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Initial Genomic Scan of the NIMH Genetics Initiative Bipolar Pedigrees: Chromosomes 3, 5, 15, 16, 17, and 22

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As part of the four-center NIMH Genetics Initiative on Bipolar Disorder we carried out a genomic scan of chromosomes 3, 5, 15, 16, 17, and 22. Genotyping was performed on a set of 540 DNAs from 97 families, enriched for affected relative pairs and parents where available. We report here the results of the initial 74 markers that have been typed on this set of DNAs. The average distance between markers (θ) was 12.3 cM. Nonparametric analysis of excess allele sharing among affected sibling pairs used the SIBPAL program of the S.A.G.E. package to test three hierarchical models of affected status. D16S2619 gave some evidence of linkage to bipolar disorder, with $P = 0.006$ for Model II (in which bipolar 1, bipolar 2 and schizoaffective-bipolar type individuals are considered affected). Nearby markers also showed increased allele sharing. A second interesting region was toward the telomere of chromosome 5q, where D5S1456 and nearby markers showed increased allele sharing; for D5S1456, $P = 0.05$, 0.015 and 0.008 as the models of affected status become more broad. MOD score analysis also supported the possible presence of a susceptibility locus in this region of chromosome 5. A pair of adjacent markers on chromosome 3, D3S2405 and D3S3038, showed a modest increased allele sharing in the broad model. Several isolated markers had excess allele sharing at the $P < 0.05$ level under a single model. D15S217 showed a MOD score of 2.37 ($P < 0.025$). Multipoint analysis flagged the region of chromosome 22 around D22S533 as the most interesting. Thus, several regions showed modest evidence for linkage to bipolar disorder in this initial genomic scan of these chromosomes, including broad regions near previous reports of possible linkage. Am. J. Med. Genet. 74:238–246, 1997.

KEY WORDS: genetic linkage; sib pair analysis; family study; unipolar recurrent depression; microsatellite markers

INTRODUCTION

As discussed in the companion article [The NIMH Genetics Initiative Bipolar Group, 1997], there is good evidence for a genetic contribution to the risk of bipolar affective disorder. Many different chromosomal regions have been reported linked to bipolar disorder, although many of these reported linkages have not been confirmed [Risch and Botstein, 1996]. Given the complex nature of the disease, with a spectrum of diagnoses whose underlying biological relationships are not clear and with no clear mode of inheritance established,

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strategies to address the genetics must be carefully designed.

To locate genes that contribute to this risk, a large collection of families with individuals diagnosed as having bipolar disorder has been assembled as part of the NIMH Genetics Initiative. From these families, an initial genome screening panel was selected that was composed primarily of affected individuals, with some common relatives to allow inference of identity by descent (IBD) where possible. Individuals were considered affected if they met the requirements of any of three hierarchical models: Model I included bipolar I (BP I) plus schizoaffective, bipolar type (SA/BP); Model II included Model I plus bipolar II (BP II); Model III included Model II plus unipolar recurrent depression (UPR).

Using this panel, we have carried out a preliminary genomic scan of chromosomes 3, 5, 15, 16, 17, and 22. This subset of chromosomes contains several loci for which there have been reports of suggestive evidence for linkage to bipolar affective disorder. Ginns et al. [1996] reported P values of 0.0003 (using SIBPAL) for D15S45 in a large pedigree from the Old Order Amish, but no evidence for linkage disequilibrium. Eiberg et al. [1993] reported some evidence for linkage of manic depressive illness to PGP, located on 16p13.3, using an autosomal dominant model of inheritance. In 1995, the same group [Ewald et al., 1995] reported a possible locus for manic depressive illness at 16p13, with the highest two-point lod score (2.52) reached with D16S510. Coon et al. [1993] presented some evidence for linkage to D5S62, in the region of DRD1 and GABRA1. Kelsoe et al. [1995, 1996] found suggestions of a susceptibility locus near the dopamine transporter gene on chromosome 5p15.3 using several modes of analysis; none reached conventional levels of significance [see Coon et al., 1993, for negative results].

This manuscript reports the initial genomic scan of chromosomes 3, 5, 15, 16, 17, and 22 at an average intermarker distance of 12.3 cM. It should be read in conjunction with the companion manuscripts in this issue [The NIMH Genetics Initiative Bipolar Group, 1997; Detera-Wadleigh et al., 1997; Rice et al., 1997; Stine et al., 1997]. The data have been analyzed by nonparametric affected sibling pair methods (using the SIBPAL program of the S.A.G.E. package), as well as by MOD scores and a multipoint method (ASPEX, D. Hinds and N. Risch, unpublished) to identify regions of interest for further study.

METHODS

Genotyping Sample and Diagnoses

The strategy for collection of the subjects and determination of diagnoses is explained in the companion article [NIMH Genetics Initiative Bipolar Group, 1997]. A set of three hierarchical diagnoses were used in all analyses, as summarized above and presented in more detail in the companion paper [NIMH Genetics Initiative Bipolar Group, 1997].

