Linkage Analysis of Fifty-Seven Microsatellite Loci to Bipolar Disorder

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The authors' goal was to screen for genetic linkage with highly informative deoxyribonucleic acid (DNA) microsatellite markers on a series of moderately sized North American bipolar disorder (BP) pedigrees. These BP pedigrees were genotyped with 57 short tandem-repeat polymorphic systems (microsatellites) that were enzymatically amplified from genomic DNA. We did not find significant evidence for genetic linkage. We found isolated LOD scores greater than 2 on chromosome 1 at two loci in individual pedigrees. Simulation studies for multiple analyses under the assumptions of linkage and nonlinkage were performed. The simulations show that LOD scores greater than 2 could be expected even when linkage is absent. Significance levels need to be considered carefully in systematic linkage studies. ***[Neuropsychopharmacology 9:31–40, 1993]***

KEY WORDS: *Mapping; Bipolar illness; Microsatellites; Genetic linkage simulations; GNAS1; Human X-linked GABA-A receptor α3-subunit gene*

A body of epidemiologic studies implies that there is a genetic component that confers susceptibility to bipolar disorder (BP) (Nurnberger et al. 1986). However, examination of the distribution of illness in families shows that it is improbable that a single defective gene completely determines whether a person shows the disease phenotype, because the mode of genetic transmission of BP does not follow simple Mendelian rules. Several plausible theories explaining the pathophysiology of BP have been proposed, but without full support from experimental evidence, and it may be argued that the field lacks solid candidate genes (Nurnberger et al. 1986). In this context, identification of susceptibility genes by the use of genetic linkage analysis strategies that do not require an a priori knowledge of the pathophysiology of BP become an attractive research alternative. Furthermore, genetic linkage methods can detect a susceptibility gene when the exact manner of transmission is not known (Clerget-Darpoux et al. 1986). The effects of misspecification of genetic parameters in pairwise analysis is generally modest: linkage is detected but the recombination fraction is biased (Clerget-Darpoux et al. 1986). The magnitude of bias will depend upon the true and the assumed values of the genetic parameters.

Berrettini et al. (1991a) described a series of 21 pedigrees with BP. Genetic linkage of illness in this pedigree series has been examined in previously published papers for 107 markers on chromosomes 1, 10q, 11q, 13, 15, and 17 (Berrettini et al. 1991b), for 24 markers on chromosome 5 in 14 of these pedigrees (Detera-Wadleigh et al. 1992), and for five markers on Xq27-28 in a subset of families in which segregation of BP is consistent with X-chromosome transmission (Berrettini et al. 1990; Gejman et al. 1990), with classic restriction fragment length polymorphism (RFLP) markers used for

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nearly all loci. Here we report linkage analyses of 57 additional markers, all microsatellites, to BP.

The power of this or any pedigree series to detect linkage, when it exists, is a function of the pedigree structures, the complexity of genetic transmission, the proportion of cases linked, and the informativeness of the marker loci. Informativeness refers to the genetic variation of the markers. It is commonly measured by the frequency of heterozygotes at a locus and expressed in terms of heterozygosity. High heterozygosity values (greater than 70%) permit a more complete genetic analysis of the affected families because the segregation of the disease and marker can be analyzed in a larger number of meioses. Deoxyribonucleic acid (DNA) markers with high heterozygosity are specially useful when the number of pedigrees that can be studied is limited, as is often the case.

The polymerase chain reaction (PCR) allows inexpensive and rapid genotyping of a large number of individuals with minimum effort. Polymerase chain reaction is used to enzymatically amplify marker loci with high-average heterozygosity, dispersed throughout the genome. These have recently become available through the discovery of dinucleotide tandem repeats (microsatellites) of the form $(dC\text{-}dA)_n\cdot(dG\text{-}dT)_n$ (Weber and May 1989). These sequences display highly variable numbers of dinucleotide repeats, which show up on denaturing polyacrylamide gels as length polymorphisms.

The recent availability of microsatellite markers, which might reasonably be expected to be randomly dispersed, offer an opportunity to add significantly to the number of markers mapped in this series of pedigrees. We decided to exploit the opportunity as a "first pass" with later incorporation of data into systematic chromosomal scanning for susceptibility loci when this is appropriate, such as when gaps in the known genetic map can be filled with these markers. In published papers, linkage analysis to microsatellites located in chromosomes not yet scanned can be considered a "first pass."

