, the families were not divided into subsamples and were not analyzed separately according to their regional descent. For the same reason, the analysis was restricted to the broad affection status definition (BP I, BP II, SA/BP, and UPR), which included the maximum number of affected individuals and produced the strongest linkage evidence within our one-dimensional scan.[4]

Genotyping

The genotyping for the genomewide analysis was conducted at the Gene Mapping Center in Berlin (procedures described in the work of Lee et al.[6]). A total of 435 STR markers were genotyped, with an average intermarker distance of 8.3 cM (deCODE Genetics map). The additional linkage markers, which covered the interacting BPAD regions, were genotyped at deCODE Genetics in Reykjavik (21 markers on chromosome 6q23-q24, with the use of procedures described in the work of Bjornsson et al.[7]) and at the Institute of Human Genetics in Bonn (21 markers on chromosomes 2q21-q24 and 15q26, with the use of procedures described in the work of Cichon et al.[8]).

Statistical Analysis

The multipoint nonparametric interaction analysis was performed according to the method described by Kong and Cox,[9] with the use of proportional family weights ($weight_{PROP}$), in accordance with the work of Cox et al.[10] In detail, for each family, multimarker NPL scores were calculated under the given trait definition at each genomewide linkage marker. The NPL score of a given family at a given marker locus called the "conditional marker" was then used as a weighting factor ($weight_{PROP}$) for the same family, and a multipoint NPL analysis at a second marker locus called the "scan marker" was performed. Only families with NPL scores >0 at the conditional locus were included for the interaction linkage analyses at the scan markers. Thus, the weighting factors used were proportional to the linkage evidence at the conditional locus. At each scan marker of the genome, two NPL scores were determined: one unweighted, called the "baseline NPL score," and one under the weighting scheme, called the "interaction NPL score." The difference between these two NPL scores is termed "ΔNPL score." The weighting scheme corresponds to the scheme called "PROP" by Cox et al.,[10] except for a slight modification: if the baseline NPL score was <0, it was set to 0 before calculating the difference. This avoids positive ΔNPL scores in a region with negative NPL scores and, thus, negative linkage evidence. Furthermore, ΔNPL scores were calculated only if interaction NPL scores were greater than baseline NPL scores. For the multipoint nonparametric locus-heterogeneity analysis, we adapted the linkage approach described above, with the exception of using another weighting scheme: at the conditional locus, only families with NPL scores <0 were included, and, for the heterogeneity linkage analysis at the scan locus, weighting factors were used that were inversely proportional to the negative NPL scores at the conditional locus. In correspondence with the procedure described above, we determined two NPL scores at the scan locus: one unweighted baseline NPL score and one under the weighing scheme, called the "heterogeneity NPL score." The difference between both NPL scores was again termed "ΔNPL score." The baseline NPL score was set to 0 if it was <0, and, as described above, ΔNPL scores were calculated only if heterogeneity NPL scores were greater than baseline NPL scores. Both the genomewide interaction and locus-heterogeneity analyses were restricted to interchromosomal markers, to avoid statistical interference. Both analyses were done by running Allegro version 2.0f.[11]

To assess the significance of our genomewide interaction and locus-heterogeneity findings, we performed genomewide permutation analyses. We randomly permuted the family weights for all conditional markers simultaneously on the same chromosome to assess their contributions to the interaction and heterogeneity NPL scores. Since ΔNPL scores reflect the differences between the baseline and the interaction/heterogeneity NPL scores, the exceeding probabilities determined by the permutation procedure refer to the *P* values for both ΔNPL scores and interaction/heterogeneity NPL scores. The permutation was applied to all conditional- and scan-marker combinations only in the case where both were located on separate chromosomes. For each combination, 10,000 permutations were done, and, for each combination, the permutation was followed by calculating the interaction and heterogeneity NPL scores under each weight. Permutation-based NPL scores that exceeded those from the original weighting procedure were counted and were then used to determine the significance of the findings. Permutation analyses were performed by using a dedicated program, which took 193 h for each weighting scheme (interaction and heterogeneity).

To enable the research community to implement all statistical methods, we created a Web site that includes all programs used in the present study (see the Institute for Medical Biometry Web site). In addition, the genotypic and phenotypic information

Table 1. Characteristics of the BPAD Sample Studied for the Genomewide Interaction Scan

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

from our genome scan and fine-mapping data set can be obtained on request.

Results

Genomewide Interaction Scan

Figure 1 presents the data from our genomewide interaction scan. Table 2 lists the top 100 genomewide ΔNPL scores, and table 3 presents the top 10 ΔNPL results.

Only two findings—between chromosomes 2q and 6q as well as 2q and 15q—belonged to the top 10 genomewide BPAD interactions and produced ΔNPL scores >5 (table 3 and fig. 1). On chromosome 2q22-q24, four adjacent markers showed ΔNPL scores >6 with use of a conditional STR on 6q23. Within the center of this region, we observed the strongest interaction, with a ΔNPL score of 6.94, at *D2S1399* and an interaction NPL score of 7.55 at *D2S2241* (table 3). This interaction is supported by the genomewide permutation procedure. *P* values between <.0001 and .0014 were observed for all implicated 2q markers (table 3). In addition, our analysis provided strong interaction evidence on chromosome 6q23 (ΔNPL scores >4) by the use of conditional markers on 2q22-q24 (fig. 1 and table 2). One of these findings belongs to the top 10 genomewide interaction results. At *D6S1009,* we observed a ΔNPL score of 4.96 and an interaction NPL score of 7.63 (table 3). This vice-versa interaction was observed only once by chance through 10,000 permutations ($P = .0001$) (table 3). The second BPAD interaction with ΔNPL scores belonging to the top 10 genomewide results was observed between 15q26 and, again, chromosome 2q22-q24 (table 3 and fig. 1). With use of *D15S642* as the conditional marker, three adjacent STRs on chromosome 2q showed ΔNPL scores >5. The strongest interaction was observed at *D2S1399,* with a ΔNPL score of 5.65 (table 3).

