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Genomewide Scan for Affective Disorder Susceptibility Loci in Families of a Northern Swedish Isolated Population

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We analyzed nine multigenerational families with ascertained affective spectrum disorders in northern Sweden's geographically isolated population of Västerbotten. This northern Swedish population, which originated from a limited number of early settlers ~8,000 years ago, is genetically more homogeneous than outbred populations. In a genomewide linkage analysis, we identified three chromosomal loci with multipoint LOD scores (MPLOD) ⩾2 at 9q31.1-q34.1 (MPLOD 3.24), 6q22.2-q24.2 (MPLOD 2.48), and 2q33-q36 (MPLOD 2.26) under a recessive affected-only model. Follow-up genotyping with application of a 2-cM density simple-tandem-repeat (STR) map confirmed linkage at 9q31.1-q34.1 (MPLOD 3.22), 6q23-q24 (MPLOD 3.25), and 2q33-q36 (MPLOD 2.2). In an initial analysis aimed at identification of the underlying susceptibility genes, we focused our attention on the 9q locus. We fine mapped this region at a 200-kb STR density, with the result of an MPLOD of 3.70. Genealogical studies showed that three families linked to chromosome 9q descended from common founder couples ~10 generations ago. In this ~10-generation pedigree, a common ancestral haplotype was inherited by the patients, which reduced the 9q candidate region to 1.6 Mb. Further, the shared haplotype was observed in 4.2% of patients with bipolar disorder with alternating episodes of depression and mania, but it was not observed in control individuals in a patient-control sample from the Västerbotten isolate. These results suggest a susceptibility locus on 9q31-q33 for affective disorder in this common ancestral region.

Introduction

Bipolar disorder (BP, also known as "manic depression" [MIM 125480]) is characterized by alternating episodes of depression and mania (BPI) or hypomania (BPII), whereas recurrent unipolar depression (UPR) is characterized by recurrent episodes of depression without episodes of mania. BP and UPR are part of a spectrum of affective disorders that also includes major depression, cyclothymia, dysthymia, and schizoaffective bipolar disorder. Affective disorders are a major public health concern, because of a high lifetime prevalence (0.5%–1.5% for BP; 9.5% for UPR) and a high rate of morbidity and mortality (Müller-Oerlinghausen et al. 2002). Twin, adoption, and family studies support a strong genetic component (Craddock and Jones 1999; Smoller and Finn 2003); the heritability of BP was estimated at 80%.

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Since the pathophysiology of affective disorders is still unknown, linkage analysis is one method of identification of chromosomal regions and genes that might be involved in the etiology of BP. A major problem in linkage studies is the high degree of genetic heterogeneity among patients with affective spectrum disorders. The complex genetic etiology of affective disorders might explain the finding in independent linkage studies of several chromosomal regions, such as 1q31-32, 4p16, 6pter-p24, 10p14, 10q21-26, 12q23-24, 13q31-32, 18p11, 18q21-23, 21q22, 22q11-13, and Xq24-28 (reviewed by Baron [2002]).

Families originating from a population isolate are predicted to reduce the genetic complexity and potentially also the environmental background (Peltonen 2000). Therefore, fewer susceptibility alleles are expected in an isolated population for any complex disorder, and environmental factors are probably more homogeneous, which improves the chance of finding susceptibility loci and genes. The advantage of studying families originating from an isolated population has been proven earlier, with the promising findings of neuregulin 1 (*NRG1*) and D-amino-acid oxidase activator (earlier referred to as "G72"), for which initial significant linkage was found on 8p and 13q in analysis of Icelandic (Stefansson

Table 1

MLINK Two-Point Linkage Results, under Dominant and Recessive Models, for Markers Showing a LOD Score ≥1.2 in at Least One Model

	LOD Score for			
	Dominant Model		Recessive Model	
Marker	Z_{max}	θ_{max}	Z_{max}	θ_{max}
D2S326	***1.91***	.01	0.80	.20
D2S2248	.51	.20	***1.94***	.00
D2S126	***1.71***	.05	***1.76***	.01
D2S396	***1.74***	.10	***1.29***	.05
D4S415	***1.43***	.01	***1.46***	.05
D5S418	***1.336***	.00	.52	.10
D6S262	−.27	.40	***1.50***	.01
D6S308	.00	.10	***2.27***	.00
D9S289	1.07	.10	***2.17***	.00
D9S155	***1.55***	.05	***2.60***	.00
D9S290	.33	.20	***2.21***	.00
D12S326	.02	.30	***1.65***	.00
D12S346	.81	.20	***1.58***	.00
D12S78	.82	.10	***1.202***	.05
D14S63	.53	.10	***1.98***	.00
D17S785	.32	.20	***1.83***	.00
D18S61	1.02	.10	***1.64***	.00
D20S100	.40	.20	***1.37***	.05

Note.—Markers with a LOD score ≥1.2 are indicated in bold italics.

et al. 2002) and French Canadian families (Chumakov et al. 2002), respectively. The families used in the present linkage-analysis study were ascertained in northern Sweden. The current northern Swedish population is an old population with geographical isolation and a low immigration rate; therefore, it is considered a population isolate (Nylander and Beckman 1991). Because of reduced genetic variation, genomewide scans in families from northern Sweden have already been successful in identification of susceptibility loci in complex diseases such as schizophrenia (SZ) (Lindholm et al. 2001). Here, we present genomewide significant linkage in nine families ascertained through a proband with BPI or UPR and several family members with phenotypes within the affective disorder spectrum.

Material and Methods

Samples

Fourteen families originating from the region of Västerbotten in northern Sweden who segregated BP or UPR were ascertained at Umeå University Hospitals. The families were ascertained through probands with BPI or UPR. Of the 14 families, 5 were included in a linkage study of UPR (Balciuniene et al. 1998). In our study, the remaining nine multigenerational families with at least one family member with BPI were selected, comprising 365 individuals, of whom 60 were affected (fig. A1 [online only]). In each family, the proband was affected with BPI or UPR. In total, 171 persons from the nine families were examined. The following affective spectrum diagnoses were obtained in 46 patients: BPI (19), BPII (6), UPR (18), and schizoaffective disorder–bipolar type (SAM) (3) (table A1 [online only]). For 116 of the 171 individuals, the structured interview revealed no lifetime history of psychiatric disorders; therefore, they were considered healthy. Nine individuals obtained a diagnosis of other psychiatric disorders. For 16 individuals, no interview could be obtained. Three spouses had an affective spectrum diagnosis: one was diagnosed with BPI, whereas two had UPR.