Genetic linkage in this pedigree series has been previously examined systematically using mapped markers on several chromosomes. Linkage analyses to microsatellites located in these chromosomal regions should be considered a "second pass" that can provide more information at particular loci or fill in gaps in the genetic maps. An example of a successful "second pass" tactic is the detection of linkage between a microsatellite locus (D4S171) and facioscapulohumeral dystrophy disease in the distal long arm of chromosome 4 after a large portion of the genome had been excluded by using traditional DNA markers (Wijmenga et al. 1990). Linkage had not been previously detected because no markers were available in the region of linkage.

METHODS

We have studied a series of moderately sized North American pedigrees, whose structures, ascertainment, diagnostic procedures, extension rules, and expected LOD scores in linkage analyses are described elsewhere (Berrettini et al. 1991a). When a LOD score appeared positive (see below), we added the "right extension" (Kelsoe et al. 1990) of the Old Order Amish BP pedigree 110 for additional information.

Affection Status Models

The following are affection status models of who is ill or well (Berrettini et al. 1991a). *Model 1*: Either Bipolar I, Bipolar II with major depression, or schizoaffective disorder. *Model 2*: Model 1 plus recurrent (more than 1 episode) unipolar disorder. *Model 3*: Model 2 plus any of the following; nonrecurrent (one episode) unipolar; suicide; cyclothymic personality; Bipolar II with minor depression; unspecified functional psychosis, suicide, hypomania, anorexia, bulimia, other psychiatric disorder (hospitalized), and schizophrenia. Persons with affective disorders associated with brain dysfunction, including cerebrovascular accident or brain tumor, are considered as phenotype unknown.

For linkage analysis, persons considered affected only under model 3 were classified as unaffected when linkage calculations were performed for models 1 and 2, and persons considered affected only under model 2 were classified as unaffected for the calculations for model 1. However, because the precise inheritance of the BP phenotype is not known with certainty, the affection classification models used by us should be considered educated working hypotheses founded in epidemiologic data. When LOD scores suggested possible genetic linkage, a variation of these models was followed: the phenotype of persons considered affected under a less stringent model was classified as unknown in the calculations.

DNA Amplification and Electrophoresis

Polymerase chain reaction was performed in a total volume of 15 μl using approximately 0.15 μg of genomic DNA, 200 μmol each of adenosine triphosphate, guanosine triphosphate, and thymidine triphosphate, 2.5 μmol of cytidine triphosphate, 0.03 μCi of cytidine triphosphate [$^{\alpha\text{-}32}$]phosphate (3000 Ci/mmol), 5 pmol of each primer, 50 mmol KCl, 10 mmol Tris (pH 8.3), 1.5 mmol $MgCl_2$, 0.01% gelatin, 0.25 mmol spermidine, and 0.10 unit of Taq polymerase (Perkin Elmer Cetus). Typically, two sets of primers were included in each reaction (multiplexing). In a few experiments, only one set of PCR primers was used. Samples were

overlaid with 20 μl of mineral oil and were processed through 20 temperature cycles (denaturation, annealing, and extension) consisting of 50 seconds at 92°C, 90 seconds at 52°C, and 90 seconds at 72°C. In the last cycle, the extension step was lengthened to 4 minutes. Polymerase chain reaction was performed in microtiter plates in a Techne thermocycler (MW2). Polymerase chain reaction aliquots were electrophoresed on 6% to 8% denaturing polyacrylamide DNA sequencing gels. Gel size standards were dideoxy sequencing ladders (M13mp18 template). Gels were fixed, dried, and autoradiographed for 1 to 4 days. Oligonucleotide primers were synthesized on a DNA synthesizer (Cyclone Plus, Milligen/Biosearch, Millipore).