The genome screening panel included all sibling pairs with individuals affected under any of the three models if DNA was available, plus biological parents when available to allow for estimation of identity by descent (IBD), and individuals affected under any of the three models if they were part of an affected relative pair such as avuncular, cousin or grandparent-grandchild. The genotyped sample consisted of 540 DNAs from 97 families. It included DNA from 232 individuals with BPI, 32 with SA/BP, 72 with BPII and 88 with UPR. The number of sibling pairs (all possible affected pairs) under the three models was 121, 197 and 282 for Models I, II and III, respectively.

Genotyping

Markers were selected from several sources, the largest of which was the Cooperative Human Linkage Center [CHLC; Sheffield et al., 1995; online updates from http://www.chlc.org/]. Other sources include GDB (TM) Genome Database [1990], Hudson et al. [1992, 1995], the Center for Medical Genetics at the Marshfield Clinic (J. Weber; online at http://genetics.mfldclin.edu/), and the Utah group (http://www-genetics.med.utah.edu/totalmap/). Primers were synthesized from published sequences by the I.U. Biochemistry Biotechnology Facility, or were purchased from Research Genetics (Huntsville, AL).

DNA samples at various concentrations were obtained from the Coriell Institute for Medical Research (Camden, NJ). DNAs were diluted to a common concentration and assembled into "panels" of 88–92 individual samples in microtiter-format plates, with families grouped within single panels. Standard CEPH DNAs were included as internal controls within the panels.

Allele sizes were determined by manual methods using incorporation of [^{35}S]dCTP during the polymerase chain reaction (PCR) to label the products. A Biomek 1000 Automated Laboratory Workstation (Beckman, Fullerton, CA) was used to distribute aliquots of a premix and of DNA into GeneAmp tubes (Perkin Elmer/Cetus, Norwalk, CT). The final 10-μl reactions contained 50 mM KCl, 10 mM Tris (pH 8.3), 1.5 mM $MgCl_2$, 0.01% gelatin, 200 μM each dATP, dGTP, dTTP, 2.6 μM [^{35}S]dCTP (38 Ci/mmol), 5 pmol of each primer, 96 ng DNA, and 0.5 units of Taq polymerase (Perkin Elmer/Cetus). The polymerase chain reaction was carried out on the Perkin Elmer/Cetus GeneAmp PCR System 9600 thermocycler; the standard program was 5 min at 94°C followed by 27–30 cycles of 94°C/30 sec, 53°C/30 sec, 72°C/45 sec, then 5 min at 72°C; for some primers the annealing temperature was altered. Products of the reactions were mixed with 10 μl of formamide/dye solution, heated to 90°C for 2 min, and 3-μl aliquots were electrophoresed on 6% denaturing polyacrylamide gels (Gel Mix-6, GIBCO/BRL, Gaithersburg, MD). Gels were dried and exposed to Kodak XAR5 film for 6 to 72 hours.

Allele sizes were determined relative to the internal standards whose sizes were previously estimated by comparison to DNA sequencing ladders or from published values. Data were independently entered as sizes in nucleotides into a Microsoft Excel spreadsheet by two people, both blind to the affection status of individuals. The data were compared using a spreadsheet program which generated a file flagging discrep-

ancies; each gel reader reexamined the autoradiograms. Corrected (or checked and unchanged) files were recompared, and only data fully agreed by the two readers were forwarded to the database. These initial readings were done without using pedigree information.

Another level of error checking was accomplished using a database program (GeneMaster, J. Rice) that detects alleles in a child which are not present in his/her parents. Such alleles were reexamined on the original autoradiograms; they include potential nonpaternities and new mutations. Changes were only made if two readers agreed.

Each completed marker was assessed for Mendelian inheritance first using CRIMAP [Green et al., 1990] and then using the USERM13 option of the MENDEL suite of programs [Boehnke, 1991; Lange et al., 1988]. Each family with an identified noninheritance was reviewed individually and the genotypic data from one or more individuals incompatible with the rest of the family were removed. A database was maintained to record all changes.

Allele Frequencies and Maps

Estimates of allele frequencies were obtained using the maximum likelihood methods developed by Boehnke [1991] and implemented in the program USERM13. The marker order was specified based upon prior data, and the sex-average distances between them estimated using CRIMAP. Locations were confirmed using the "flip" option in CRIMAP. The distances are given in Table I.

Analytical Methods

Initial analysis was by a nonparametric affected sibling pair method [Blackwelder and Elston, 1985]. For each marker, the mean proportion of marker alleles identical by descent was calculated and a one-sided t-test was applied to determine if this value was significantly greater than 0.50. All calculations were performed using the SIBPAL routine (version 2.7.2) in the S.A.G.E. package [S.A.G.E., 1994]. This program calculates identity by descent for all possible pairs of affected siblings. While this may result in an inflated contribution from sibling pairs with a large number of individuals, in this sample fewer than 10 families had four or more affected individuals under models I and II. Half-sibs were not included in these analyses, to avoid overweighting their contribution.

