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A genome screen for genes predisposing to bipolar affective disorder detects a new susceptibility locus on 8q

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Bipolar affective disorder (BPAD), also known as manic depressive illness, is a severe psychiatric disorder characterized by episodes of mania and depression. It has a lifetime prevalence of ~1% in all human populations. In order to identify chromosomal regions containing genes that play a role in determining susceptibility to this psychiatric condition, we have conducted a complete genome screen with 382 markers (average marker spacing of 9.3 cM) in a sample of 75 BPAD families which were recruited through an explicit ascertainment scheme. Pedigrees were of German, Israeli and Italian origin, respectively. Parametric and non-parametric linkage analysis was performed. The highest two-point LOD score was obtained on 8q24 (D8S514; LOD score = 3.62), in a region that has not attracted much attention in previous linkage studies of BPAD. The second best finding was seen on 10q25–q26 (D10S217; LOD score = 2.86) and has been reported in independent studies of BPAD. Other regions showing 'suggestive' evidence for linkage localized to 1p33–p36, 2q21–q33, 3p14, 3q26–q27, 6q21–q22, 8p21, 13q11 and 14q12–q13. In addition, we aimed at detecting possible susceptibility loci underlying genomic imprinting by analyzing the autosomal genotype data with the recently developed extension of the GENEHUNTER program, GENEHUNTER-IMPRINTING. Putative paternally imprinted loci were identified in chromosomal regions 2p24–p21 and 2q31–q32. Maternally imprinted susceptibility genes may be located on 14q32 and 16q21–q23.

INTRODUCTION

Bipolar affective disorder (BPAD), also known as manic depressive illness, is a severe psychiatric disorder characterized by episodes of mania and depression. The disorder is common with a lifetime prevalence of ~1% in all human populations. The etiology is multifactorial. Family, twin and adoption studies provide strong support for an important genetic component (1). Theories concerning the possible involvement of multiple genes of modest effect and/or the occurrence of major allelic effects in epistasis have been advanced. Concurrently, environmental factors must also play a role since the concordance rate in monozygotic twins is 70–80%.

In the absence of substantial molecular pathophysiological knowledge, linkage analysis is one of the best available methods to identify chromosomal regions harboring genes that

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contribute to the development of BPAD. Mainly due to problems concerning diagnosis, ascertainment of pedigrees, and the use of statistical methods derived from work on monogenic traits, early linkage findings on chromosome 11p15 (2) and the X chromosome (3–5) could subsequently not be replicated (6–10). However, these experiences have led to major methodological improvements, including advances in diagnostic procedures, ascertainment procedures, statistical methods for linkage analysis of complex genetic traits and high-throughput molecular genetic techniques. In the past few years, several groups worldwide have been undertaking linkage studies in a large series of families with BPAD. Promising findings, most of which have been reported in at least two independent studies, are beginning to emerge. Chromosomal regions of interest include 4p16 (11–13), 4q35 (14), 10p (15), 10q25–q26 (16,17), 12q23–q24 (18–21), 13q32 (13), 18p11.2-cen (22–25), 18q21–q23 (23,24,26–29), 21q22 (30–32) and 22q (16).

We have previously reported on results of screens of chromosomes 10 and 18. Our data provided evidence for linkage of BPAD to markers in chromosomal region 10q25–q26 (17) and 18p11.2 (24). Here, we report the results of a complete genome screen at an average marker distance of 9.3 cM in a set of 75 BPAD families from Germany, Israel and Italy. The family sample comprises 194 possible affected sib pairs under a broad disease model and represents one of the largest BPAD sib pair samples worldwide. Our data provide strong evidence for susceptibility loci for BPAD on 8q24 and 10q25–q26 that are to some extent supported by prior linkage findings. Other regions of interest were identified on 1p, 2q, 3p, 3q, 6q, 8p, 13q and 14q.

Further, we addressed the question whether imprinted loci might play a role in the development of BPAD, as has been suggested from clinical (33–35) and molecular studies (23,24,36). For this purpose, linkage analyses were conducted with a recently developed extension of the GENEHUNTER program, GENEHUNTER-IMPRINTING (37). This method allowed to perform the first systematic study to scan the whole autosomal genome for imprinted disease loci in BPAD. We found evidence for possible susceptibility loci underlying genomic imprinting in chromosomal regions 2p, 2q, 14q and 16q.

RESULTS

A total of 382 highly polymorphic microsatellite markers covering the whole genome at an average spacing of 9.3 cM were genotyped. Two genetic transmission models (dominant and recessive) and two affection status models [narrow (ASM I) and broad (ASM II)] were deliberately chosen prior to linkage analysis. Thus, two-point LOD scores were calculated under four models. In addition, parametric multipoint LOD scores were calculated for a dominant, recessive, maternal imprinting and paternal imprinting model, using GENEHUNTER-IMPRINTING, for the broad and narrow affection status model. This results in eight models examined by multipoint analysis. Non-parametric linkage (NPL) scores were calculated for the broad and narrow affection status model. This is an exploratory approach to linkage analysis and aims at modeling the phenotype–genotype relation for BPAD as closely as possible.

The results of the two-point LOD score analysis are depicted in Table 1. The Table lists all markers that yielded a LOD score of ≥1.0 under at least one of the four models examined. Twelve markers on chromosomes 1, 2, 3, 8, 10, 13 and 14 provided 'suggestive' linkage evidence that surpassed a proposed threshold for genome-wide screens of 1.9 (38): D1S234, D1S220, D2S368, D2S141, D2S326, D3S1565, D8S382, D8S514, D10S1757, D10S217, D13S175 and D14S276 (given in 'bold' in Table 1). The results of the NPL analyses are presented in Figure 1.

The strongest evidence for linkage to BPAD in the entire genome screen was observed at 8q24. Under the dominant narrow model, D8S514 (located at 130 cM from the top of chromosome 8) yielded a two-point LOD score of 3.619 and a multipoint HLOD score of 3.01 ($\alpha = 0.66$) (data not shown). The NPL score (narrow model) showed a maximum at the same location (3.56; $P = 0.00029$). The two-point LOD score obtained at this locus surpasses the threshold for 'significant' linkage proposed by Lander and Kruglyak (38).

A second region on chromosome 8, 8p21, provided 'suggestive' evidence for linkage under the recessive narrow model. Consistently, a two-point LOD score of 2.303, a multipoint HLOD score of 2.64 ($\alpha = 0.47$), and an NPL score of 2.34 ($P = 0.01$) indicated a region at marker D8S382 (located at 51 cM).