A sample of 182 unrelated patients with BPI and 182 unrelated control individuals, originating from the same Västerbotten region, were randomly ascertained at Umeå University Hospitals in northern Sweden. The sex ratio of the patient group was 96 females:86 males, the mean age at inclusion was 56.3 years, and the mean age at disease onset was 30.8 years. The control group was matched with the patient group according to age and sex. These individuals stem from the Betula project, a large population-based prospective study described in detail elsewhere (Nilsson et al. 1997). In the present study, individuals with a lifetime history of psychotic disorders were excluded. The sex ratio of the control group was 90 females:92 males, and the mean age was 59.5 years.

All individuals were aged ≥18 years and gave informed consent, after approval of the study by the Medical Ethical Committee of the University of Umeå. Medical records were available for all patients; records were examined by experienced psychiatrists and classified according to DSM-IV criteria. The diagnoses were confirmed using the relevant chapters of the Schedules for Clinical Assessment in Neuropsychiatry (Wing et al. 1990). The medical ethical committees of the University of Umeå and the University of Antwerp approved the inclusion of the patients, their relatives, and control individuals in genetic studies.

Genotyping

From each participant, a venous blood sample was obtained, and genomic DNA was extracted using standard procedures. DNA of 187 individuals of the nine families with BP was included in a 10-cM density genomewide scan by use of 380 fluorescent-labeled STR markers (described in detail by Lindholm et al. [2001]). On the basis of genetic distances (sex averaged) of the Marshfield map (Center for Medical Genetics), the mean marker distance was 9.34 cM.

Genetic fine mapping of the candidate chromosomal

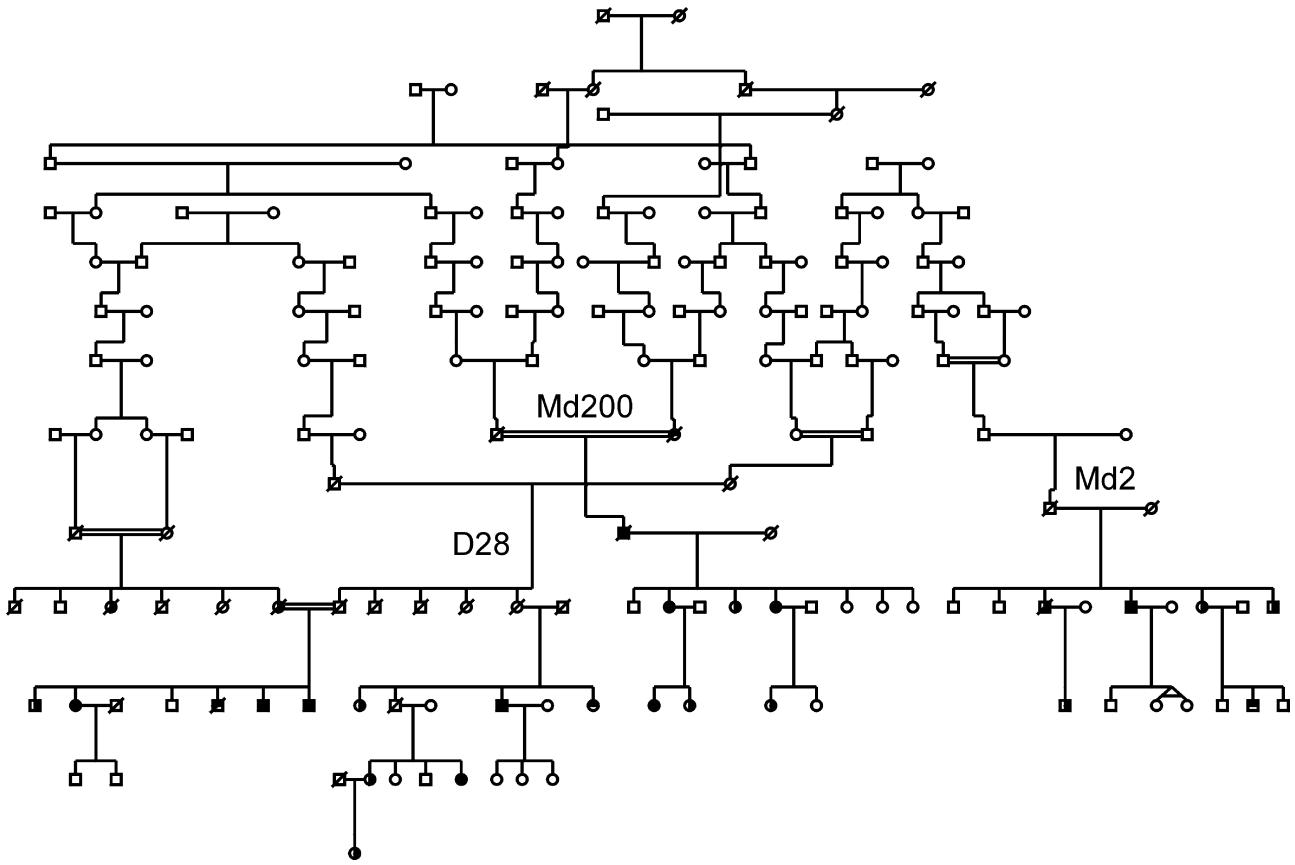


Figure 1 Genealogical ~10-generation pedigree linking the three families—MD200, MD2, and D28—to common founders. Blackened symbols represent patients.

regions was performed with STR markers selected from the Marshfield (Center for Medical Genetics) or the DECODE Icelandic genetic maps (Kong et al. 2002). In the first 2-cM fine-mapping step, map order and distances between markers were based on the genetic maps. A second fine-mapping step was performed for chromosome 9 with 15 novel STR markers, identified using the Sputnik program, to identify haplotype sharing (table A2 [online only]). Map order and distances between these markers are based on physical distances. All PCR amplifications were performed under standard reaction conditions in a total volume of 20 μl on a Primus HT, by use of fluorescent-labeled primers. After 4–9 markers were pooled, PCR products were sized on an ABI 3700 or ABI 3730 Sequencer (Applied Biosystems), and genotypes were assigned using GENOTYPER version 2.1 or Genemapper version 3.0, respectively. The STR markers used in the fine mapping were genotyped for 91 northern Swedish control individuals, to estimate population allele frequencies. Also, 25 STR markers in the LOD−1 interval of the 9q-region were genotyped in the northern Swedish patient-control–association sample, to investigate allelic and genotypic distributions and to further investigate the shared haplotype among unrelated patients and healthy individuals in the northern Swedish population.