Table 2. Top 100 Genomewide Interaction ΔNPL Scores in BPAD, Ordered according to the Conditional Chromosomes

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

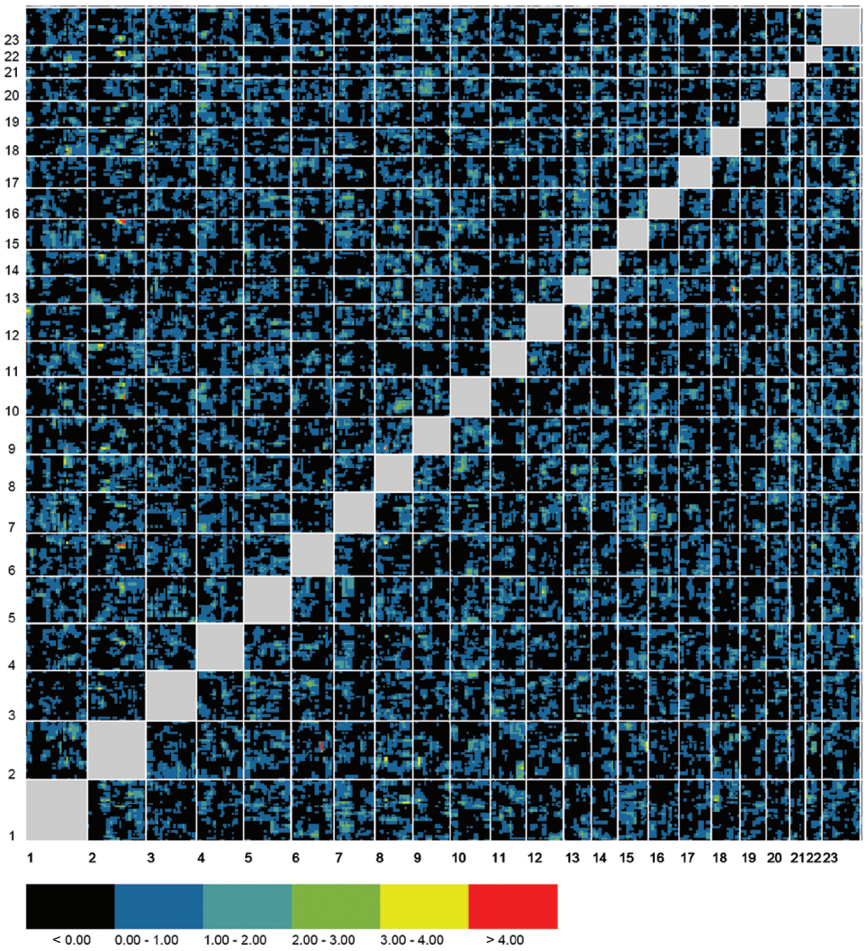

Figure 1. Genomewide interaction scan for BPAD. The different levels of ΔNPL scores are presented using different colors: red indicates ΔNPL scores >4; yellow indicates ΔNPL scores >3; green indicates ΔNPL scores >2; light blue indicates ΔNPL scores >1; dark blue indicates ΔNPL scores >0; black indicates ΔNPL scores <0.

Table 3. Genomewide Top 10 ΔNPL Scores in BPAD, Ordered according to Interacting Regions

	Conditional Marker			Scan Marker						
Rank	Chromosome	Marker	Position[a]	Chromosome	Marker	Position[a]	Baseline NPL	Interaction NPL	ΔNPL	P[b]
1	6	*D6S1009*	138.76	2	*D2S1399*	158.20	.38	7.32	6.94	.0001
2	6	*D6S1009*	138.76	2	*D2S2241*	163.27	1.06	7.55	6.48	<.0001
3	6	*D6S1009*	138.76	2	*D2S1334*	148.76	.75	6.94	6.20	.0005
4	6	*D6S1009*	138.76	2	*D2S1353*	167.91	.23	6.24	6.01	.0008
5	6	*D6S1009*	138.76	2	*D2S114*	146.86	.67	6.57	5.89	.0014
9	2	*D2S2241*	163.27	6	*D6S1009*	138.76	2.67	7.63	4.96	.0001
6	15	*D15S642*	133.69	2	*D2S1399*	158.20	.38	6.03	5.65	.0061
7	15	*D15S642*	133.69	2	*D2S1353*	167.91	.23	5.72	5.50	.0044
8	15	*D15S642*	133.69	2	*D2S2241*	163.27	1.06	6.26	5.20	.0057
10	15	*D15S642*	133.69	2	*D2S1334*	148.76	.75	5.67	4.92	.0127

NOTE.—For each scan marker, the ΔNPL and interaction NPL scores are presented. In addition, the baseline NPL score observed in the one-dimensional genomewide scan is presented.

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

[b] P values are determined through 10,000 genomewide permutations.

The genomewide permutation showed that one would expect this interaction in 61 of 10,000 replicates by chance (P = .0061) (table 3). The highest interaction NPL score of 6.26 was observed at *D2S2241* (table 3). Although none of the 15q markers showed ΔNPL scores belonging to the top 10 interaction results, there was some evidence of epistasis in both directions. By use of 2q STRs as conditional markers, ΔNPL scores >3 were observed on chromosome 15q26 (best ΔNPL score of 3.92 at *D15S642*, rank 38, P = .0022) (table 2).

Detailed Interaction Linkage Analysis on Chromosomes 2q22-q24 and 6q23-q24

In addition to the genomewide scan markers, 15 further STR markers on chromosome 2q (interval *D2S347–D2S376*, average intermarker distance 2.06 cM) and 21 further STRs on chromosome 6q were genotyped (interval *D6S407–D6S494*, average intermarker distance 1.21 cM) (table 4). The most centromeric and telomeric markers were located at a distance of >10 cM from the STRs, which showed ΔNPL scores within the genomewide top 10 range. On chromosome 2q, 113 marker combinations showed ΔNPL scores >5 with use of conditional STRs on 6q. The 10 best ΔNPL findings—scores between 5.97 and 6.69—are presented in table 5 and were observed at four adjacent markers (table 5 and fig. 2*A*). The strongest interaction NPL score of 6.70 was observed at *D2S222* (table 5). In the vice-versa direction, a total of 133 marker combinations showed ΔNPL scores >4 on chromosome 6q. The 10 best ΔNPL findings—scores between 4.67 and 4.84—are located in the same region as those found when chromosome 6 STRs were used as conditional markers for the chromosome 2q scan (table 5 and fig. 2*B*). The strongest interaction NPL score of 7.66 was observed at *D6S403* (table 5).