The second best evidence for linkage was obtained for chromosomal region 10q25–q26. At marker D10S217, a two-point LOD score of 2.86 (dominant broad model) (Table 1) and an NPL score of 2.36 ($P = 0.01$) (Fig. 1) were observed. Genotyping of additional markers in this region and inclusion of the data in the multipoint analysis yielded a maximum NPL score of 3.12 ($P = 0.0013$) between markers D10S1483 and D10S217 (17).

Other regions showing 'suggestive' evidence for linkage to BPAD were identified on 1p, 2q, 3p, 3q, 13q and 14q.

On 1p33–p36, GENEHUNTER localized a possible disease locus between D1S197 and D1S220 (NPL score of 3.09; $P = 0.0016$) under the broad disease model. The multipoint HLOD peaked at 2.04 ($\alpha = 0.56$) in that region (dominant broad model). On 2q21–q33, a maximum multipoint HLOD score of 2.76 ($\alpha = 0.63$) under the recessive narrow model and an NPL score of 2.64 ($P = 0.0047$) between D2S368 and D2S141 supported the 'suggestive' two-point LOD scores. Two possible BPAD loci may be located on chromosome 3: on 3p14, a maximum NPL score of 3.70 ($P = 0.0004$) was found between markers D3S1285 and D3S659 using the broad model. The multipoint HLOD for the dominant broad model was 1.99 ($\alpha = 0.47$) at the same position, thus ~8 cM apart from the maximum two-point LOD score (1.882 for D3S3614 located at 101.6 cM). The second linkage signal on this chromosome was obtained at 3q26–q27 near marker D3S1614 positioned at 177.8 cM with an NPL score of 2.44 ($P = 0.008$) under the narrow model. Another region of interest is located close to the centromere of chromosome 13. The dominant narrow model yielded a two-point LOD score of 2.337 for D13S175 (positioned at 6.0 cM from the top of the chromosome) for the dominant narrow model. Evidence for linkage was supported by multipoint HLOD analysis. The peak HLOD score was 3.426 ($\alpha = 0.99$), but was placed 'off the marker map' at –12.8 cM which most likely indicates a wrong specification of the genetic transmission model at this locus, a problem which is notorious in multipoint linkage analysis. A maximum NPL score of 3.28 ($P = 0.00069$) was obtained at

Table 1. Results of two-point LOD score analysis

Marker	Position[a]	Recessive broad LOD (θ_{max})	Recessive narrow LOD (θ_{max})	Dominant broad LOD (θ_{max})	Dominant narrow LOD (θ_{max})
D1S468	004.2		1.261 (0.15)		
D1S228	029.9		1.108 (0.20)		1.267 (0.15)
D1S234	**055.1**	1.377 (0.10)		**2.028 (0.10)**	
D1S255	065.5	1.540 (0.10)			
D1S220	**087.3**			**2.154 (0.10)**	
D1S424	126.2		1.311 (0.15)		1.385 (0.10)
D1S484	169.7			1.443 (0.15)	
D2S281	014.1	1.081 (0.10)			
D2S367	055.0			1.063 (0.15)	
D2S286	094.1	1.207 (0.10)			
D2S368	**144.5**		**1.977 (0.15)**		
D2S141	**161.3**	1.260 (0.10)	**1.961 (0.15)**	**1.995 (0.15)**	1.460 (0.15)
D2S326	**177.5**			**2.052 (0.15)**	
D2S364	186.2		1.197 (0.20)		1.441 (0.15)
D2S117	194.5				1.467 (0.15)
D3S1304	022.3	1.353 (0.10)			
D3S3614	101.6	1.166 (0.15)	1.292 (0.20)	1.882 (0.10)	
D3S1565	**186.0**	**1.954 (0.05)**	**2.090 (0.15)**	1.065 (0.10)	1.388 (0.15)
D3S1262	201.1				
D4S394	014.5			1.107 (0.20)	
D4S1599	023.1		1.000 (0.20)		
D4S419	032.6		1.322 (0.20)		
D4S418	043.9	1.522 (0.10)			
D4S1575	132.1		1.019 (0.20)		1.548 (0.15)
D4S1548	153.5			1.175 (0.15)	1.000 (0.15)
D5S674	047.1	1.247 (0.15)		1.239 (0.15)	
D5S424	082.0		1.365 (0.20)		
D5S617	095.4		1.424 (0.20)		
D6S443	025.1			1.071 (0.15)	
D6S261	120.4	1.281 (0.10)			1.258 (0.20)
D6S262	130.0				1.061 (0.20)
D6S311	148.2				1.244 (0.15)
D6S1961	150.0				1.629 (0.20)
D6S305	166.6				1.134 (0.20)
D7S645	080.4			1.297 (0.15)	
D7S530	134.6	1.069 (0.15)			
D7S684	147.2				1.216 (0.20)
D8S258	041.6				1.501 (0.15)
D8S382	**051.2**	1.517 (0.10)	**2.303 (0.15)**		
D8S260	079.4	1.672 (0.10)	1.252 (0.20)		1.079 (0.20)
D8S514	**130.0**		**2.743 (0.15)**		**3.619 (0.05)**
D8S284	143.8		1.016 (0.20)		1.272 (0.15)
D9S171	042.7	1.267 (0.10)			
D9S161	051.8		1.292 (0.20)		
D9S1832	112.6				1.132 (0.20)

Table 1. *Continued.*

Marker	Position[a]	Recessive broad LOD (θ_{max})	Recessive narrow LOD (θ_{max})	Dominant broad LOD (θ_{max})	Dominant narrow LOD (θ_{max})
D10S192	124.3				1.101 (0.20)
D10S597	128.7		1.057 (0.20)		
D10S1757	**141.7**			**2.121 (0.10)**	1.492 (0.15)
D10S587	147.6			1.480 (0.15)	
D10S217	**157.9**			**2.862 (0.05)**	
D11S1313	058.4				1.260 (0.15)
D11S987	067.5	1.116 (0.15)			
D13S175	**006.0**		1.464 (0.15)		**2.337 (0.10)**
D13S221	012.9				1.067 (0.20)
D13S1246	020.4				1.285 (0.15)
D14S261	006.5		1.501 (0.20)		
D14S288	047.5	1.018 (0.15)	1.046 (0.20)		1.134 (0.15)
D14S276	**065.4**	**1.998 (0.05)**	1.004 (0.20)	1.424 (0.15)	1.592 (0.15)
D15S130	098.0				1.199 (0.15)
D16S405	027.0	1.316 (0.15)			
D17S938	014.7				1.166 (0.20)
D17S808	082.6			1.297 (0.15)	
D17S802	106.8				1.574 (0.15)
D17S784	116.9				1.308 (0.15)
D20S118	039.3			1.074 (0.15)	1.389 (0.15)
D20S119	061.8	1.442 (0.10)			
D22S274	051.5		1.232 (0.20)		1.457 (0.15)

[a]Map positions (cM from top of the chromosome) were taken from the Marshfield sex-averaged map (59).