Statistical Analysis

Simulation analysis was performed using the SLINK program (Ott 1989; Weeks et al. 1990), in which a marker with eight equally frequent alleles was hypothesized. We calculated the number of false positives; that is, the number of times that the LOD score exceeded the LOD score threshold of 1–6 under the assumption of no linkage ($\theta = 0.50$) in 10,000 replicates. The data indicated that a suggestive LOD score $\geqslant$1.2 could be expected once in a genome scan and a significant LOD score $\geqslant$2.4 once in 20 genome scans. To estimate the statistical power of the family set, we performed four simulation studies with 10,000 replicates, each under dominant and recessive models, with the disease gene located at recombination fraction $\theta = 0.05$ from the simulated marker, assuming either genetic homogeneity or 10% genetic heterogeneity. An average maximum LOD score of 2.80 was obtained under dominant and

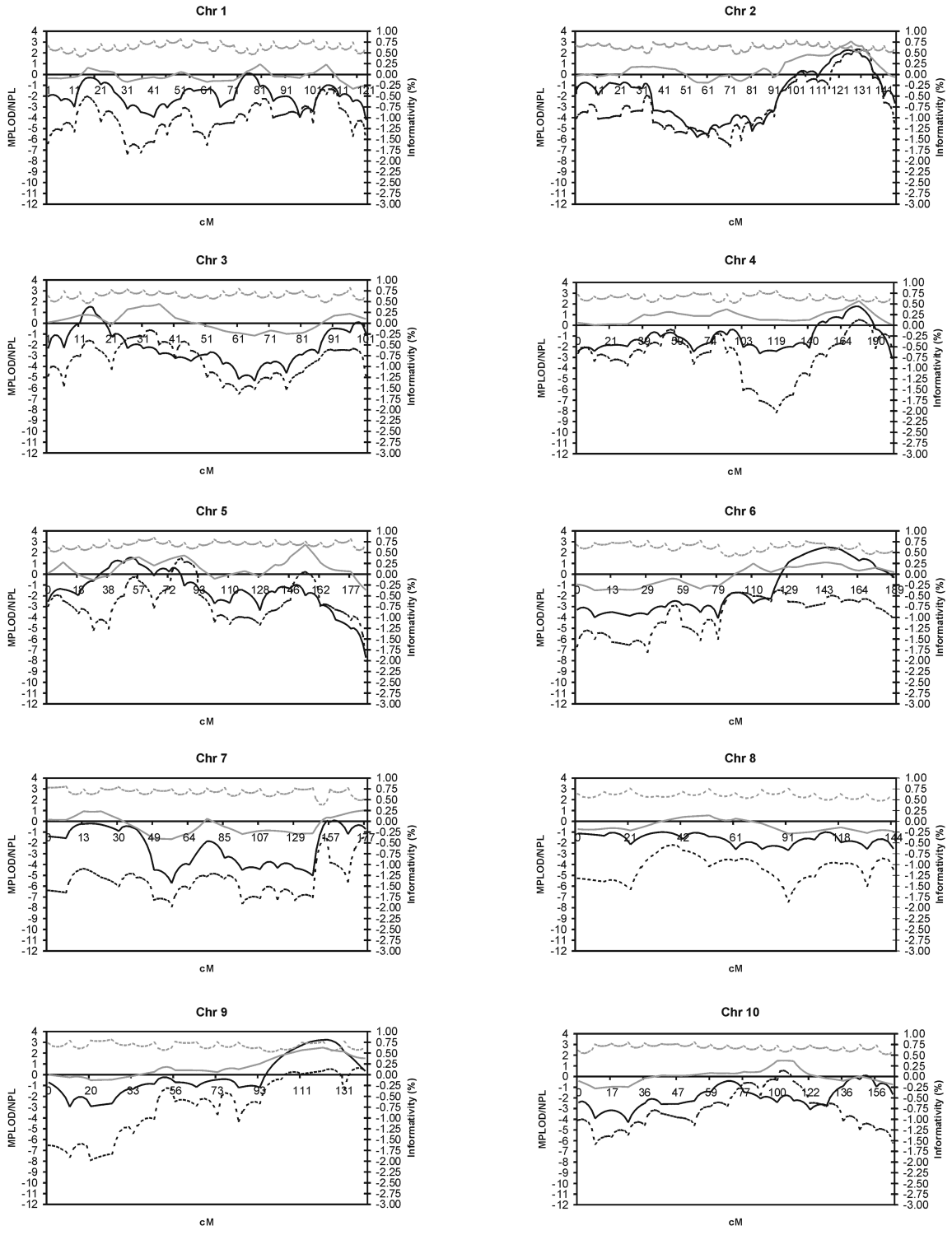
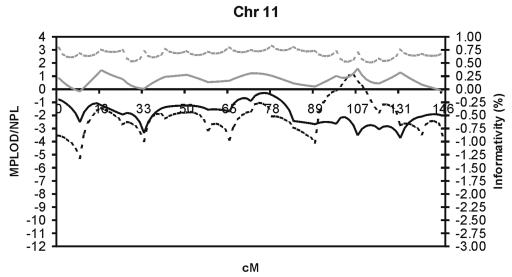
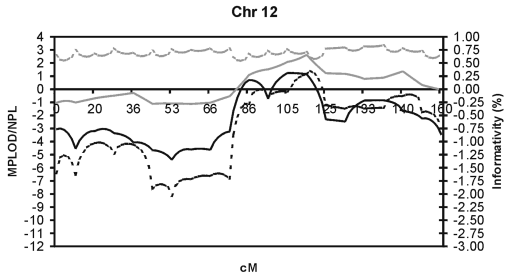


Figure 2 Multipoint linkage data of the genomewide scan for each chromosome. The black dashed curve represents MPLODs under the dominant model, the solid black curve MPLODs under the recessive model; the gray curve represents NPL scores; and the gray dashed curve represents the STR marker informativity. Genetic distances are based on the Marshfield genetic map (Center for Medical Genetics).