The robustness of our finding is implicated not only by the permutation analysis but also by the fact that a high proportion of the families contributes to the interaction. With use of STRs on 6q23-q24 as conditional markers, ~69% (n = 36) of the 52 families contributed to the interaction on 2q, and ~36% (n = 19) were thereby attributed with a $\text{weight}_{\text{PROP}}$ factor >1, indicating that their linkage contribution to the interaction findings increased by a factor >1 compared with the baseline study (factor = 1) (fig. 3*A* and 3*B*). Similarly, a majority, ~61% (n = 32), of families contributed to the interaction on 6q with use of conditional markers at 2q22-q24, and ~36% (n = 19) were attributed with a $\text{weight}_{\text{PROP}}$ factor >1 (fig. 3*A* and 3*B*). The symmetry of our finding is also indicated by the distribution of families contributing to the vice-versa interaction: ~42% (n = 22) of families were included in the study through their overlapping linkage evidence at conditional loci 2q and 6q, and ~21% (n = 11) were thereby attributed with a $\text{weight}_{\text{PROP}}$ factor >1 (fig. 3*A* and 3*B*).

Detailed Interaction Linkage Analysis on Chromosomes 2q22-q24 and 15q26

The second-best BPAD interaction was observed between chromosomes 2q22-q24 and 15q26. Whereas the 2q region was already covered by 21 STR markers for the 6q fine mapping, 6 additional markers were genotyped on the 15q26 (interval *D15S130–STR15-1002*, average intermarker distance 2.83 cM) (table 4). Since *D15S642*—which showed the strongest genomewide interaction—is located only 0.18 Mb from the telomeric end of chromosome 15, little information about additional STRs within this region is available in public databases. We therefore performed a marker discovery analysis, using the tandem repeat finder program by Benson et al.,[12] and identified three hitherto

Table 4. Fine-Mapping Linkage Markers Ordered according to Their Interacting Regions

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

Table 5. ΔNPL Scores for the BPAD Interaction between Chromosome 2q22-q24 and 6q23-q24

Chromosome and Rank	Conditional Marker		Scan Marker				
	Marker	Position[a]	Marker	Position[a]	Baseline NPL	Interaction NPL	ΔNPL
2q22-q24:							
1	*D6S1009*	138.76	*D2S1399*	158.20	−.04	6.69	6.69
2	*D6S1587*	141.16	*D2S1399*	158.20	−.04	6.69	6.69
3	*D6S1009*	138.76	*D2S381*	157.19	−.03	6.67	6.67
4	*D6S1009*	138.76	*D2S222*	158.35	.06	6.70	6.64
5	*D6S1587*	141.16	*D2S381*	157.19	−.03	6.63	6.63
6	*D6S1587*	141.16	*D2S222*	158.35	.06	6.69	6.63
7	*D6S1626*	136.31	*D2S1399*	158.20	−.04	6.09	6.09
8	*D6S1626*	136.31	*D2S381*	157.19	−.03	6.07	6.07
9	*D6S1626*	136.31	*D2S222*	158.35	.06	6.10	6.03
10	*D6S1009*	138.76	*D2S2275*	161.36	.84	6.81	5.97
6q23-q24:							
1	*D2S1353*	167.91	*D6S1587*	141.16	2.67	7.51	4.84
2	*D2S1353*	167.91	*D6S403*	143.66	2.91	7.66	4.79
3	*D2S1353*	167.91	*D6S1699*	143.59	2.89	7.64	4.74
4	*D2S321*	163.27	*D6S1587*	141.16	2.67	7.39	4.72
5	*D2S2241*	163.27	*D6S1587*	141.16	2.67	7.39	4.72
6	*D2S381*	157.19	*D6S1587*	141.16	2.67	7.38	4.71
7	*D2S1353*	167.91	*D6S1009*	138.76	2.85	7.55	4.70
8	*D2S1399*	158.20	*D6S1587*	141.16	2.67	7.36	4.69
9	*D2S222*	158.19	*D6S1587*	141.16	2.67	7.34	4.67
10	*D2S2950*	165.18	*D6S976*	133.57	2.70	7.37	4.67

NOTE.—The first set of 10 rankings represents the strongest ΔNPL and interaction NPL scores on chromosome 2q22-q24 with the use of STRs on 6q as conditional markers. The second set of 10 rankings represents the strongest ΔNPL and interaction NPL scores on chromosome 6q23-q24 with the use of STRs on 2q as conditional markers. In addition, for each scan marker, the baseline NPL score is presented.

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

unknown markers (*STR15-980, STR15-994,* and *STR15-1002*), which were analyzed together with three annotated markers. The most centromeric marker (*D15S130*) was located at a distance of >10 cM from *D15S642*. On chromosome 2q, we observed at 11 marker combinations ΔNPL scores >5, using conditional STRs on 15q. The 10 best ΔNPL findings—scores between 5.08 and 5.62—were found in a circumscribed region with use of two adjacent 15q conditional markers (table 6 and fig. 4*A*). The strongest interaction NPL score of 6.00 was observed for *D2S2950* (table 6). In the vice-versa direction, a total of 23 marker combinations showed ΔNPL scores >3 on chromosome 15q. The same two STRs that produced the strongest interaction evidence when used as conditional markers for the 2q scan were implicated by the top 10 ΔNPL findings on chromosome 15q (scores between 3.40 and 3.74) (table 6 and fig. 4*A*). The strongest interaction NPL score on 15q26 was 3.86 and is located at *D15S642* (table 6).

Although less impressive when compared with the BPAD interaction between 2q and 6q, the interaction between 2q and 15q was observed in a substantial proportion of families. With use of STRs on 15q26 as conditional markers, ~59% ($n = 31$) of families contributed to the interaction on 2q, and ~21% ($n = 11$) were thereby attributed with a $\text{weight}_{\text{PROP}}$ factor >1 (fig. 3*C* and 3*D*). With use of conditional markers at 2q22-q24, ~61% of families contributed to the interaction on 15q, and ~36% were attributed with a $\text{weight}_{\text{PROP}}$ factor >1 (fig. 3*C* and 3*D*). Furthermore, fewer families than those in the 2q-6q interaction contributed symmetrically to the epistasis between 2q and 15q: ~32% ($n = 17$) of families were included in the study because of their overlapping linkage evidence at both loci, and a moderate number, ~13% ($n = 7$), of families were thereby attributed with a $\text{weight}_{\text{PROP}}$ factor >1 (fig. 3*C* and 3*D*).