0.0 cM. In chromosomal region 14q12–q13, a multipoint HLOD score of 1.70 (α = 0.58) under the dominant narrow model and an NPL score of 2.57 (P = 0.0057) indicated a region between markers D14S70 and D14S288 (positioned at 40.1 and 47.5 cM, respectively).

For a small number of loci, multipoint linkage analyses yielded marked differences to the two-point LOD score results. In this respect, the most interesting region is located on 6q. Over a region of >40 cM, two-point LOD scores between 1.0 and 1.6 were observed under the dominant narrow model. The multipoint HLOD score reached 3.75 (α = 0.60) at marker D6S262, the highest HLOD score observed in the entire genome screen. The NPL score under the narrow model was 2.99 (P = 0.0017) at the same locus. Other regions with two-point LOD scores below the threshold for 'suggestive' evidence for linkage but stronger support for linkage from NPL scores were: 4q (NPL score 2.6; P = 0.005 at D4S430), 5q (NPL score 2.54; P = 0.007 at D5S674), 15q (NPL score 2.77; P = 0.003 between D15S127 and D15S130) and 16p (NPL score 2.57; P = 0.0057 between D16S405 and D16S420).

Another goal of this study was the identification of imprinted BPAD loci. As an analytical tool, we used GENEHUNTER-IMPRINTING. For this purpose, HLOD scores generated under four models (paternal imprinting narrow, paternal imprinting broad, maternal imprinting narrow and maternal imprinting broad), each characterized by a penetrance vector of four elements, were compared to the regular three-penetrance HLOD scores. The following chromosomal regions provided substantially higher HLOD scores under the imprinting models: on chromosome 2, there are two peaks for the paternal imprinting model, one between D2S305 and D2S165 [HLOD score 2.12 (α = 0.60); broad model] and one between D2S396 and D2S206 [HLOD score 2.20 (α = 0.53); narrow model] (Fig. 2). On 4p16, the region initially reported by Blackwood *et al.* (11), two-point LOD scores of 1.107 (dominant broad) for D4S394 and 1.0 for D4S1599 (recessive narrow) were seen. For a paternal imprinting model, a maximum HLOD of 1.89 (α = 0.50) was obtained at D4S394. A second region on chromosome 4, close to marker D4S1566, showed a peak HLOD score of 1.87 (α = 0.51) under the same model. On 11p, close to D11S1313, the paternal imprinting model yielded an HLOD score of 1.646 (α = 0.50) under the broad model.

Evidence for the existence of maternally imprinted loci was seen on 14q and 16q (Fig. 2). On 14q, the HLOD score maximized between D14S65 and D14S78 (2.476, α = 0.69). The highest HLOD on 16q (2.099, α = 0.49) localized at D16S516.

DISCUSSION

We have conducted a complete genome screen with 382 markers in a sample of 75 systematically ascertained families with BPAD that were of German, Israeli and Italian origin. The