Genotyping and Linkage Analysis

Members of 19 families in the pedigree series had amplified DNA arranged on a gel in a fixed order by family. Each autoradiogram was uniquely identified with preprinted labels. The bands were read independently by two persons, and the readings recorded on the indicated column on two separate sheets. We carefully examined the quality of each autoradiogram generated with every experiment before deciding to include it in the database of genotypes. If this first level of scrutiny was successfully passed, the two separate readings were entered into a computer database that detects the presence of differences between the entries. Resolution of inconsistencies was done by reinspecting the autoradiograms, and in instances where they remained unresolved, the genotypes (of individuals, sibships, or families at a given locus) were considered unknown. Whenever a sample of genomic DNA was found to systematically give genotypes inconsistent with the rest of the family, this batch was discarded and another one was obtained.

A subset of seven families in which the segregation of BP is consistent with X-chromosome, transmission (0016, 0024, 0065, 0068, 0278, and 0643) was typed with the human X-linked gamma-aminobutyric acid-A receptor α3-subunit gene (see, Gejman et al. 1990; Berrettini et al. 1990 for description of families and absence of male-to-male transmission).

Linkage analyses were performed with the LINKAGE package version 5.03 (Lathrop et al. 1985). The assumed genetic transmission model of disease was dominant with a susceptibility allele frequency of 0.01. Penetrance values varied according to age: six susceptibility classes were considered. Penetrances for disease gene carriers varied from 17% to 85%. It could be argued that other genetic models should be examined because the mode of transmission of BP is unknown. Families in our sample were selected because they have a large number of affecteds in successive generations. Thus, the assumption of the dominant mode of inheritance seems reasonable when screening markers for linkage. Other models may be analyzed in a future publication. The penetrance for noncarriers was set to vary from 0.001 to 0.01. This implies that the assumed rate of phenocopies increases with age and was different from the penetrances previously assumed (Berrettini et al. 1991a). At young ages, the rate of phenocopies is 22%, but at older ages the rate of phenocopies increases up to 37%. Our model, with some particular family structures that include affected individuals located in the higher phenocopy penetrances class, would lead to less extreme LOD scores than previously calculated (Berrettini et al. 1991b). We assumed equal recombination in males and females for the initial analysis.

LOD score calculations were done under the three affection status models, with recombination fractions (θ) from 0 to 0.3. In a pedigree series such as ours, where heterogeneity may be present, we examined the total LOD score in pairwise analysis, followed by examination of the individual LOD scores in each pedigree.

RESULTS

Table 1 shows the chromosomal location and the polymorphism information content (PIC) of microsatellites used in these analyses and the total LOD scores under homogeneity at each locus ($\theta = 0$, $\theta = 0.10$, and $\theta = 0.20$).

Although all individuals had DNA amplified, not all persons yielded technically acceptable genotypes. An average of 261.2 individual genotypes (81% of individuals) per microsatellite system with a standard deviation of 67.6 were entered into the data base. A strict criterion for selecting data to be included in the calculations accounts for the individuals who were not typed (see section on genotyping and linkage analysis). Twelve loci had more than 300 individuals genotyped (19 families typed per locus). Less than 100 individuals have been typed with one microsatellite system, D8S87 (87 individuals typed in six families). Between 110 and 150 individuals were typed at seven loci (D17S250, D19S47, D19S48, D2S72, D22S156, D4S171, and D4S74). We have excluded genetic linkage under homogeneity at most of the loci at a distance of 10 to 20 cM.

We computed the power to detect linkage under heterogeneity using the program SIMLINK (Smith 1963; Ploughman and Boehnke 1989) for three different sample sizes corresponding to the number of individuals actually genotyped in three representative experiments. We assumed the same genetic parameters as those used for the pairwise analyses and that half of the families had an illness gene linked to a marker. For a marker with PIC = 0.7 and $\theta = 0.01$, the power