Genomewide Locus-Heterogeneity Scan

In addition to the identification of interacting BPAD loci, we were interested in the pattern of locus heterogeneity in our family data set. Therefore, we performed a genomewide locus-heterogeneity analysis. Those BPAD-affected families that showed negative linkage evidence at each conditional marker were assigned a weight proportional to the absolute value of the NPL score. Table 7 lists the top 10 heterogeneity findings, representing the ΔNPL scores >2.5 (see table 8 for the top 100 heterogeneity findings). The reason for these rather moderate linkage findings and the fact that they were assessed as significant by our permutation procedure (see table 7) can be explained by the small proportion of contributing families. Although many families ($n = 24$–26) were included in the analysis of the top 10 heterogeneity results with a $\text{weight}_{\text{PROP}}$ factor

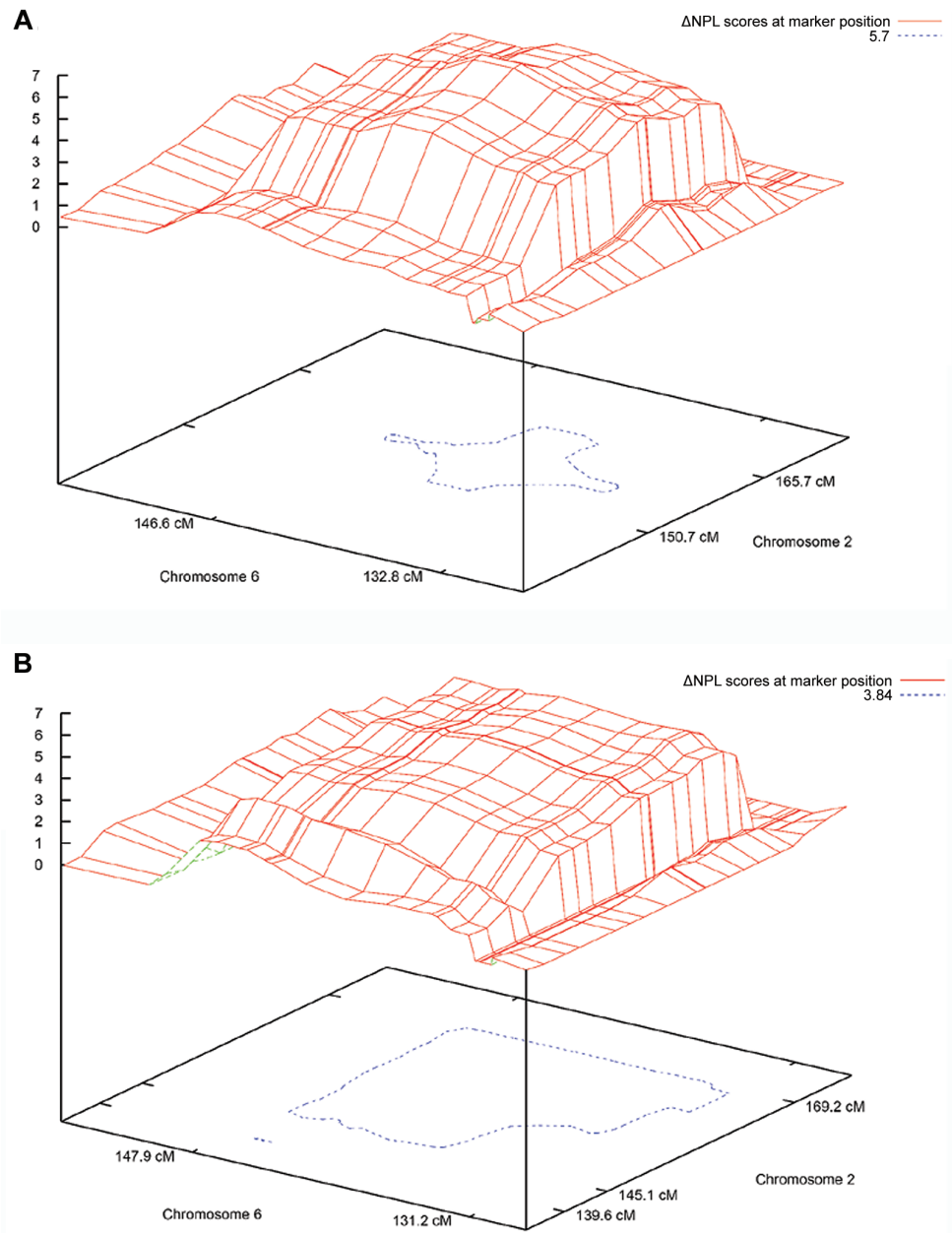

Figure 2. Three-dimensional ΔNPL plot for the BPAD interaction between chromosomes 2q22-q24 and 6q23-q24. The 1-LOD intervals are given at the bottom of the plot, in blue. Genetic-marker positions are determined from the deCODE Genetics sex-averaged map, and ΔNPL scores are indicated by red lines. *A,* Interaction on chromosome 2q22-q24, presented using STRs on 6q as conditional markers (highest ΔNPL score 6.69). *B,* Interaction on chromosome 6q23-q24, presented using STRs on 2q as conditional markers (highest ΔNPL score 4.84).

>0, only a few families (n = 1–6) contributed to these findings with a weight$_{PROP}$ factor >1. Table 9 provides detailed information about the permutation results and families included.

Although none of the regions listed in table 7 was implicated twice as a top 10 finding, four appeared to be of particular interest when adjacent markers at both sides were included—the conditional and at the scan locus. All ΔNPL scores belonged hereby to the top 100 heterogeneity findings (see table 8). In detail, the use of negative NPLs as inversely proportional weight at four adjacent STRs on 11p13-p15 increased the linkage evidence at two neighboring markers on chromosome 6p24-p25 (ΔNPL scores >2.27) (see table 8), and the strongest heterogeneity evidence was observed at *D6S477* (ΔNPL score of 2.65, heterogeneity NPL score of 3.19, rank 1; P = .0004) (table 7). Five neighboring conditional markers at a second locus on chromosome 11—at 11p12-q13—increased the linkage

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

Figure 3. Proportions of families contributing to the BPAD interaction. The legend is available in its entirety in the online edition of *The American Journal of Human Genetics*.

evidence at four adjacent STRs on 9p21-q21 (ΔNPL scores >2.13) (see table 8), and the best finding was observed for *D9S1122* (ΔNPL score of 2.56, heterogeneity NPL score of 2.56, rank 7; $P = .0001$) (table 7). Interestingly, inversely proportional NPL weights at 13q31 increased the linkage evidence at 2q22-q24, one of our BPAD-interaction regions. The use of two adjacent conditional markers on 13q resulted in ΔNPL scores >2.22 at three neighboring STRs on 2q22-q24 (see table 8), and the strongest ΔNPL score of 2.57 was observed for *D2S1399* (heterogeneity NPL score of 2.94, rank 6; $P = .0096$) (table 7). Evidence of locus heterogeneity was also observed on 8q24. The linkage findings increased at two adjacent markers on 8q when two neighboring STRs on chromosome 16q21 were used as conditional markers (see table 8). The best ΔNPL score of 2.59 was found for *D8S1128* (heterogeneity NPL score of 3.00, rank 4; $P = .0004$) (table 7).