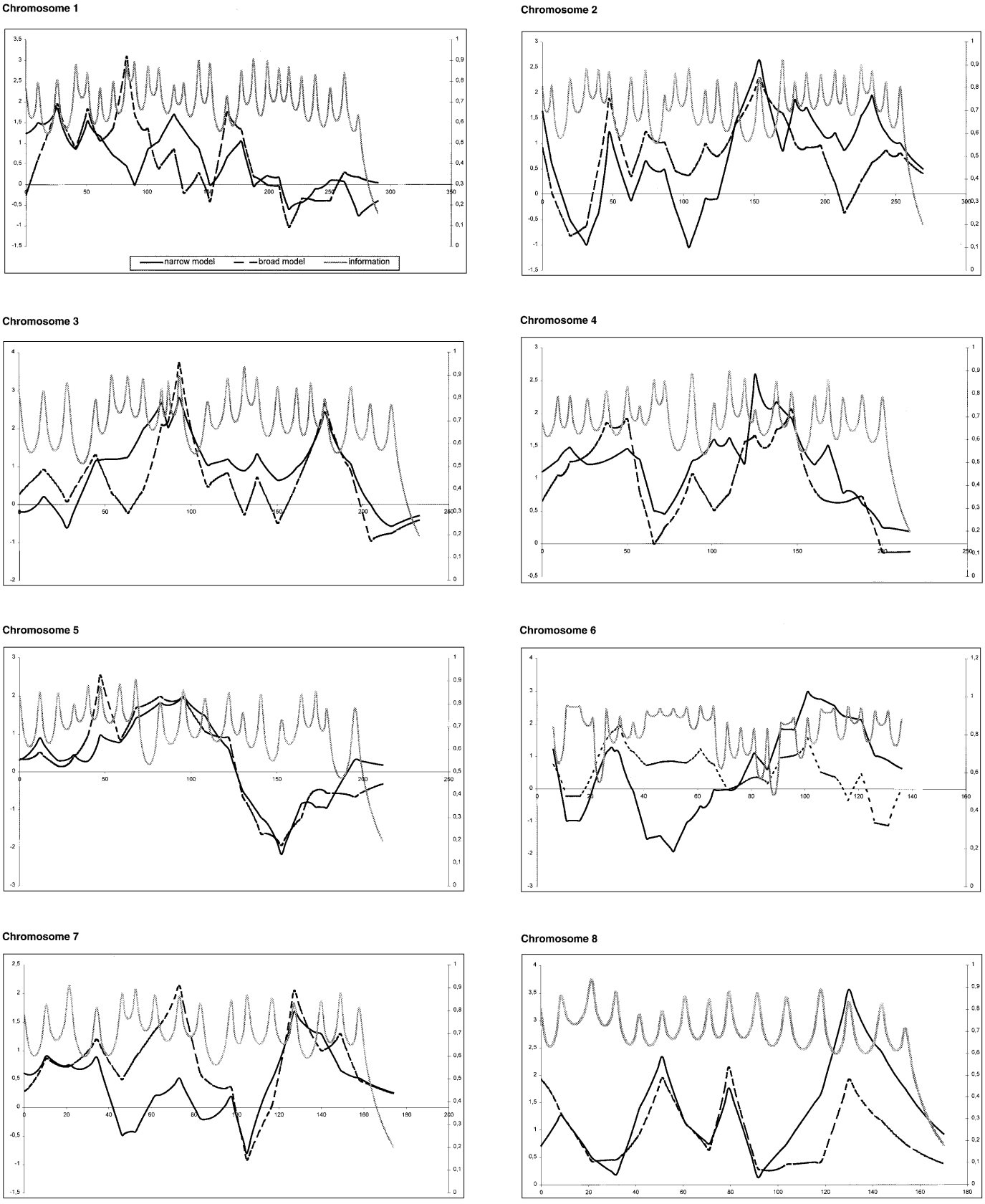


Figure 1. Results from GENEHUNTER analysis. Each chromosome is separately plotted. The information content (scale on right side *y*-axis) at each marker and between markers is shown (grey line). Also shown are the NPL scores (scale on the left side *y*-axis) for the narrow (continuous black line) and broad (dashed black line) disease model. On the *x*-axis, positions from the top of the chromosome are given in cM.

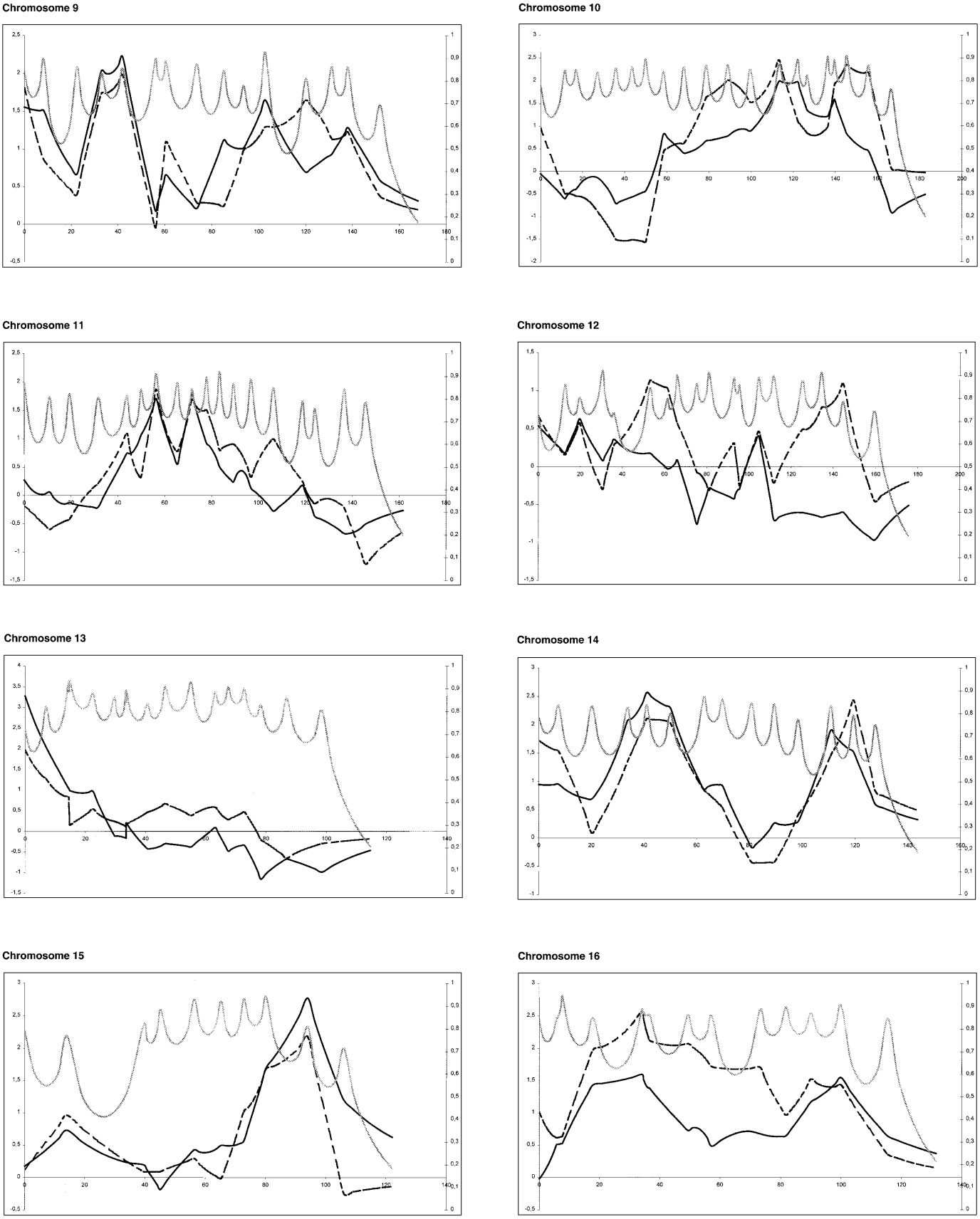
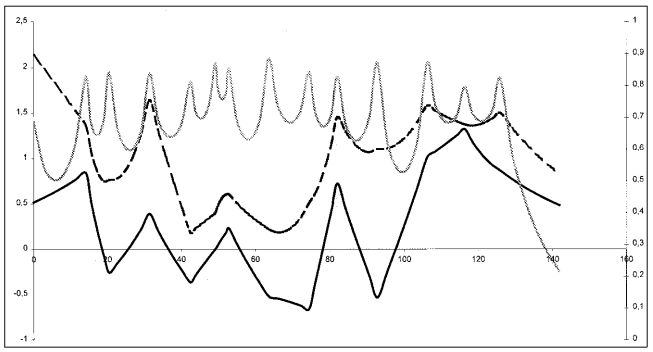
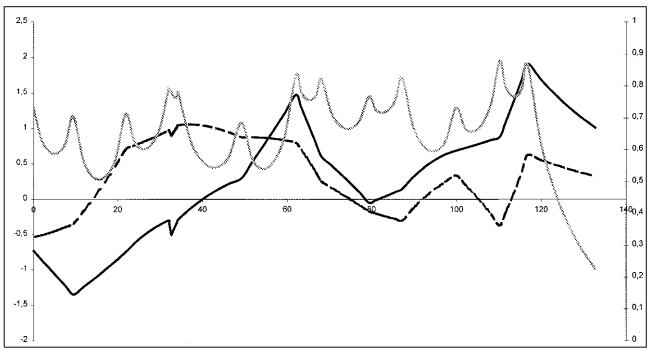