Chr 11
MPLOD/NPL
Informativity (%)
cM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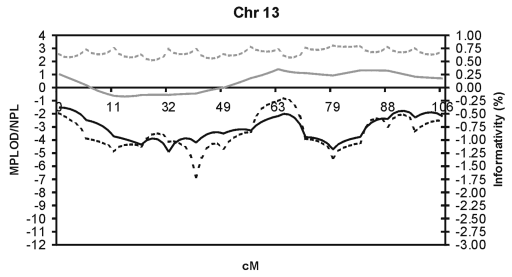
Informativity (%)
cM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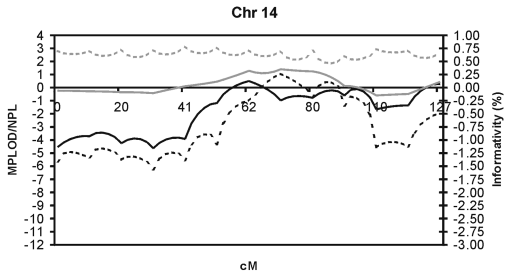
Informativity (%)
cM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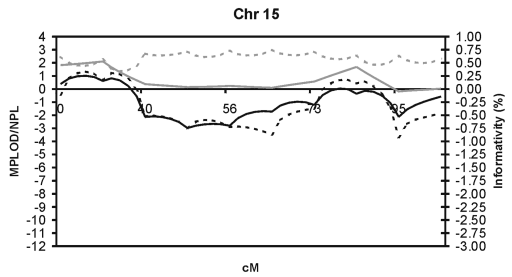
MPLOD/NPL
Informativity (%)
cM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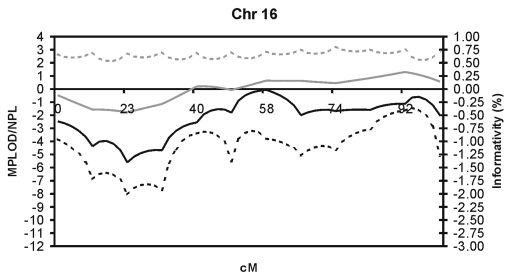
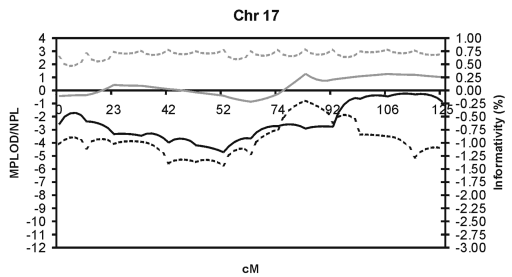
MPLOD/NPL
Informativity (%)
cM


Chr 16
MPLOD/NPL
Informativity (%)
cM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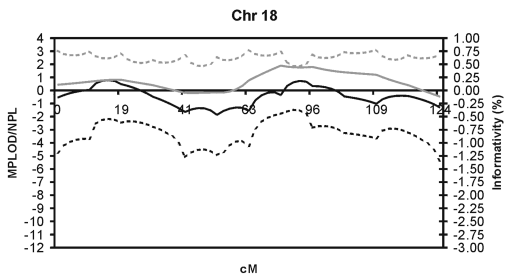
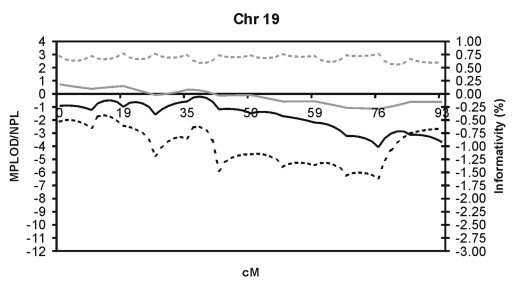
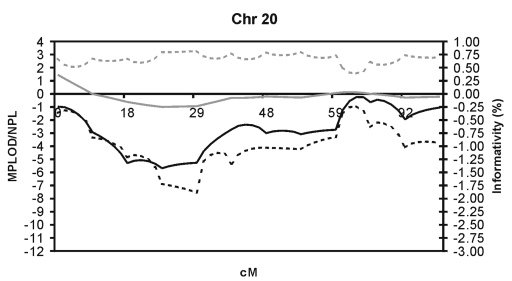
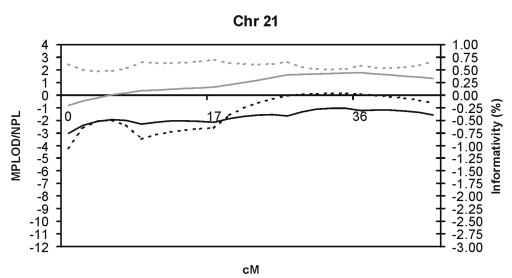
MPLOD/NPL
Informativity (%)
cM