Locus Heterogeneity in BPAD-Interaction Regions

We were particularly interested in the pattern of locus heterogeneity at the identified BPAD-interaction regions. The strongest evidence of locus heterogeneity was observed at 2q22-q24 with the use of 13q31 conditional markers (see above). One other finding belonged to the top 10 results and was supported by adjacent STRs on the scan side. Using *D22S1169* on 22q11 as the conditional STR produced ΔNPL scores >2.27 at three markers within the BPAD 2q21-q22 interval (see table 8). The strongest result was observed at *D2S1334* (ΔNPL score of 2.55, heterogeneity NPL score of 3.29, rank 8; $P = .0154$) (table 7). Although there was some further evidence of locus heterogeneity on 2q when markers on 3q29 and 7q35-q36 were used as conditional markers and on 19q13 with use of a 2q STR as a conditional marker (table 8), no other interacting STR—including markers on 6q and 15q—was highlighted by the top 100 heterogeneity findings or by the inclusion of heterogeneity results at adjacent markers.

Discussion

BPAD-Interaction Evidence between Chromosomes 2q22-q24 and 6q23-q24

Whereas chromosome 6q23-q24 already showed linkage evidence within our one-dimensional linkage scan (NPL

Table 6. ΔNPL Scores for the BPAD Interaction between Chromosomes 2q22-q24 and 15q26

	Conditional Marker		Scan Marker				
Chromosome and Rank	Marker	Position[a]	Marker	Position[a]	Baseline NPL	Interaction NPL	ΔNPL
2q22-q24:							
1	*STR15-1002*	133.69	*D2S1399*	158.20	−.04	5.62	5.62
2	*STR15-1002*	133.69	*D2S381*	157.19	−.03	5.59	5.59
3	*D15S642*	133.60	*D2S1399*	158.20	−.04	5.58	5.58
4	*D15S642*	133.60	*D2S381*	157.19	−.03	5.56	5.56
5	*STR15-1002*	133.69	*D2S222*	158.53	.06	5.62	5.56
6	*D15S642*	133.60	*D2S222*	158.53	.06	5.58	5.52
7	*STR15-1002*	133.69	*D2S2334*	153.41	.63	5.89	5.26
8	*D15S642*	133.60	*D2S2334*	153.41	.63	5.86	5.23
9	*STR15-1002*	133.69	*D2S2950*	165.18	.88	6.00	5.12
10	*D15S642*	133.60	*D2S2950*	165.18	.88	5.96	5.08
15q26:							
1	*D2S1353*	167.91	*D15S642*	133.60	.11	3.86	3.74
2	*D2S1353*	167.91	*STR15-1002*	133.69	.11	3.84	3.73
3	*D2S2395*	169.58	*D15S642*	133.60	.11	3.79	3.68
4	*D2S2950*	165.18	*D15S642*	133.60	.11	3.78	3.67
5	*D2S2395*	169.58	*STR15-1002*	133.69	.11	3.77	3.67
6	*D2S2950*	165.18	*STR15-1002*	133.69	.11	3.77	3.66
7	*D2S321*	163.27	*D15S642*	133.60	.11	3.52	3.41
8	*D2S2241*	163.27	*D15S642*	133.60	.11	3.52	3.41
9	*D2S321*	163.27	*STR15-1002*	133.69	.11	3.50	3.40
10	*D2S2241*	163.27	*STR15-1002*	133.69	.11	3.50	3.40

NOTE.—The first set of 10 rankings represents the strongest ΔNPL and interaction NPL scores on chromosome 2q22-q24 with the use of STRs on 15q as conditional markers. The second set of 10 rankings represents the strongest ΔNPL and interaction NPL scores on chromosome 15q26 with the use of STRs on 2q as conditional markers. In addition, for each scan marker, the baseline NPL score is presented.

[a] Determined from the deCODE Genetics sex-averaged map. No genetic map information is available for markers *D15S107, STR15-980,* and *STR15-994*. For these markers, genetic map positions were calculated by interpolating their physical positions (according to the UCSC Genome Browser) with the nearest marker listed by the deCODE Genetics sex-averaged map.