Figure 1. *Continued.*

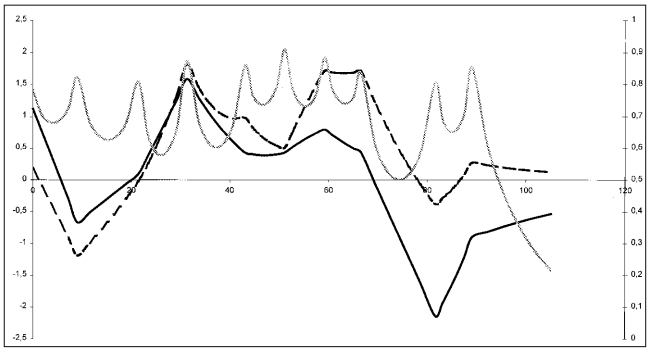
Chromosome 17



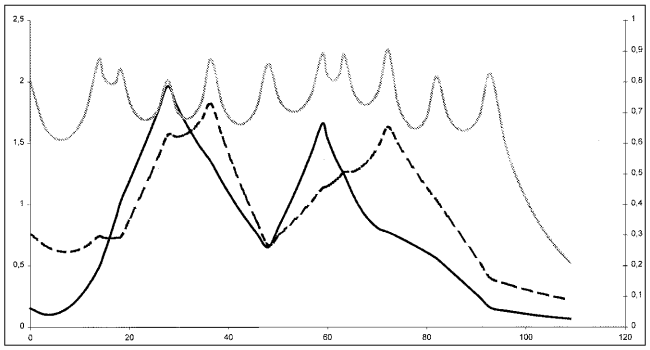
Chromosome 18



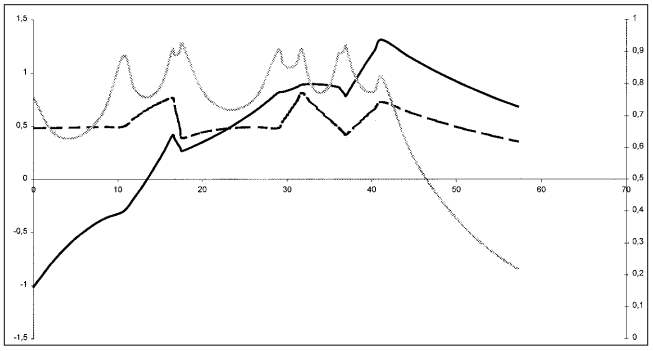
Chromosome 19



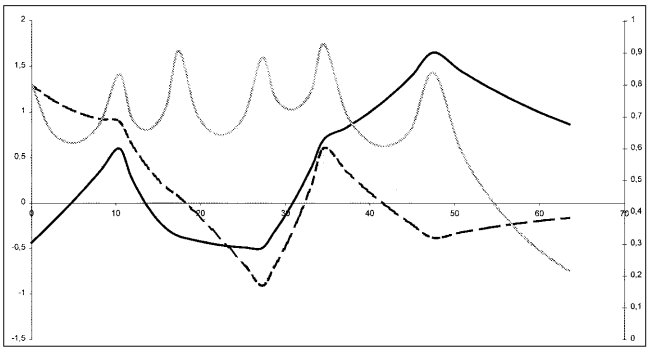
Chromosome 20



Chromosome 21



Chromosome 22



Chromosome X

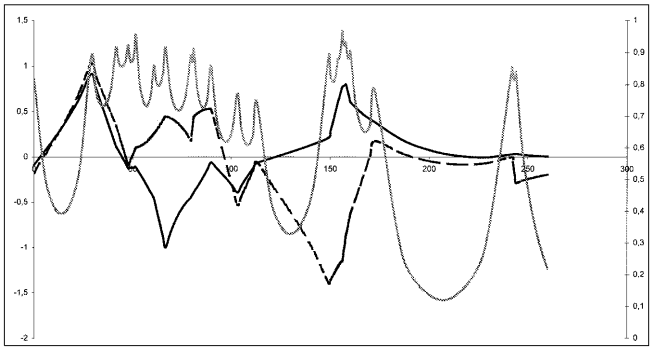


Figure 1. *Continued.*

aim of this study was the identification of chromosomal regions containing genes that play a role in determining susceptibility to this psychiatric condition. To achieve this goal, parametric and NPL methods were applied. A relatively prudent approach to parametric two-point and multipoint LOD score analysis was used, in particular 'affecteds-only' analysis and assumption of parameters that allow for phenocopies. Specification of the models followed the aim to best cover the 'true' mode of inheritance with a minimum number of models tested. Hence, LOD scores were not maximized with respect to disease model parameters. Although multipoint analysis extracts more linkage information than does two-point analysis, we give particular weight to the presentation of two-point LOD scores because they are less sensitive to misspecification of the genetic trait model (39). In addition to conventional LOD score analysis, the autosomal genotype data were analyzed with GENEHUNTER-IMPRINTING (37) to detect possible susceptibility loci underlying genomic imprinting. Furthermore, NPL analysis was conducted which might have superior power to detect linkage if the models specified for parametric analysis are not sufficiently close to the true mode of inheritance of BPAD.

The highest linked region in the genome screen, 8q24, provides a consistent picture in both parametric and non-parametric analyses (two-point LOD score of 3.6 at D8S514; multipoint HLOD of 3.01, $\alpha = 0.66$, and NPL score of 3.56, $P = 0.00029$). The positive linkage region is large and extends between 118 and 154 cM on the Marshfield map. Although 8q24 has not attracted particular attention in previous linkage studies of BPAD, a review of results from published genome screens provides at least moderate support for this locus. Interestingly, some evidence for this locus comes from a study by Friddle *et al.* (40) who concluded to have failed to detect linkage in a genome screen of 50 BPAD families. Their conclusion was based on the absence of significant LOD score results (≥3.3). Nevertheless, three regions produced HLOD scores ≥2.0. The best region was 8q, where an HLOD of 2.39 ($\alpha = 0.42$) under a dominant genetic model was obtained between D8S256 and D8S272 (positioned at 148 and 154 cM, respectively). Although the distance between the HLOD peaks in the genome screen by Friddle *et al.* and ours is ~20 cM, apparently there is an overlap of the positive linkage regions between both studies. Therefore, they may detect the same putative disease locus on 8q24. Some evidence for linkage with 8q24 was also found in a genome screen in 20 North American families reported by Kelsoe *et al.* (16).