Chr 18
MPLOD/NPL
Informativity (%)
cM


Chr 19
MPLOD/NPL
Informativity (%)
cM


Chr 20
MPLOD/NPL
Informativity (%)
cM


Chr 21
MPLOD/NPL
Informativity (%)
cM

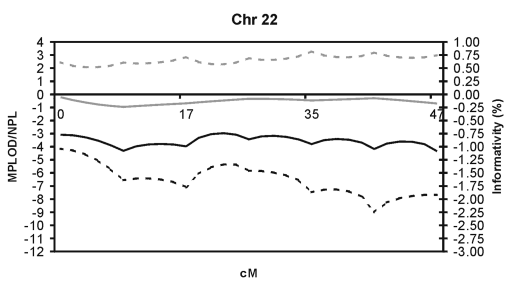

Chr 22
MPLOD/NPL
Informativity (%)
cM

Table 2

Genomewide Multipoint Parametric and Maximum NPL Scores

Chromosome	Region (cM)	Findings for: Parametric Linkage, Dominant	Parametric Linkage, Recessive	Nonparametric Linkage, NPL	Nonparametric Linkage, *P*
2q33-q35	32	***2.35***	***2.26***	***2.93***	.0085
6q22.2-q24.2	29	<0	***2.48***	1.08	.1219
9q31.1-q34.1	26	.53	***3.24***	***2.51***	.0157

NOTE.—LOD scores ⩾2.2 are indicated in bold italics.

recessive models, under the assumption of genetic homogeneity. There was 55% and 85% power to obtain significant (LOD score ⩾2.4) and suggestive (LOD ⩾ 1.2) linkage, respectively. Under the assumption of 10% genetic heterogeneity, average maximum LOD scores of 2.35 and 2.39 were obtained, under dominant and recessive models, respectively. Under the dominant model, we obtained 44% and 75% power, whereas, under the recessive model, we obtained 45% and 79% power to detect significant and suggestive evidence for linkage, respectively.

Two-point parametric linkage analysis was performed using the MLINK program (Lathrop and Lalouel 1984). Nonparametric allele-sharing statistics (nonparametric LOD [NPL] scores and corresponding *P* values), multipoint parametric linkage analysis, and haplotype reconstruction were performed using the GENEHUNTER software package beta version 2.1_r2 (Kruglyak et al. 1996; Kruglyak and Lander 1998). Linkage analysis was performed under an affected-only model, with patients phenotypes among the BP spectrum disorders. Relatives without psychiatric symptoms, with other psychiatric disorders, or who were not interviewed were considered "unknown." The phenocopy rate or percentage of patients with phenotypes unrelated to the genetic defect in a family was defined differently for BPI and SAM (1%), BPII (2%), and UPR (5%), in agreement with the population frequencies of those disorders. The disease-allele frequency in the general population was set at 0.01 and 0.10 for the dominant and recessive models, respectively.

In the genomewide linkage analysis, STR-allele frequencies were estimated on the basis of married-in individuals in the pedigree, whereas, in the candidate regions, STR allele frequencies were estimated on the basis of 91 healthy unrelated individuals ascertained in the Västerbotten region. Allelic and genotypic distribution of the 25 STR markers, in the LOD−1 interval of the 9q-region, in the northern Swedish association sample was investigated using GENEPOP version 3.3 (Raymond and Rousset 1995). EHPLUS (Zhao et al. 2000) was used to estimate haplotype frequencies in the patient-control association sample.

Results

Linkage Analysis

The families used in the 10-cM density genomewide linkage study were ascertained in northern Sweden, a region that is characterized by geographical isolation and low immigration rate and considered a population isolate (Nylander and Beckman 1991). Moreover, church records and national registers were available, and genealogy studies showed that three families—MD200, MD2, and D28—descended from common founders ~10 generations ago (fig. 1).

Linkage analysis resulted in suggestive two-point LOD scores ⩾1.2 with 18 markers on 10 chromosomes—2q, 4q, 5q, 6q, 9q, 12q, 14q, 17q, 18q, and 20q (table 1). *D9S289, D9S155,* and *D9S290* were neighboring markers only on chromosome 9 and showed LOD scores ⩾2. The multipoint linkage plots for nonparametric and parametric (under dominant and recessive models) affected-only analyses are shown per chromosome in figure 2. In eight chromosomal regions—2q, 3p, 4q, 5q, 6q, 9q, 12q, and 15p—suggestive multipoint LOD scores (MPLOD) ⩾1.2 were calculated. Chromosomes 6q and 9q had a significant MPLOD ⩾2.4, under the recessive linkage model, with highest scores at chromosome 9q, which showed an MPLOD of 3.24 corresponding to a candidate region of 26 cM (115–141 cM from 9pter) within the LOD−1 interval (table 2). Chromosome 6q had an MPLOD of 2.48, with a LOD−1 candidate region extending over 29 cM 133–162 cM from 6pter. A near-significant MPLOD of 2.26 was also observed for chromosome 2q, with a LOD−1 interval of 32 cM 198–229 cM from 2pter.

The chromosome 2q, 6q, and 9q regions were further analyzed, using additional STR markers selected from public genetic maps, to an overall marker density of 2 cM (fig. 3), and were statistically analyzed under the recessive affected-only parametric model. This resulted in (1) an MPLOD of 3.22 at 124 cM from 9pter and a candidate region of 10 cM between *D9S289* and *D9S258* (121–131 cM) (fig. 3*A*), (2) an MPLOD of 3.25 at 146 cM from 6pter and a 3-cM candidate region between *D6S310* and *D6S1654* (144–147 cM) (fig. 3*B*), and (3) an MPLOD of 2.16 at 200 cM from 2pter and a candidate region of 23 cM between *D2S374* and *D2S2197* (198–221 cM) (fig. 3*C*).

Identical-by-Descent Mapping at Chromosome 9q31.1-q34.1

Families MD200, MD2, and D28, who descended from common founder couples (fig. 1), contributed a considerable part to the linkage peak at chromosome 9q (table 3). To identify haplotype sharing among these families,