Table 1. Assumed Genetic Model of Disease*

Locus Name	Chromosome	Regional Localization	PIC or Heterozygosity	Affection Status Model	LOD Scores: Recombination Fraction θ = 0	θ = 0.10	θ = 0.20
D1S103	1		0.78	1	−15.70	−3.98	−1.15
				2	−15.62	−1.93	0.83
D1S104	1	1q21-q23	0.66	1	−6.44	−1.34	−0.02
				2	−15.01	−3.80	−1.05
CRP	1	1q21-q23	0.53	1	−15.39	−5.13	−0.86
				2	−20.12	−4.53	−0.34
D1S117	1	1q23-q25	0.77	1	−10.41	−3.79	−1.32
				2	−24.42	−8.68	−3.53
D2S72	2		0.71	1	−9.15	−3.66	−1.74
				2	−7.99	−2.76	−1.21
D3S196	3		0.68	1	−13.23	−3.39	−1.04
				2	−16.21	−3.91	−1.20
D3S240	3		0.30	1	−1.58	−0.50	0.10
				2	−2.71	−1.10	−0.31
GLUT2	3	3q26.1-q26.3		1	−14.35	−4.53	−1.54
				2	−17.45	−5.42	−2.22
D4S174	4		0.86	1	−5.93	−1.08	−0.02
				2	−9.00	−0.97	0.70
FABP2	4	4q28-q31	64%	1	−15.73	−5.89	−2.90
				2	−15.84	−4.41	−1.57
D5S108	5		0.45	1	−3.09	0.03	0.56
				2	−6.25	−0.74	0.24
D5S117	5		0.62	1	−8.10	−4.71	−2.32
				2	−15.53	−9.08	−4.39
D5S118	5		0.48	1	−9.82	−2.26	−0.32
				2	−16.53	−4.10	−1.03
D5S119	5		0.50	1	−8.95	−3.06	−1.38
				2	−14.33	−4.83	−1.81
D5S107	5	5q11.2-q13.3	0.78	1	−11.01	−3.80	−1.63
				2	−20.33	−6.23	−2.73
CFS1R	5	5q33.3-34	0.85	1	−27.00	−8.96	−3.81
				2	−36.33	−12.65	−5.60
D6S87	6		0.53	1	−9.16	−1.81	−0.31
				2	−18.84	−5.16	−1.67
D7S435	7		0.53	1	−16.25	−5.32	−2.46
				2	−22.70	−5.55	−1.95
D8S87	8	8p12	0.71	1	−3.38	−1.48	−0.33
				2	−4.96	−1.97	−0.33
D8S84	8	8q12-q13	0.58	1	−18.05	−6.07	−2.60
				2	−20.75	−6.23	−2.21
D9S43	9		0.74	1	−9.09	−1.60	−0.06
				2	−14.82	−4.80	−2.05
ASS	9	9q34	64%	1	−20.82	−6.28	−2.28
				2	−32.59	−9.37	−3.72
D10S89	10		0.71	1	−14.41	−3.61	−1.24
				2	−22.34	−5.97	−2.18
D11S419	11		0.43	1	−6.31	−1.37	−0.10
				2	−8.92	−2.76	−0.99
D11S35	11	11q22	0.79	1	−13.30	−3.71	−1.30
				2	−10.89	−1.63	−0.03
CD3D	11	11q23	0.69	1	−16.22	−5.56	−2.11
				2	−19.18	−5.74	−1.99
D11S420	11	11q23.3-q24	0.66	1	−14.62	−5.25	−2.39
				2	−19.15	−6.49	−2.93
D12S43	12		0.71	1	−13.83	−5.87	−3.08
				2	−20.39	−7.71	−3.91
PLA2	12		0.73	1	−18.65	−5.70	−2.17
				2	−24.90	−7.80	−2.92
IGF1	12	12q22-q24.1	0.53	1	−8.15	−2.97	−1.51
				2	−9.42	−2.62	−1.04
D13S71	13		0.67	1	−13.83	−5.87	−3.08
				2	−20.39	−7.71	−3.91
FLT1	13	13q12	0.49	1	−5.39	−0.83	0.05
				2	−7.40	−0.55	0.49