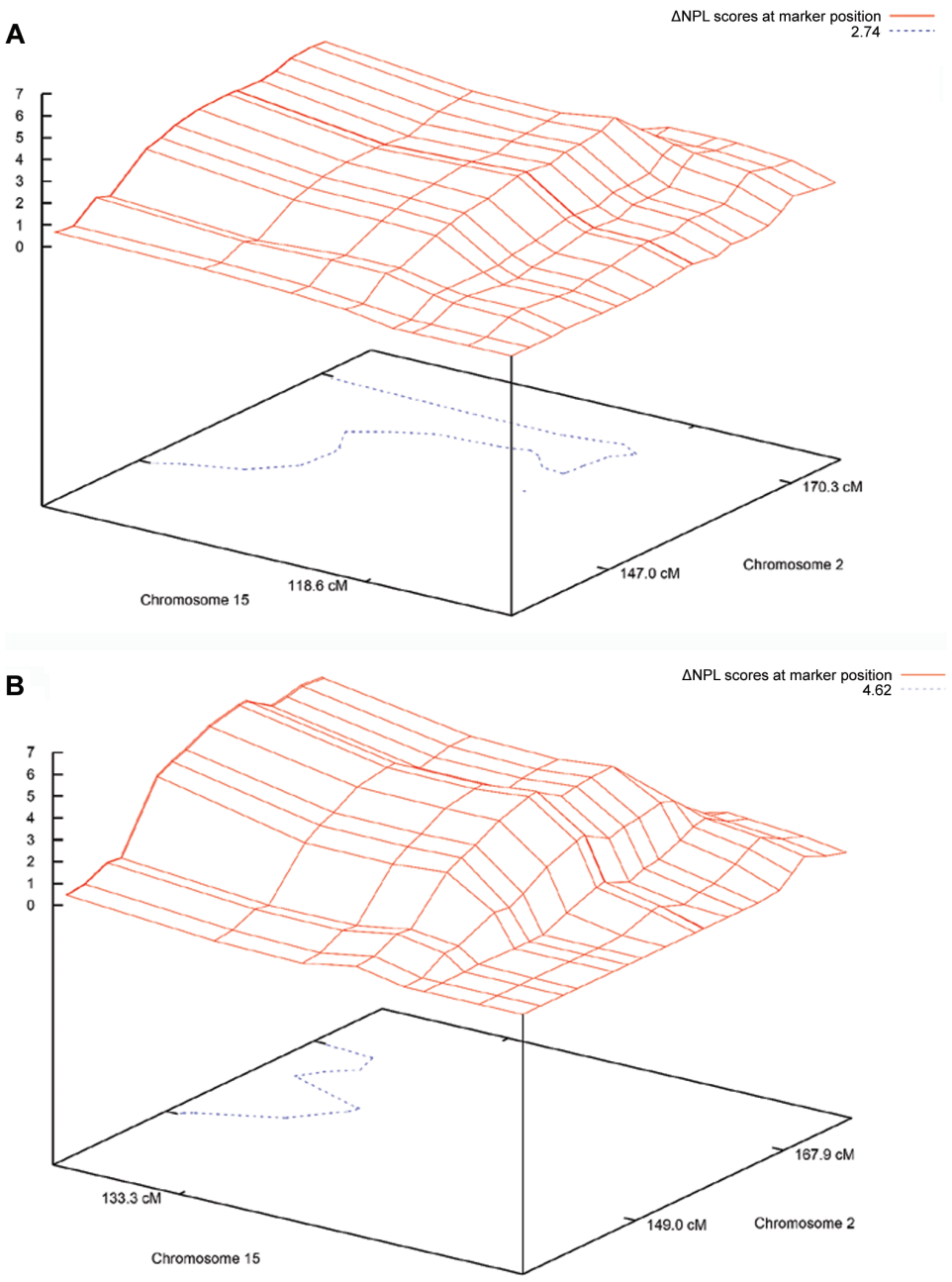

Figure 4. Three-dimensional ΔNPL plot for the BPAD interaction between chromosome 2q22-q24 and 15q26. The 1-LOD intervals are given at the bottom of the plot, in blue. Genetic-marker positions are determined from the deCODE Genetics sex-averaged map, and ΔNPL scores are indicated by red lines. *A,* Interaction on chromosome 15q26, presented using STRs on 2q as conditional markers (highest ΔNPL score 3.74). *B,* Interaction on chromosome 2q22-q24, presented using STRs on 15q as conditional markers (highest ΔNPL score 5.62).

score of 2.67 at *D6S1009*) (see table 3 and the work of Schumacher et al.[4]), chromosome 2q22-q24 showed no linkage evidence within this study (NPL scores 0.23–1.06) (see table 3). This BPAD locus was detectable only by the performance of a two-dimensional linkage scan. With use of a 1-LOD interval, the underlying BPAD gene on 2q is located between 150 and 166 cM (fig. 2*A*), corresponding to ~137 and ~157 Mb, respectively, according to National Center for Biotechnology Information (NCBI) build 36.1. Although this region has not been listed among the confirmed BPAD-linkage regions so far (as reviewed in the work of Craddock and Forty[2]), evidence of a BPAD gene within this interval comes from independent studies. At 145 Mb, Middleton et al.[13] observed the second-best result—NPL score of 3.09—within their genomewide scan of 25 multiplex families with BPAD. At 147 Mb (marker *D2S151*), Ewald et al.[14] found an NPL score of 4.24 in one multiplex family with BPAD, and Fallin et al.[15] reported

Table 7. Genomewide Top 10 ΔNPL Scores in BPAD, Ordered according to Regions of Locus Heterogeneity

	Conditional Marker			Scan Marker						
Rank	Chromosome	Marker	Position[a]	Chromosome	Marker	Position[a]	Baseline NPL	Heterogeneity NPL	ΔNPL	*P*[b]
1	11	*D11S1981*	25.59	6	*D6S477*	9.18	.54	3.19	2.65	.0004
2	6	*D6S1613*	96.90	X	*DXS7108*	18.37	.01	2.60	2.59	<.0001
3	11	*D11S912*	137.90	3	*D3S4545*	23.45	−.14	2.59	2.59	.0005
4	16	*D16S3396*	61.64	8	*D8S1128*	135.57	.41	3.00	2.59	.0004
5	21	*D21S2052*	29.48	22	*D22S689*	32.92	.08	2.65	2.57	.0001
6	13	*D13S265*	80.80	2	*D2S1399*	152.04	.38	2.94	2.57	.0096
7	11	*D11S1279*	57.39	9	*D9S1122*	74.35	−.17	2.56	2.56	.0001
8	22	*D22S1169*	68.82	2	*D2S1334*	145.08	.75	3.29	2.55	.0154
9	9	*D9S910*	101.60	1	*D1S549*	239.66	.79	3.32	2.53	<.0001
10	22	*D22S1169*	68.82	11	*D11S1998*	119.99	−.08	2.52	2.52	.0001

NOTE.—For each scan marker, the ΔNPL and the heterogeneity NPL scores are presented. In addition, the baseline NPL score observed in the one-dimensional genomewide scan is presented.

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

[b] *P* values are determined through 10,000 genomewide permutations.

an NPL score of 2.16 in 41 families with BPAD. At 159 Mb (at *D2S1353*), Cheng et al.[16] observed a 2-point LOD score of 2.07 in 154 families with BPAD. In addition, the BPAD genomewide association study by Ophoff et al.[17] identified two adjacent three-STR-marker haplotypes starting at 154 Mb, which were associated in 109 patients.

Furthermore, chromosome 6q23-q24 is implicated as harboring a BPAD gene by independent studies (reviewed by Craddock and Forty[2]). The 1-LOD interval indicates that the BPAD susceptibility locus is located between 131 and 148 cM on chromosome 6 (fig. 2*B*), corresponding to ~132 and ~147 Mb, respectively, according to NCBI build 36.1. Within this region, Venken et al.[18] found their second-best linkage result with a multipoint LOD score of 3.25 between 142 and 149 Mb (*D6S310* and *D6S1654*) in nine multiplex families with BPAD. At 137 Mb (marker *D6S1009*), Ewald et al.[19] reported a 2-point LOD score of 2.49 in two multiplex families with BPAD, and Rice et al.[20] observed a (moderate) 2-point LOD score of 2.08 in 97 families with BPAD. *D6S1009* is the conditional marker that produced the strongest interaction on 2q within our genomewide scan (ΔNPL score 6.94 at *D2S1399*) (table 3). In addition, the identified region on 6q23-q24 overlaps with the most significant implicated BPAD locus in the linkage meta-analysis by McQueen et al.[21] They combined the data sets of 11 individual BPAD-linkage studies and found genomewide significant linkage evidence on chromosome 6q, with a peak LOD score of 4.19 at 108 Mb.