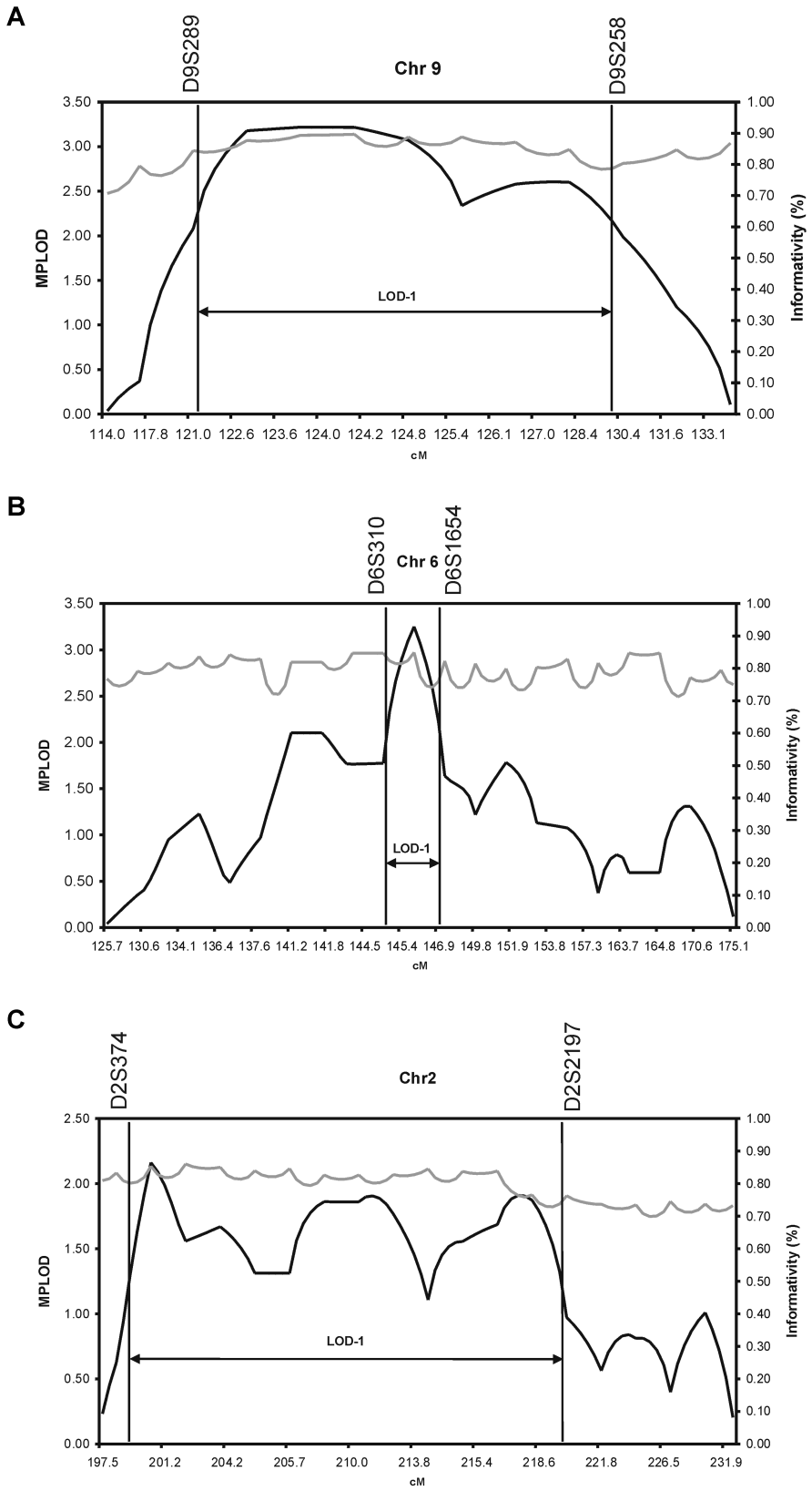


Figure 3 Multipoint linkage analyses for 2-cM STR density genotyping in the 9q (*A*), 6q (*B*), and 2q (*C*) regions, showing the MPLOD curve for the recessive model (*black curve*). For the genetic fine mapping on chromosomes 9q, 6q, and 2q, we used an additional 16, 19, and 21 STR markers. STR markers flanking the LOD−1 candidate region on each chromosome are indicated. The gray curve indicates the informativity of the STR markers in the region.

Table 3

Results of Fine Mapping Chromosomal Loci 9q, 6q, and 2q under a Recessive Model

		Results of Fine Mapping under a Recessive Model													
		Parametric Multipoint Linkage												Nonparametric Linkage	
Chromosome	Peak (cM)	MD15	MD200	MD17	MD2	MD205	MD5H	MD5U	D28	D29	MPLOD	α[a]	HLOD[b]	NPL	*P*[c]
9q31.1-q34.1	124	.12	.88	−.29	.63	.71	.24	.00	.46	.47	3.22	1.00	3.22	1.68	.05
6q22.2-q24.2	146	.09	.87	.44	1.02	.70	.17	.00	−.49	.45	3.25	1.00	3.25	1.44	.08
2q33-q35	200	.09	−.73	.50	.99	.70	.24	.00	.70	−.32	2.16	.90	2.20	2.09	.03

Note.—Families MD200, MD2, and D28 descend from common founders ~10 generations ago.

[a] α = homogeneity parameter of the estimated proportion of families linked to a locus.

[b] HLOD = heterogeneity LOD score.

[c] *P* value at linkage peak.

18 additional STR markers were selected, using the Sputnik program, to obtain a marker density of 200 kb in and around the 10-cM candidate region. Statistical analysis under the recessive model resulted in an MPLOD of 3.70 at *TV9S011*, located 122.9 cM from 9pter (fig. 4). The candidate region further decreased to 4 cM between markers *TV9S001* at 122 cM and *TV9S002* at 126 cM. Haplotypes were reconstructed for all individuals in the families, to identify haplotype sharing among patients in these families. Figure 5 shows the patients' haplotypes in the LOD−1 interval at chromosome 9q. This enabled the identification of the 5-marker haplotype 1-1-2-5-1, shared identical by descent by 18 of the 21 patients, between *TV9S014* and *TV9S017* in a 1.6-Mb region. Incomplete sharing (11/24 [46%] chromosomes) was observed at *D9S754*, mapped between *D9S1802* and *TVS016*.