(continued

Table 1. *(continued)*

Locus Name	Chromosome	Regional Localization	PIC or Heterozygosity	Affection Status Model	LOD Scores: Recombination Fraction θ = 0	θ = 0.10	θ = 0.20
D14S43	14	14q24.3	0.72	1	−9.95	−3.22	−1.33
				2	−10.78	−3.42	−1.49
D15S87	15		0.85	1	−13.01	−4.12	−1.68
				2	−17.23	−6.10	−2.29
D16S260	16		0.43	1	−7.53	−2.91	−1.36
				2	−9.97	−3.00	−1.34
D16S261	16		0.66	1	−12.21	−3.15	−0.49
				2	−15.15	−4.14	−1.21
D16S265	16		0.75	1	−10.04	−3.78	−1.29
				2	−18.32	−4.68	−1.48
D16S266	16		0.54	1	−10.69	−2.94	−0.67
				2	−15.24	−5.05	−1.88
D16S267	16		0.47	1	−12.73	−5.30	−2.67
				2	−16.17	−5.73	−2.57
D17S250	17	17q11.2-q12	0.82	1	−15.55	−5.56	−2.62
				2	−13.03	−4.43	−2.14
MPO	17	17q21-23	0.45	1	−14.33	−4.70	−1.85
				2	−18.73	−7.19	−3.26
D18S35	18	18q	0.65	1	−12.44	−4.05	−1.43
				2	−17.39	−4.97	−1.66
D19S48	19		0.42	1	−4.61	−0.93	−0.13
				2	−7.21	−1.83	−0.54
D19S49	19	19q12-q13.1	0.79	1	−20.64	−7.34	−3.37
				2	−31.04	−10.71	−5.04
D19S75	19	19q12-q13.1	0.61	1	−12.21	−4.75	−2.1
				2	−22.78	−7.65	−3.5
APOC2	19	19q12-q13.2	0.79	1	−10.63	−4.91	−2.35
				2	−19.72	−7.18	−3.4
D19S47	19	19q13.1	0.69	1	−5.01	−2.33	−1.22
				2	−16.03	−4.55	−1.88
D20S32E	20		43%	1	−4.24	−1.84	−0.73
				2	−10.31	−2.83	−1.91
D20S27	20	20p12	0.64	1	−12.60	−2.5	−0.56
				2	−12.25	−3.29	−1.14
GNAS1	20	20q13.3	58%	1	−10.52	−3.74	−1.37
				2	−11.93	−4.31	−1.55
D21S172	21	21q11.2	0.58	1	−21.52	−6.90	−2.54
				2	−26.09	−7.39	−2.38
D21S13E	21	21q11.2	0.69	1	−11.01	−3.80	−1.43
				2	−16.06	−4.46	−1.39
D21S156	21	21q22.3	0.79	1	−17.19	−3.53	−0.46
				2	−27.27	−3.92	−2.13
D21S168	21	21q22.3	0.73	1	−8.50	−2.14	−0.61
				2	−14.68	−5.97	−2.84
D22S156	22		0.64	1	−4.44	−2.28	−1.27
				2	−6.34	−2.65	−1.54
SIS	22		0.57	1	−10.69	−3.04	−0.78
				2	−14.40	−4.02	−1.2
GABA-A	X	Xq28	0.29	1	−8.16	−2.16	−0.75
				2	−10.86	−1.59	−0.85

[a] Dominant transmission, susceptibility allele frequency of 0.01, variable age of onset, with a maximum penetrance value of 85% for individuals age 50 carrying either one or two susceptibility alleles, and 1% maximum penetrance for those who do not.

[b] We assume equal recombinations in males and females for the initial analysis. θ is recombination fraction. Heterozygosity is expressed as a percentage.

[c] Microsatellite mapping information and informativeness was obtained from: Buckle et al. 1989; Decker et al. 1992; Dracopoli et al. 1991; Gyapay et al. 1991; Granqvist et al. 1991; Guo et al. 1990a,b; Hazan et al. 1992; Hicks et al. 1991; Kwiatkowski et al. 1992; Lewis et al. 1992; Litt et al. 1990; Luo et al. 1990; Martinez and Goldin 1990; Mills et al. (in press); Patel et al. 1991; Patterson et al. 1990; Polymeropoulos et al. 1990a,b; 1991a–c; Sharma and Litt 1991; Sharma et al. 1991a,b; Wang and Weber (1992); Weber and May 1989; 1990a–g; Weber et al. 1990a–q; Wilkie et al. 1992; Yamada et al. 1991; Yuille et al. 1990.