Collectively, the data provide strong evidence of BPAD genes between 137 Mb and 157 Mb on chromosome 2 and between 132 Mb and 147 Mb on chromosome 6, which contribute epistatically to BPAD. According to the RefSeq Genes track (University of California–Santa Cruz [UCSC] Genome Browser), the genomic intervals on chromosomes 2q and 6q contain 32 and 70 known genes, respectively. Although speculative, there are some genes within both regions that act through the same or related pathways. For example, several genes involved in inflammatory processes are located on 2q22-q24 (*TNFAIP6* [MIM 600410] and *NMI* [MIM 603525]) and on 6q23-q24 (*TNFAIP3* [MIM 191163], *IL20RA* [MIM 605620], *IL22RA2* [MIM 606648], and *IFNGR1* [MIM 107470]). These genes are interesting, since some studies point to an inflammatory pathomechanism in BPAD (reviewed in the work of Liu et al.,[22] Kaufman,[23] and O'Brien et al.[24]). In addition, lithium, the medication of first choice for the long-term treatment of BPAD, is known to have inflammation-modulating effects (see the work of Maes et al.,[25] Bournat et al.,[26] and Nemeth et al.[27]). However, systematic SNP-based linkage disequilibrium (LD) mapping should lead to the identification of the BPAD genes within both regions. The consideration of the underlying epistasis and the application of conditional LD studies may be crucial for the successful positional cloning of these genes.

BPAD-Interaction Evidence between Chromosomes 2q22-q24 and 15q26

Similar to chromosome 2q22-q24, which has not been listed among the confirmed BPAD-linkage regions so far (see above), chromosome 15q26 has attracted less attention in BPAD. This may reflect the limited power of one-dimensional linkage scans to detect loci that act through epistasis. However, four independent studies that used samples from families affected with BPAD or combined BPAD and schizophrenia reported linkage evidence within the 15q26 interaction region. Defined by the 1-LOD criterion, the present results indicate that the interesting 15q interval is located between 118 cM and the telomere (~133.7 cM) (fig. 4*B*), corresponding to 95 Mb and 100.3 Mb, respectively, according to NCBI build 36.1. Within this region, Maziade et al.[28] observed a maximized LOD score of 4.55 at 96 Mb (at *D15S1014*) in 21 multiplex families affected by BPAD and/or schizophrenia. Using the same phenotype definition, Vazza et al.[29] found an NPL score of 3.05 in 16 families with BPAD or schizophrenia at the same marker (*D15S1014*). At *D15S642,* which was the strongest implicated 15q marker in our interaction

Table 8. Top 100 Genomewide Heterogeneity ΔNPL Scores in BPAD, Ordered according to the Conditional Chromosomes

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

scan, Park et al.[30] observed a 2-point LOD score of 1.96 in 40 families with BPAD. *D15S642* (at 100 Mb) also belongs to one of the 21 markers that showed significant LD in the genomewide BPAD-association study by Ophoff et al.[17] In addition, 15q26 is implicated in major depressive disorder (MDD). Holmans et al.[31] reported a multipoint LOD score of 3.73 at *D15S652* (at 90 Mb) in their first-phase MDD sample of 297 families and confirmed this finding with a multipoint *Z* likelihood-ratio score of 3.05 between 90 Mb and 93 Mb in their full MDD sample of 656 families with MDD.[32]

However, since only a moderate proportion of families contributed to our interaction evidence on 2q and 15q and since the observed epistasis between both loci appeared to be less impressive than the interaction seen for 2q and 6q, our 2q-15q BPAD interaction finding should be interpreted more carefully and requires confirmation. It therefore seems premature to discuss whether our interaction findings between 2q22-q24 and 6q23-q24 and between 2q22-q24 and 15q26 point to a multidimensional epistasis involving all three loci. However, in our genomewide scan, we observe a ΔNPL score of 4.53 at *D6S1009,* using *D15S642* as a conditional marker (rank 15) and a ΔNPL score of 4.39 in the vice-versa direction (rank 20) (table 2), which may support the idea of a higher-dimensional epistasis.

Independent Interaction Linkage Studies in BPAD

Two studies that apply interaction linkage analysis to BPAD have been previously published; both used preselected markers. McInnis et al.[33] selected five conditional STRs that showed NPL scores between 2.2 and 3.3 in their baseline linkage scan and performed an interaction analysis with 153 families with BPAD-affected sib pairs. They observed linkage increases (ΔNPLs) in the range 1.7–2.7 (interaction NPL scores between 2.3 and 3.1) at five different marker combinations. None of these findings showed ΔNPL scores ⩾2 within our genomewide scan (data not shown), which, together with the level of their interaction evidence, may indicate that their results represent more-moderate epistatic effects. Furthermore, the families in the study of McInnis et al.[33] were assigned using the $\text{weight}_{1\text{-}0}$ method, whereas we applied the $\text{weight}_{\text{PROP}}$ method of Cox et al.[10] In construction of the $\text{weight}_{1\text{-}0}$ family weighting, families are assigned weight 0 if their NPL score at the conditional locus is ⩽0 and weight 1 if their NPL score is >0. In contrast, within the $\text{weight}_{\text{PROP}}$ method, more-complex family-specific weights proportional to the evidence of linkage at the conditional locus are used, and it has been shown that both weighting methods can lead to different results.[10] The second BPAD-interaction study analyzed 18 preselected markers across chromosome 6 in 245 families with affected sib pairs and pointed to an epistatic effect between 6p22 and 6q16-q21.[34] Our study design was restricted to the analysis of interchromosomal epistasis only. Although this represents a limitation of our study, we used this design to avoid false-positive results by analyzing STRs, which are on the same chromosome and may therefore segregate dependently.