Association Analysis at Chromosome 9q31.1-q34.1

For chromosome 9, allelic and genotypic distribution of 25 STR markers in and around the LOD−1 interval of the 9q-region, with a spacing of ~200 kb, were investigated in the northern Swedish BPI-association patient-control sample; *P* values are shown in table 4. Markers *D9S1824* and *D9S18642*, separated by a distance of ~6.5 cM, yielded *P* values ⩽.05. Allele 5 of *D9S1864* is contained in the shared haplotype segregating in families MD200, MD2, and D28. *D9S1802*, also contained in the shared haplotype, shows a trend toward association ($P = .06$). EHPLUS was used to estimate the frequency of five-marker haplotype 1-1-2-5-1 in the association sample. The haplotype was present in 4.2% of the patients with BPI but was absent in 182 unrelated control individuals (frequency <0.5%).

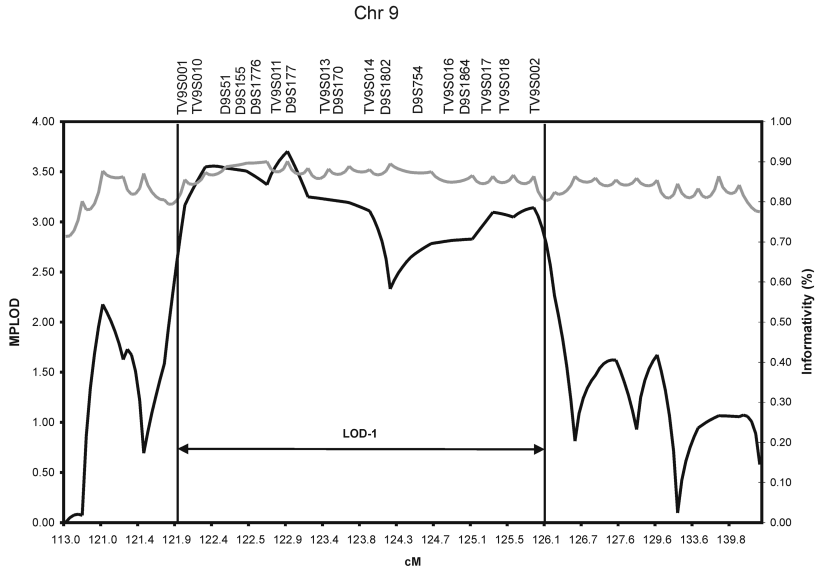


Figure 4 Multipoint linkage data for the 200-kb STR fine mapping in the 9q region, showing MPLODs for the recessive model (*black curve*). The gray curve indicates the STR marker informativity.

MPLOD	Marker	kb	cM	freq	Patient No																					
					MD200										MD2				D28							
					4		14	15	6	7		16	13		7	9	18	11	7	9	11	13	14		17		18	
					BP1		BP2	UP	UP	BP1		UP	UP		BP1	UP	SAM	UP	UP	BP1	SAM	UP	BP1		BP1		BP1	
3.17	TV9S001	19158	122.1		2	1	2	2	1	2	1	1	2	1	3	3	3	3	2	3	2	2	2	2	2	2	2	2
3.55	TV9S010	19379	122.3		3	1	3	3	1	3	1	1	3	1	3	3	3	3	3	3	3	2	3	2	3	2	3	2
3.54	D9S51	19515	122.5		4	5	4	4	5	4	5	5	4	5	4	4	4	4	4	5	4	4	5	4	5	4	5	4
3.51	D9S155	19556	122.5		5	4	5	5	4	5	4	4	5	4	4	4	4	4	6	2	6	6	5	6	5	6	5	6
3.37	D9S1776	19617	122.6		5	5	5	5	5	5	5	5	5	5	7	7	7	7	1	3	1	1	2	1	2	1	2	1
3.7	TV9S011	19939	122.9		6	6	6	6	6	6	6	6	6	6	8	8	8	8	5	11	5	5	8	5	8	5	8	5
3.25	D9S177	20117	123.1		9	6	9	9	6	9	6	6	9	6	2	2	2	2	3	5	3	3	5	3	5	3	5	3
3.22	TV9S013	20524	123.5		5	3	5	5	3	5	3	3	5	3	3	3	3	3	3	4	3	3	4	3	4	3	4	3
3.19	D9S170	20725	123.7		3	2	3	3	2	3	2	2	3	2	5	5	5	5	7	5	7	7	5	7	5	7	5	7
3.11	TV9S014	20985	123.9	0.34	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2.33	D9S1802	21281	124.2	0.46	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2.6	D9S754	21728	124.7	0.36	1	4	1	1	4	1	4	4	1	4	2	2	2	2	1	2	1	1	3	1	3	1	3	1
2.78	TV9S016	21979	124.9	0.91	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
2.8	D9S1864	22145	125.1	0.15	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	0	5	5	5	5	5	5	5	5
2.83	TV9S017	22412	125.4	0.67	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2.99	TV9S018	22557	125.5	0.12	6	6	6	6	6	6	6	6	6	6	8	8	8	8	7	7	7	7	6	7	6	7	6	7
3.06	TV9S002	22768	125.7		2	3	2	2	3	2	3	3	2	3	6	6	6	6	5	7	5	5	7	5	7	5	7	5
2.78	D9S1848	23395	126.3		1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Figure 5 Haplotype analysis in the LOD−1 interval on chromosome 9q. Only haplotypes with allele sharing between patients of the three families MD200, MD2, and D28 in the multigenerational pedigree are shown (indicated by gray shading). MPLODs corresponding to physical distance (kb) and allele frequencies (freq) of the shared alleles are given.

Discussion

We present genomewide linkage results for affective spectrum disorders in nine multigenerational families with BP ascertained in the population isolate of Västerbotten in northern Sweden. On the basis of the simulated significance LOD-score thresholds in the nine families with BP, two loci, one on 9q (MPLOD 3.24) and one on 6q (MPLOD 2.48), showed MPLODs of genomewide significance (≥2.4), whereas one locus at 2q was close to significance (MPLOD 2.26). After fine mapping at 2-cM STR density, the two susceptibility regions on 9q and 6q showed MPLODs ≥3.2 that are close to the Lander and Kruglyak (1995) levels of genomewide significance (MPLOD ≥3.3). The LOD−1 candidate regions were 10 cM for 9q31.1-q34.1 (MPLOD 3.22) and 3 cM for 6q23-q24.3 (MPLOD 3.25). The third region at 2q33-2q36 remained suggestive, with an MPLOD of 2.2 and a LOD−1 candidate region of 23 cM. For all three loci, the highest LOD scores were obtained under the recessive affected-only model, which might reflect the high degree of inbreeding in this isolated population rather than a true recessive inheritance. This is, for example, evidenced by the observation that the chromosome 9q shared haplotype appeared homozygous as well as heterozygous in different patients of the ~10-generation genealogical pedigree (figs. 1 and 5).