[d] CRP = C-reactive protein gene; GLUT2 = human liver/islet glucose transporter gene; FABP2 = human intestinal fatty acid binding protein gene; CFS1R = human c-fms proto-oncogene for the CFS-1 receptor; ASS = human argininosuccinate synthetase gene; CD3D = human gene encoding the delta subunit of the CD3 T-cell receptor complex; PLA2 = human pancreatic phospholipase A-2 gene; IGF1 = insulin-like growth factor 1 gene; FLT1 = human fms-related tyrosine kinase gene; MPO = light and heavy chains of myeloperoxidase glycoprotein gene; APOC2 = apolipoprotein CII gene; GNAS1 = human Gs-alpha subunit gene; SIS = human c-sis proto-oncogene; GABA-A = human X-linked GABA-A receptor α3-subunit gene; PIC = polymorphism information content.

to detect linkage for sample sizes of genotyped individuals of 194, 244, and 305 is 69%, 80%, and 90%, respectively. As the recombination fraction increases or the proportion of linked families decreases, the power to detect linkage is lower.

The highest maximum LOD scores for the whole series of pedigrees were at D1S103 with affection status model 2 at 0.3 recombination fraction (1.19); LOD scores are negative with models 1 and 3.

For this locus, D1S103, as shown in Table 2, family 1482 at $\theta = 0$ had Z_{max} (maximum LOD score) = 1.99 and 2.39 under models 1 and 2, respectively. These were the highest LOD scores for an individual family in the pedigree series. Other positive LOD scores in individual pedigrees on chromosome 1q were noted: pedigree 1505, locus D1S117, Z_{max} = 2.1 and 0.52 under models 1 and 2, respectively; family 1512, locus CRP, Z_{max} = 0.17 and 1.58 under models 1 and 2. Other analyses of loci on the long arm of chromosome 1 did not reveal similar positive scores (Table 3). Our two-point scores

Table 2. LOD Scores for Locus D1S103 Affection Status Models 1 and 2

	Recombination Fraction							
Family No.	**0.0**	**0.01**	**0.05**	**0.1**	**0.15**	**0.2**	**0.3**	**0.4**
Model 1								
16	−1.32	−1.24	−0.96	−0.69	−0.49	−0.34	−0.14	−0.03
48	−1.73	−1.32	−0.78	−0.49	−0.32	−0.20	−0.07	−0.02
65	0.10	0.10	0.07	0.05	0.03	0.02	0.01	0.00
68	−1.69	−1.41	−0.90	−0.58	−0.38	−0.24	−0.08	−0.01
92	−1.49	−1.16	−0.69	−0.45	−0.32	−0.24	−0.13	−0.07
137	−2.70	−2.26	−1.45	−0.95	−0.64	−0.43	−0.17	−0.04
278	−2.51	−2.28	−1.63	−1.20	−0.94	−0.76	−0.48	−0.23
441	0.04	0.04	0.03	0.02	−0.00	−0.02	−0.02	−0.01
488	0.75	0.73	0.64	0.53	0.42	0.33	0.17	0.06
643	−0.41	−0.40	−0.35	−0.29	−0.24	−0.18	−0.09	−0.02
1442	−0.90	−0.86	−0.58	−0.27	−0.07	0.04	0.10	0.04
1482	1.99	1.94	1.76	1.54	1.31	1.08	0.63	0.24
1483	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00	−0.00
1484	0.55	0.67	0.86	0.87	0.82	0.73	0.50	0.26
1505	−1.12	−0.88	−0.49	−0.29	−0.18	−0.11	−0.04	−0.01
1512	−2.73	−2.12	−1.37	−0.94	−0.66	−0.46	−0.19	−0.05
1520	−1.11	−0.78	−0.29	−0.04	0.09	0.14	0.14	0.06
1536	−1.68	−1.56	−1.22	−0.93	−0.73	−0.58	−0.35	−0.18
9000	0.13	0.15	0.18	0.18	0.17	0.14	0.08	0.02
Total	−15.82	−12.67	−7.17	−3.94	−2.14	−1.06	−0.12	0.00
Model 2								
16	−1.32	−1.24	−0.96	−0.69	−0.49	−0.34	−0.14	−0.03
48	−2.15	−1.82	−1.06	−0.60	−0.34	−0.18	−0.03	0.01
65	−1.46	−0.54	−0.10	0.09	0.14	0.15	0.10	0.03
68	−0.61	−0.36	0.03	0.19	0.25	0.24	0.15	0.05
92	−3.28	−2.80	−2.23	−1.83	−1.46	−1.12	−0.58	−0.23
137	−2.70	−2.26	−1.45	−0.95	−0.64	−0.43	−0.17	−0.04
278	−1.11	−1.00	−0.54	−0.19	0.01	0.13	0.21	0.15
441	0.42	0.41	0.38	0.33	0.26	0.20	0.07	0.00
448	0.75	0.73	0.64	0.53	0.42	0.33	0.17	0.06
643	−1.82	−1.64	−1.19	−0.84	−0.60	−0.41	−0.17	−0.04
1442	−1.46	−1.10	−0.58	−0.27	−0.08	0.02	0.08	0.03
1482	2.39	2.34	2.15	1.90	1.64	1.39	0.86	0.36
1483	0.06	0.06	0.05	0.04	0.03	0.02	0.01	0.00
1484	0.42	0.55	0.75	0.78	0.73	0.65	0.46	0.24
1505	−1.28	−1.21	−0.79	−0.44	−0.25	−0.14	−0.04	−0.01
1512	−1.43	−0.35	0.22	0.38	0.40	0.37	0.22	0.06
1520	0.36	0.40	0.51	0.55	0.54	0.50	0.32	0.11
1536	−1.77	−1.72	−1.42	−1.08	−0.83	−0.64	−0.39	−0.19
9000	0.97	0.96	0.87	0.76	0.65	0.53	0.31	0.13
Total	−15.02	−10.72	−4.73	−1.35	0.41	1.28	1.47	0.70