Another region of interest in our genome screen is 10q25–q26. A maximum two-point LOD score of 2.86 was obtained at D10S217 using a dominant genetic model and a broad definition of the affection status. D10S587 and D10S1757, located 10 and 16 cM proximal, respectively, produced LOD scores of 1.48 and 2.12. We have genotyped additional markers at an average marker distance of 1.7 cM in an ~30 cM region between markers D10S190 and D10S212 (17). Including this information, GENEHUNTER localized a putative susceptibility locus within a 15 cM interval between markers D10S1483 and D10S217 with a maximum NPL score of 3.12 ($P = 0.0013$). Supportive evidence for an involvement of this region in BPAD comes from two other studies: Kelsoe *et al.* (16) observed a LOD score of 2.27 for D10S1223 and 1.74 for D10S217. Ewald *et al.* (41) performed a genome screen in two large Danish families with BPAD and reported a LOD score of 2.17 for D10S217. It should be noted that linkage findings for schizophrenia have been reported for the same region (42).

To some extent, other regions providing suggestive evidence for linkage in our genome screen are supported by independent studies: 1p22.3–p21 has been implicated by the NIMH Genetics Initiative on BPAD (43). Multipoint sib pair analysis localized a putative locus between D1S224 and D1S1648, ~15 cM proximal to our linkage peak. The region on 3p14 yielded the highest NPL score in our study (3.7, $P = 0.0004$) and also provided evidence for linkage in the studies by McInnes *et al.* (28) (LOD score of 2.59 at D3S1285; 2 cM distal to our peak) and Kelsoe *et al.* (16) (LOD score of 2.01 at D3S4542; 3 cM distal to our peak). Furthermore, our analyses indicate that there might be a second locus for BPAD on chromosome 3, within 3q26–q27. Again, support comes from the family sample investigated by Kelsoe *et al.* (16) where several markers yielded modest LOD scores in a region that overlaps with the region implicated by our study. The highest LOD score found by Kelsoe *et al.* was 2.66 at D3S2398.

Another interesting region in our study is 13q11. D13S175 yielded a two-point LOD score of 2.34 under the dominant narrow model, HLOD and NPL scores reached 3.43 ($\alpha = 0.99$) and 3.28 ($P = 0.00069$), respectively. A small positive LOD score for the same marker was reported by McInnes *et al.* (28). Other genome screens have detected greater evidence for linkage of 13q with BPAD, although these studies probably detect a distinct locus located ~30–90 cM further distal (13,16,44).

The new tool GENEHUNTER-IMPRINTING has enabled us to perform the first systematic study covering the autosomal genome for putatively imprinted disease loci in BPAD. This was originally stimulated by clinical data suggesting a parent-of-origin effect operating in the inheritance of BPAD (33–35) and has been supported by molecular genetic studies (23,24,36). A possible explanation for the results of these studies is the existence of a paternally imprinted, i.e. maternally expressed, susceptibility locus on 18p11.2. Due to the lack of efficient statistical tools to detect imprinting effects, the only possible way to investigate such an effect was a subdivision of the samples into families inheriting BPAD through the paternal or the maternal lineage. However, this leads to a substantial loss of power because usually only a small fraction of the families can be reliably grouped. Further, if linkage is detected in 'maternal' or 'paternal' subgroups, imprinting is only one possible explanation for such an effect. The use of GENEHUNTER-IMPRINTING now provided the tool to directly model imprinting and extract information from all BPAD families. It should be noted that, in terms of significance, when a predefined disease model is used, LOD scores obtained with GENEHUNTER-IMPRINTING are directly comparable to standard three-penetrance LOD scores. In chromosomal regions 2p24–p21, 2q31–q32, 4p16, 4q31, 11p11–p12, 14q32 and 16q21–q23, HLOD scores under the imprinting models were substantially higher than those obtained with non-imprinting models. However, of these, only HLOD scores in the regions on 2p, 2q, 14q and 16q exceeded the threshold for suggestive evidence for linkage. Replication in other studies will be necessary to assess the validity of these findings and we strongly encourage other research groups to analyze their genome screen data using methods that