Locus Heterogeneity in BPAD

Compared with our BPAD-interaction findings, the locus-heterogeneity analysis produced a less consistent picture. Although several of the ΔNPL scores were significant by permutation, inspection of the family-specific data re-

Table 9. Permutation Analysis of the Top 10 Locus-Heterogeneity ΔNPL Scores

Conditional Locus		Scan Locus[a]			Permutation Analysis[b]				
Marker	No. of Families with Weight[c] >0, >1	Marker	Baseline NPL	Heterogeneity NPL	Heterogeneity NPL Exceeded (*P*)	Average NPL[d]	Min. NPL[e]	Max. NPL[e]	SD[f]
D11S1981	24, 1	*D6S477*	.5441	3.1911	4 (.0004)	.279	−2.2587	3.6576	.8239
D6S1613	36, 6	*DXS7108*	.0076	2.5962	0 (<.0001)	.0026	−2.1758	2.2011	.6125
D11S912	34, 5	*D3S4545*	−.1400	2.5872	5 (.0005)	−.0945	−2.4013	3.1298	.7696
D16S3396	33, 3	*D8S1128*	.4134	2.9987	4 (.0004)	.2493	−2.2675	3.2651	.8035
D21S2052	26, 3	*D22S689*	.0809	2.6527	1 (.0001)	.0538	−1.9114	2.9359	.6929
D13S265	33, 3	*D2S1399*	.3762	2.9446	96 (.0096)	.2359	−2.5743	4.2921	1.0330
D11S1279	28, 1	*D9S1122*	−.1708	2.5562	1 (.0001)	−.1065	−2.4815	2.6951	.7140
D22S1169	31, 1	*D2S1334*	.7477	3.2931	154 (.0154)	.4218	−2.1720	5.4990	.0831
D9S910	36, 4	*D1S549*	.7892	3.3150	0 (<.0001)	.5184	−1.7529	3.1184	.7245
D22S1169	31, 1	*D11S1998*	−.0844	2.5228	1 (.0001)	−.0574	−2.2374	2.6153	.6446

[a] Results of the heterogeneity analysis obtained at the scan locus.
[b] Results of the permutation analysis.
[c] Number of families included in the heterogeneity analysis, with use of $\text{weight}_{\text{PROP}}$ factors >0 and $\text{weight}_{\text{PROP}}$ factors >1.
[d] Average NPL score observed from the permutation procedure.
[e] Minimal (Min.) and maximal (Max.) NPL score observed from the permutation procedure.
[f] SD from the average NPL.

vealed that each of these results was attributable to a small number of families (between 1 and 6 families each). Therefore, these results should be viewed with caution and need further confirmation. However, our findings on chromosomes 2q, 6p, 13q, and 22q showed a more congruent picture of locus heterogeneity, and they have been proposed to harbor BPAD risk genes by independent studies. For example, *BDNF* (MIM 113505), one of the most implicated candidate genes in BPAD (as reviewed in the work of Craddock and Forty[2]), is located in our heterogeneity region on 11p13-p15. Furthermore, our STRs on 6p24-p25 represent the closest genomewide scan markers to *DTNBP1* (MIM 607145), which also has attracted attention in BPAD.[35–38] Although speculative, our results may provide evidence that families with BPAD who share no *BDNF* risk variants at 11p are more susceptible to BPAD risk variants at 6p or *DTNBP1*. In addition, chromosomal regions 13q31 and 22q13 were both highlighted by one of the linkage meta-analyses applied to BPAD so far[39] and by individual linkage studies. Detera-Wadleigh et al.[40] and Kelsoe et al.[41] observed strong BPAD-linkage evidence on 13q and 22q, which overlap with our findings on both chromosomes. Furthermore, BPAD-linkage evidence on chromosome 13q was reported by Shaw et al.[42] Thus, the present study provides evidence that families who share no BPAD risk genes at the linkage loci 13q and 22q could be more susceptible to BPAD risk genes on 2q22-q24, which represents a BPAD-linkage region as well (see above). Convincing linkage evidence has been independently reported for some of the other BPAD heterogeneity regions—for example, for chromosome 8q24 (as reviewed in the work of Craddock and Forty[2]). However, the corresponding conditional or scan regions have not attracted attention to BPAD so far. Although this does not necessarily exclude them as heterogeneity loci, the probability for a true-positive finding might be higher when independent studies have already reported BPAD linkage at both sides—the conditional and the scan region.

Conclusions and Outlook

Our study represents the first systematic genomewide interaction and locus-heterogeneity analysis applied to BPAD. With use of this approach, chromosome 2q22-q24, which showed no linkage evidence in our one-dimensional linkage scan, has been strongly implicated as harboring a BPAD gene, which interacts epistatically with a second risk locus on 6q23-q24. Although multidimensional linkage scans involve multiple testing, making it crucial to control the overall type I error (e.g., see the work of Frankel and Schork[43]), we suppose that the 2q-6q interaction represents a true-positive finding. This is implicated by the strength of interaction evidence (NPL scores 7.55 on 2q and 7.63 on 6q), the results through 10,000 permutations ($P < .0001$ and $P = .0001$, respectively), and the fact that a high proportion of our families contribute to this interaction. In addition, both loci have been implicated independently in BPAD, and it seems rather unlikely to find by chance evidence of a BPAD epistasis between both regions when applying a systematic interaction approach.

Several studies propose that the consideration of gene-gene interaction at the association level offers great potential in the identification of risk genes for complex disorders (e.g., the work of Carlson et al.[44] and Lin et al.[45]). However, in the absence of established risk genes, only hypothesis-free genomewide interaction linkage data can provide systematic insights into the framework of epistasis. This is the strength of interaction linkage scans, which could be of importance in forthcoming genomewide association studies. The loci identified through interaction linkage scans should lead to more-comprehensive strategies in the analysis of genomewide LD data, and the application of conditional LD analyses may facilitate the identification of the underlying risk and interacting genes.

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Web Resources

The URLs for data presented herein are as follows:

deCODE Genetics, http://www.decode.com (for information about the genetic map)

Institute for Medical Biometry, Informatics and Epidemiology, University of Bonn, http://mendel.meb.uni-bonn.de/~rfuerst/supplementary/ (for statistical programs used in the present study)

NCBI, http://www.ncbi.nlm.nih.gov/ (for information about BPAD and the candidate genes)

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for BPAD, *TNFAIP6, NMI, TNFAIP3, IL20RA, IL22RA2, IFNGR1, BDNF,* and *DTNBP1*)

UCSC Genome Browser, http://genome.ucsc.edu/ (for information about the marker positions and RefSeq Genes track)

World Health Organization, http://www.who.int/whr/2002/whr2002_annex3.pdf (for World Health Report 2002)

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