Interestingly, Lindholm et al. (2001) found significant genomewide linkage for an SZ susceptibility locus at 6q25, slightly more telomeric than our region 6q23-q24. The patients of the 12-generation family ascertained in the same geographic region in northern Sweden inherited a common ancestral haplotype (Lindholm et al. 2004). Patients received diagnoses within the "broad spectrum of SZ," including SZ, psychoses not otherwise specified, schizoaffective disorder depressed type, and schizoaffective disorder bipolar type. Also in our study, 72% of the patients with BP and schizoaffective patients had psychotic features. Therefore, we cannot exclude sharing at the 6q locus between this extended pedigree with SZ and some of the nine families with BP included in our study. Linkage of BP and SZ to 6q has also been observed in several other ethnic population—including isolated populations of Portuguese and Arab Israeli origin, as well as outbred populations of American, Danish, and Austrian origin—which indicates that a gene or genes at 6q might contribute considerably to the risk of psychiatric disease (Bailer et al. 2002; Ewald et al. 2002; Dick et al. 2003; Lerer et al. 2003; Middleton et al. 2004). Also, chromosome 2q has been linked to BP as well as SZ in a number of

Table 4

Results of Chromosome 9q Association Study

Locus	*P* Value for	
	Allelic Differentiation	Genotypic Differentiation
D9S1824	***.01526***	***.0218***
TV9S008	.4773	.544
TV9S001	.25324	.3693
TV9S010	.18651	.1741
D9S51	.30601	.3401
D9S155	.71646	.6744
D9S1776	.86383	.8759
TV9S011	.45454	.4252
D9S177	.71948	.7162
TV9S013	.44365	.4505
D9S170	.62938	.6487
TV9S014	.9547	.961
D9S1802	.09107	.0601
D9S754	.42837	.4322
TV9S016	.89654	.8938
D9S1864	***.0271***	***.0401***
TV9S017	.40054	.3447
TV9S002	.48055	.5029
D9S1848	.17911	.0908
TV9S020	.78411	.7702
TV9S003	.09762	.0697
D9S258	.43362	.3625
TV9S004	.58749	.5954
TV9S005	.40008	.3469
D9S1682	.0964	.0917

NOTE.—*P* values ≤.05 are indicated in bold italics.

studies in regions that are either centromeric (Cichon et al. 2001; Ewald et al. 2003) or telomeric (Paunio et al. 2001; Bennet et al. 2002; Wijsman et al. 2003) to the region we identified in the Swedish families with BP.

In a first step toward identifying the underlying susceptibility genes, we focused our attention first on the chromosome 9q31.1-q34.1 region that had the highest two-point and multipoint LOD scores in the genomewide scan. Also, our chromosome 9q signal overlaps with previous BP findings (Detera-Wadleigh et al. 1997; Badenhop et al. 2002; Liu et al. 2003; Shink et al. 2004). Further, significant evidence for linkage was found in Icelandic families, where patients diagnosed with anxiety and panic disorder showed a high comorbidity with mood disorders (Thorgeirsson et al. 2003). Though the data for the nine families were included, the 9q region was not supported by the two meta-analyses of genomewide scan data (Badner and Gershon 2002; Segurado et al. 2003). This does not exclude our linkage observation, since affective disorders have a complex etiology with a high degree of genetic heterogeneity that differs between populations.

In the northern Swedish population isolate, different genes might be responsible for the expression of the disease phenotype. In fact, in this isolated population, it is more likely that families have a similar underlying genetic defect that helps in the detection of a particular gene. This is exemplified by the observation that three of the families that contributed most to the 9q linkage finding are descendants of a few founding couples ~10 generations ago (fig. 1).

We further narrowed the 9q region by linkage analyses, using known and novel STR markers at a 200-kb density. A significant MPLOD of 3.70 was obtained, and the candidate region was reduced to a region of 4 cM cytogenetically located on 9q31-q33. In the three families that descended from common founders, a shared five-marker haplotype between *TV9S014* and *TV9S017* was observed in 18 of the 21 (85.7%) patients, which narrows the candidate region to 1.6 Mb. Of the eight patients with BPI, five were homozygous for the shared haplotype and two were heterozygous. The remaining patient with BPI, his sister with UPR (family D28), and one other patient with UPR (family MD2) did not segregate the shared haplotype. This might be attributed to the complexity of the affective spectrum phenotypes, with difficulties in diagnostic classification and specification of spectrum boundaries. Further, one marker, *D9S754,* did not contribute to the haplotype sharing. Careful examination of the data, as well as retyping, excluded genotype errors. This finding might argue against a shared haplotype, but the absence of the shared haplotype, estimated in a population of 182 unrelated control individuals from Västerbotten by use of EHPLUS (Zhao et al. 2000), makes it unlikely that this is a sharing by chance rather than a true observation. Furthermore, EHPLUS analysis of the shared haplotype in a sample of 182 patients showed that this haplotype was present in 4.2% of the patients with BPI. Therefore, the most likely explanation is that *D9S754* is meiotically unstable or that it is located in a recombinational hotspot region, so that multiple mutations have occurred (Strand et al. 1993; Majewski and Ott 2000; Ellegren 2004).

In summary, we identified three susceptibility loci for affective disorders with significant linkage at 9q31-q33 and 6q23-q24 and with suggestive linkage at 2q33-q36 in a northern Swedish population isolate. Furthermore, we were able to find a shared ancestral haplotype at 9q31-q33.

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Electronic-Database Information

The URLs for data presented herein are as follows:

Center for Medical Genetics, http://research.marshfieldclinic.org/genetics/ (for the Marshfield genetic map)
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for bipolar disorder)
Sputnik, http://espressosoftware.com/pages/sputnik.jsp

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