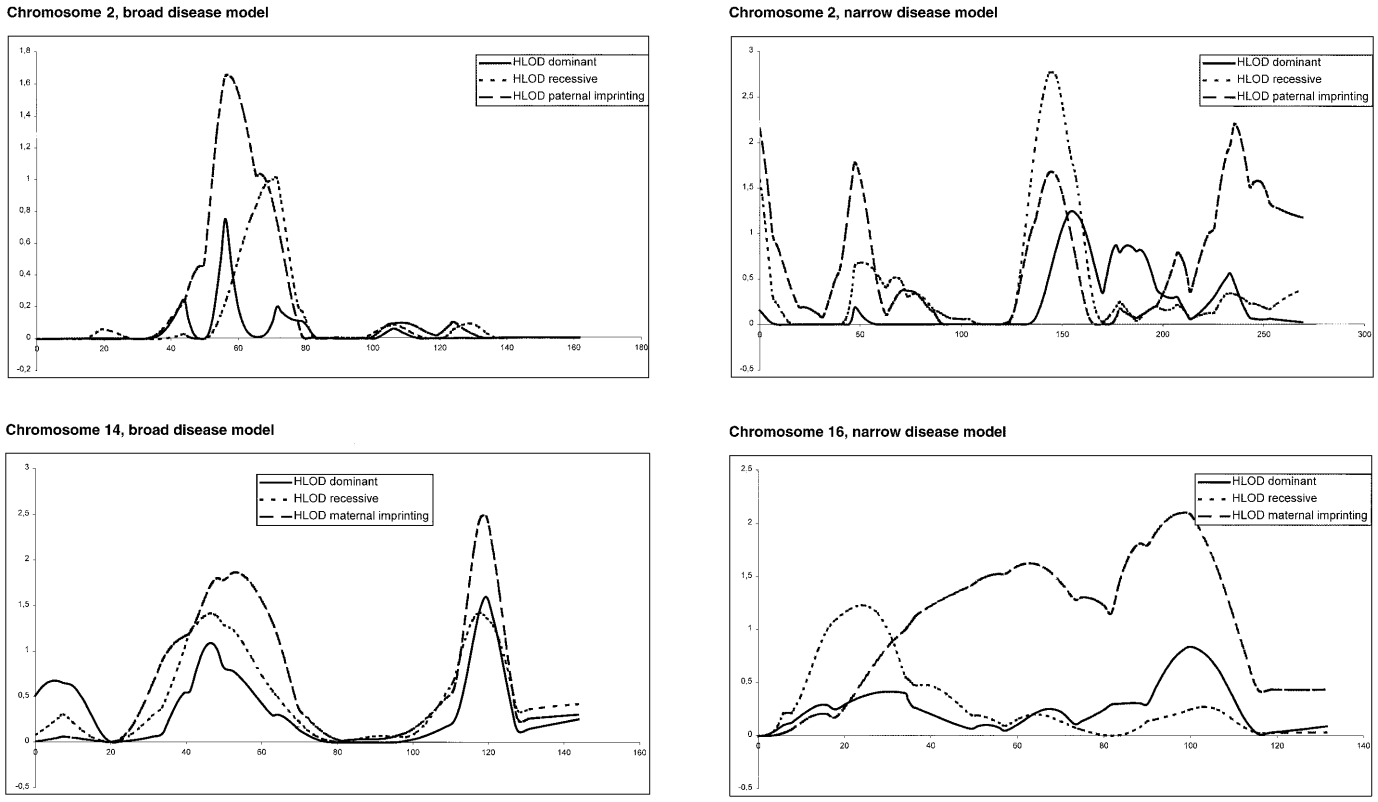


Figure 2. Results from GENEHUNTER-IMPRINTING analysis for chromosomes 2, 14 and 16. HLOD scores (scale on the left side *y*-axis) are depicted for the maternal or paternal imprinting model (dashed line). For comparison, non-imprinting HLOD scores, i.e. dominant (continuous line) and recessive (dotted line), are also shown.

adequately take a parent-of-origin effect into account, such as GENEHUNTER-IMPRINTING.

In an earlier linkage study, 14 'paternal' families that are part of the family sample investigated in this study, had provided evidence for linkage to 18p11.2 (24). A possible explanation for this finding was the presence of a paternally imprinted locus in this region. However, GENEHUNTER-IMPRINTING analysis of the data in the present study did not confirm the hypothesis of an imprinted locus on 18p11.2. Interestingly, when applying the regular GENEHUNTER version, a multipoint HLOD of 1.79 ($\alpha = 0.36$) was obtained under a recessive narrow model. Thus, by grouping the 'paternal' families in our previous study, we may have separated a genetically more homogeneous portion of the family sample that has a susceptibility locus on 18p11. However, taking into account the results of this study, this locus does not appear to be subject to genomic imprinting. An alternative explanation would be that our initial linkage finding on 18p11.2 simply occurred by chance.

The number and statistical significance of the loci identified in this genome screen exceeds expectations of chance findings in such a study for a given model and type of analysis (38). Here, in the context of a disease that definitely is genetically complex, we regarded the type II error of not finding a disease gene as more serious than the type I error of claiming linkage where it is not present. Therefore, we have performed exploratory linkage analysis under a couple of different models. This certainly increases the overall probability of a type I error. Still, it is very likely that at least a portion of the identified loci represents 'true' signals. Yet, it is difficult to judge whether a linkage peak represents a true signal or simply noise. It is of course possible that the largest effects observed in a genome screen may be false positives, whereas true genetic factors may only give modest signals. As noted by Kruglyak and Lander (45), a 10 cM map only extracts ~70% of the total information about identical-by-descent (IBD) status at a marker and ~60% between markers. To some extent, genotyping of additional markers around our hot spots should clarify the importance of some of these regions. Further evidence has to come from replication by independent studies or from meta-analyses of several genome scans, as proposed by Wise *et al.* (46).

In conclusion, we hold the position that the results of this genome screen are a valid basis for the identification of susceptibility genes in BPAD using a positional cloning strategy, as has successfully been performed for other complex diseases such as type II diabetes mellitus (47) and Crohn's disease (48,49).

MATERIALS AND METHODS

Family ascertainment

Seventy-five families were included in the genetic analyses, comprising of 66 families from Germany, eight families from Israel and one family from Italy. Informed consent was obtained from all individuals included in the study.

Standard diagnostic definitions for index case ascertainment and pedigree extension procedures are the following: bipolar I (BPI), schizoaffective, bipolar type (SA/BP), unipolar, recurrent (UPR), unipolar, single episode (UPS) and other minor psychiatric disorders are defined by DSMIII-R criteria (50); bipolar II (BPII) by Research Diagnostic Criteria (RDC) (51) with the modification that it requires recurrent episodes of depression. The diagnosis of BPII cannot be made in DSMIII-R, but is made through RDC. We additionally specified that depression be recurrent because of concerns about the reliability of hypomania and single-episode major depression (52).

Inclusion criteria for the systematically ascertained BP families were: (i) a proband with BPI and admission to one of the treatment facilities; (ii) a secondary affected sib with either BPI, BPII, SA/BP or UPR; (iii) availability of both parents or, if only one parent was accessible, availability of at least two more sibs from the sib-ship of the proband.

All individuals were interviewed by an experienced psychiatrist using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L) (53). Best estimate diagnoses were based on the combination of interview, review of all available clinical records and family history information.

Description of families

The families included in this study had been successively collected by a collaborative project funded by the Deutsche Forschungsgemeinschaft (DFG) between 1989 and 1999. The maximum number of families included in the genome screen was 75, consisting of 445 individuals. In the course of the genome screen, some families had to be replaced by more recently collected families because DNA samples were exhausted. The minimum number of families investigated for a single chromosome was 63. Exact figures of the number of investigated families for each single chromosome are given in Supplementary Material (Table 2).