Pairwise Analysis of BP and D1S103.
Pedigree 9000 is Old Order Amish 110 right extension (Kelsoe et al. 1990).
See Table 1 legend for genetic parameters.

Table 3. Simulation of Maximum Values of LOD Score with Multiple Analyses

	No Linkage (θ = 0.5)		Linkage (θ = 0.01)	
LOD Scores	**F1505**	**F1482**	**F1505**	**F1482**
Model 1				
Average Z_{max}	0.20	0.35	1.21	1.92
P ($Z_{max} > 2$)	0.35%	0.7%	22.3%	43.8%
P ($Z_{max} > 3$)	0.15%	0.5%	0.0%	20.4%
Model 2				
Average Z_{max}	0.25	0.40	1.36	2.53
P ($Z_{max} > 2$)	0.4%	0.4%	28.2%	61.0%
P ($Z_{max} > 3$)	0.0%	0.25%	0.0%	36.0%

Frequency of maximum LOD score (Z_{max}) for families 1482 and 1505 under disease Model 1 and 2, when there is no linkage (θ = 0.5) and true linkage (θ = 0.01) based on 2000 replicates.

between all markers on 1q in our data are consistent with the published map order as described by Dracopoli et al. (1991) (results not shown).

When penetrance was decreased to 50% for locus D1S103, the Z_{max} for all the families was 0.37 at θ = 0.30 for model 1 and 1.32 at θ = 0.25 for model 2. In the same analysis, family 1482 at θ = 0 had Z_{max} = 1.77 and 2.34 under models 1 and 2, respectively.

In some analyses at this locus, individuals affected under a lower classification model (model 1 is highest and model 3 is lowest) were considered unknown. The Z_{max} for all the families was 0.001 at θ = 0.40 and 1.32 at θ = 0.25 under models 1 and 2, respectively.

We have also analyzed the Old Order Amish pedigree 110 with D1S103 and D1S117. LOD scores were slightly positive at D1S103 under model 1 and model 2 and negative at D1S117 (see Table 2). When linkage analysis of the Amish pedigree is added the maximum LOD score (under homogeneity) for the whole series of pedigrees at D1S103 is 1.47 at θ = 0.30 recombination fraction.