Seventy out of the 75 families were two-generation families and five were three-generation families. Results from the complete family sample have previously been published for markers on chromosome 10 (17), and results from a subsample have been published for markers on chromosomes 12 (19) and 18 (24). The mean number of individuals per pedigree was 5.9 and the mean number of affected individuals (according to the broad affection status model) per pedigree was 2.9. The distribution of diagnoses for the 445 individuals was as follows: 128 individuals with BPI, including the index cases, 40 individuals with BPII, 14 individuals with SA/BP, 40 individuals with UPR, 51 individuals with a minor psychiatric diagnosis and two individuals with a diagnosis of schizophrenia. 170 of the genotyped individuals were unaffected. There were 60 sib-ships with two affected siblings, 17 sib-ships with three affected siblings, five sib-ships with four affected siblings, two sib-ships with five affected siblings and two with six affected siblings. Depending on the affection status model (see Linkage analyses), a maximum number of 83 (under ASM I) or 194 (under ASM II) possible affected sib pairs were included in the analyses.

Except five families (WUE49, WUE67, WUE78, MAI18, HAA117), there were no families where both parents of the index case had a psychiatric diagnosis. In family WUE49, the father had a diagnosis of BPI and the mother had a diagnosis of UPR. The father of family WUE78 displayed an adjustment disorder with depressed mood, the mother had UPS. The father of family WUE67 had UPR, the mother was BPI. In family MAI18 the father was diagnosed with UPS, the mother with BPII. Both father and mother of family HAA117 had a personality disorder. According to the affection status models applied in this study, only families WUE49 and WUE67 display bilineal transmission of the disease.

DNA isolation and cell lines

EDTA anticoagulated venous blood samples were collected from 445 individuals who were available for the study. Leukocyte DNA was isolated as described by Miller *et al.* (54). Whenever possible, leukocytes were isolated and transformed using Epstein–Barr virus in order to establish permanent cell lines.

Genotyping

A total of 382 highly polymorphic microsatellite markers, taken from the ABI Prism Linkage Mapping Set Version 1.0 (Applied Biosystems) as well as from the Genome Data Base (GDB; http://www.gdb.org/gdb/) Version 5.6 were genotyped. According to the Marshfield Genetic Map (accessible at http://research.marshfieldclinic.org/genetics/), the spacing of the markers ranged from 0 to 25.4 cM and the average spacing was 9.3 cM. Individual DNA samples were arrayed in 96-well microtiter plates and subjected to amplification by PCR, with individual marker amplicons. The PCR reaction for each marker was carried out in a 10 µl volume containing 40 ng genomic DNA, 5 pmol of each primer, 200 µM of each dNTP, 50 mM KCl, 10 mM Tris–HCl pH 8.3, 1.5–2.5 mM $MgCl_2$ and 0.6 U *Taq* polymerase (Life Technologies). After an initial denaturation of 5 min at 95°C, 33 cycles of amplification of 15 s at 94°C, 15 s at 55–62°C and 30 s at 72°C were performed in a Perkin-Elmer 9600 thermocycler. The resulting amplified products were separated on 4.5% denaturing polyacrylamide gels on an automated DNA sequencer (Model 377; Applied Biosystems). Allele sizes were determined relative to an internal size standard in each lane using Genescan Analysis Version 2.1.1 and Genotyper Version 2.0 software (Applied Biosystems). In addition, a reference individual with known genotype was loaded on each gel. All gels were scored independently by two individuals who were blind to the disease status. Each marker was tested for Mendelian segregation in every family.

Linkage analyses

Two models of affection were used in the linkage analyses: ASM I (narrow definition of the phenotype) included only individuals with BPI as affected, all other psychiatric diagnoses were coded as 'unknown'; ASM II (broad definition) included all individuals with a diagnosis of BPI, BPII, SA/BP and UPR with UPS and other minor psychiatric disorders coded 'unknown'.

Two-point LOD scores were calculated by means of MLINK and ILINK programs of the LINKAGE package version 5.2 (55). All analyses were conducted using an 'affecteds-only' approach, where the disease status was set to zero for all unaffected individuals and individuals with psychiatric diagnoses not included in the broad affection status model. For both

disease definitions the LOD scores were calculated assuming both a dominant and a recessive mode of inheritance. Under the broad affection model we assumed a phenocopy rate of 3.2% and a penetrance of 50% under both genetic models. The frequency of the disease allele was set to 24.5 and 3% for the recessive and dominant genetic model, respectively. For the narrow affection model the phenocopy rate was set to 0.1%, penetrance was assumed to be 50% and the frequency of the disease allele was set to 13.4% under recessive and to 0.9% under dominant mode of inheritance. These assumptions correspond to a lifetime morbid risk of 6% for diseases included in ASM II and of 1% for BPI (ASM I) (56). An age-dependency of the penetrance was not taken into account which diminishes the effect of quantifying the correct penetrance.

To obtain a maximum of linkage information from the pedigrees, multipoint LOD scores as well as non-parametric multipoint analyses were performed using the GENEHUNTER program Version 2.0B (57,58). For the multipoint LOD score analysis, the same models as for the two-point analysis were used (dominant and recessive mode of inheritance, narrow and broad disease definition). Overall LOD scores and HLOD scores were calculated, the latter allowing for locus heterogeneity between pedigrees. To perform LOD score analysis that models a parent-of-origin effect, Strauch *et al.* (37) have incorporated a four-penetrance formulation into GENEHUNTER-IMPRINTING. By specification of two heterozygote penetrance parameters, paternal and maternal origin of the disease allele can be treated differently in terms of probability of expression of BPAD. Under the broad affection model, paternal imprinting was modeled by assuming a heterozygote penetrance of 3.2% for having inherited the disease allele from the father and a heterozygote penetrance of 50% for having inherited the disease allele from the mother. Maternal imprinting was modeled vice versa. Similar to the dominant and recessive models, the homozygous wild-type and homozygous mutant penetrances were 3.2 and 50%, respectively. The frequency of the disease allele was set to 12.5%. Under the narrow model, paternal imprinting was specified by a heterozygote penetrance of 0.1% for a paternally inherited and 50% for a maternally inherited disease allele. Accordingly, maternal imprinting was specified by a penetrance of 50% for heterozygotes having inherited a paternal disease allele and a penetrance of 0.1% for heterozygotes having inherited a maternal disease allele. The frequency of the disease allele was set to 5%. These parameterizations conform to the aforementioned prevalence constraints.

For non-parametric multipoint linkage analysis, IBD allele sharing among all affected family members was calculated for the narrow and broad affection status model using the score function S_{all} of GENEHUNTER.

Marker allele frequencies were estimated from the genotypes of the founder individuals of all pedigrees.

SUPPLEMENTARY MATERIAL

For Supplementary Material, please refer to HMG Online.

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