Genetic heterogeneity at D1S103 was tested using the admixture test (one-sided test and type I error of 5%). As previously described by Martinez and Goldin (1990), we have modified the MLINK program (V5.03) to maximize the LOD score as a function of θ and of the proportion of linked families (heterogeneity rate), α. Analysis of linkage under heterogeneity (Amish pedigree not included) revealed a maximum LOD score of 1.98 at θ = 0.10, α = 0.35 (heterogeneity test X_1^2 = 2.35, p = 0.063), affection status model 2, and a maximum LOD score of 0.75 at θ = 0, α = 0.10, (heterogeneity test X_1^2 = 3.45, p = .031), affection status model 1. Genetic heterogeneity is thus (weakly) supported, but evidence for genetic linkage is not significant.

For families 1482 and 1505, we estimated the frequency of positive maximum LOD scores greater than 2 that could arise by chance alone and when there is a true tight linkage between the marker and the trait locus. Using the SLINK program (Ott 1989; Weeks et al. 1990), 2000 replicates of each family were simulated under disease models 1 and 2. We have considered a marker locus with four alleles equally frequent either unlinked (θ = 0.5) or tightly linked (θ = 0.01) to the disease locus and maximized the individual pedigree LOD scores (Table 3). We have investigated the possibility that multiple linkage analyses would inflate the LOD scores (Clerget-Darpoux et al. 1990; Weeks et al. 1990). Thompson (1984) derived the equivalence of multiple analyses from the significance level of the data with a single linkage test. In a report such as this one, we can consider that 20 to 50 independent markers would provide information in these two families. Assuming that these analyses are independent and that there is no disease locus linked to the tested markers for family 1482, the probability of observing a LOD score greater than 2 varies from 13% to 30% (disease model 1) and from 8% to 18% (disease model 2). For family 1505, this probability varies from 7% to 16% (disease model 1) and from 8% to 18% (disease model 2). Thus, when no disease locus exists, there is still a considerable probability of observing at least one LOD score greater than 2 when this many independent analyses are performed.

DISCUSSION

The problems of detecting a single locus for susceptibility in the psychiatric disorders include conditions of complex inheritance, which may also be present in numerous inherited common diseases. These include variable penetrance (by which is meant that people may have the disease genetic vulnerability but not themselves be ill), genetic linkage heterogeneity, oligogenic inheritance, and density and informativeness of the human genetic map. Nonetheless, linkage may be detectable under conditions of complex inheritance that are compatible with reasonable assumptions based on the observed familial recurrence risks in BP (Goldin et al. 1991).

Polymorphism information content is defined as the probability that an offspring will be informative at a given marker locus. Polymorphism information content values range from 0 (absence of heterozygosity) to 1 (informative in any given meiosis); one can think of a rough numerical equivalence of PIC and of average heterozygosity. Most of the classic RFLP markers consist of biallelic systems that have low PIC values. Dinucleotide repeats generate allele systems formed by more than two alleles (systems of more than 10 alleles are not infrequent) and have high PIC values. Currently, some microsatellite systems fill gaps in the existing genetic map, thus improving its informativeness and resolution (Decker et al. 1992; Dracopoli et al. 1991;

Hazan et al. 1992; Kwiatkowski et al. 1992; Lewis et al. 1990; Mills et al. [in press]; Wang and Weber 1992; Wang et al. [unpublished data]; Wilkie et al. 1992).

In this paper, a "second pass" with microsatellites generated some isolated positive LOD scores in chromosome 1q. Although LOD scores obtained at locus D1S103 seemed encouraging at first, they could have arisen by chance, given the number of linkage tests performed. Furthermore, in the previous published analysis of this region in these pedigrees (Berrettini et al. 1991b), the nearby markers did not suggest linkage. However, it is worth mentioning that positive LOD scores on chromosome 1q had been previously reported in the Old Order Amish pedigree 110 at loci in the same area (Pakstis et al. 1991).

Among the markers studied here, there are two possible candidate genes, Gs-alpha subunit-1 and gamma-aminobutyric acid-A (which is in a region previously analyzed [Berrettini et al. 1990]). Our results do not support a causative relationship between these loci and BP.

The availability of a large number of microsatellite systems evenly spanning the human genome, their informativeness, the rapid creation of genetic maps based on them, and the feasibility of multiplexing should make these systems the core of psychiatric genetic mapping in the upcoming years.

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