We analyzed the extended pedigrees using the MOD score method, a technique introduced by Risch [1984] and modified by Clerget-Darpoux et al. [1986]. Based upon the criteria for selecting individuals for genotyping [NIMH Genetics Initiative Bipolar Group, 1997], the most complete data for MOD score analysis are available for Model I. MOD scores were computed using the program MODLINK [R. Neuman, personal communication], which maximizes the lod score rather than the likelihood, under the assumption that the trait is determined by a single diallelic locus with recombination fraction theta and parameters for gene frequency and three penetrances associated with the trait locus. Significance is determined by multiplying the MOD score by 4.6 and comparing it to a chi-square with three degrees of freedom.

ASPEX, a multipoint program developed by D. Hines and N. Risch (unpublished) was used for multipoint sib pair analysis as well as for two-point IBD analysis. It uses marker information from parents and all sibs in each sibship to estimate IBD for individual sib pairs. Extended pedigrees were broken into nuclear families and data were analyzed using the sib_ibd program that examines all possible (n(n-1)/2) pairs in each nuclear family. Sib_ibd includes in the analysis only sibships with both parents genotyped; it does not use marker allele frequencies to estimate parental genotype. Therefore, the affected sibling pair results for individual markers using ASPEX provide an unbiased estimate of *pure* IBD sharing in a smaller portion of the dataset, sibling pairs with both parents genotyped. This differs from SIBPAL, which uses all sibling pairs and infers IBD for sibling pairs with missing parent(s) by using allele frequencies, and was used to confirm findings.

RESULTS

We report the analysis of 74 markers from chromosomes 3, 5, 15, 16, 17, and 22 that have been typed on this set of 540 DNAs (Table I). This represents an average distance between markers (θ) of 12.3 cM (averages range from 10.5 to 14.0 cM on the individual chromosomes). The markers averaged 9.28 alleles each, with an average heterozygosity of 0.704.

Initial analysis examined allele sharing in affected siblings, using the SIBPAL program. There were no overall biases in the data: overall allele sharing averaged 0.501, 0.502 and 0.501 for Models I, II and III, respectively, with standard deviations of 0.025, 0.021 and 0.019, respectively. Approximately half of the markers (39, 36 and 38, respectively) showed allele sharing below 0.500, very close to the 37 expected by chance. Ten of the 74 markers showed an excess of allele sharing (at $P < 0.05$) for at least one Model. Data are presented in Table I.

On chromosome 3, two adjacent markers (D3S2403 and D3S3038) showed an increase in allele sharing with Model III, significant at the $P = 0.05$ level. The sib_ibd module of ASPEX confirmed excess allele sharing for all three models in the region from the telomere to D3S3038, but it only reached significance for D3S2387 (Models II and III, $P < 0.01$ and 0.05, respectively) and D3S3038 (Model III, $P < 0.005$). MOD score analysis (Fig. 1A) showed a peak of 1.75 ($P < 0.05$) at D3S1764, and lower peaks in the region of D3S2387, D3S2403 and D3S2418 ($P > 0.1$ for all three markers). The multipoint maps (Fig. 2A) showed the strongest peak from the telomere to D3S2387 (Model II, mLOD = 1.76), with two smaller peaks in Model III, one at D3S2387 (mLOD = 1.08) and one at D3S3038 (mLOD = 1.19).

On chromosome 5, D5S1456 showed significantly increased allele sharing with all three models ($P = 0.05$, 0.015, 0.008 for Models I, II, III, respectively). A lesser degree of allele sharing was found at other markers in the region. The adjacent marker, GABRA1, showed

TABLE I. Results of SIBPAL Analyses for Three Models of Affection Status

		Model I BP1, SA/BP			Model II BPI, SA/BP, BPII			Model III BPI, SA/BP, BPII, UPR		
Marker	Dist[a]	Pairs[b]	IBD[c]	*P*-value*	Pairs	IBD	*P*-value	Pairs	IBD	*P*-value
Chromosome 3										
D3S2387	0.0	118	.520	0.243	193	.530	0.094	272	.517	0.186
D3S3050	8.2	100	.495	1.000	158	.494	1.000	225	.497	1.000
D3S2405	25.2	121	.490	1.000	197	.490	1.000	281	.500	1.000
D3S2403	37.5	100	.520	0.243	167	.515	0.245	244	.532	**0.029**
D3S3038	51.0	104	.524	0.249	172	.519	0.216	253	.541	**0.020**
D3S2432	62.1	110	.526	0.195	184	.520	0.200	266	.522	0.122
D3S2409	74.2	121	.480	1.000	197	.506	0.380	282	.500	1.000
D3S1766	82.6	101	.501	0.484	168	.481	1.000	236	.485	1.000
D3S2454	101.2	101	.523	0.192	167	.493	1.000	240	.498	1.000
D3S2386	116.4	112	.460	1.000	181	.444	1.000	256	.475	1.000
D3S1215	129.1	113	.488	1.000	188	.491	1.000	269	.493	1.000
D3S2460	139.0	119	.483	1.000	195	.493	1.000	278	.501	0.486
D3S1764	162.1	105	.465	1.000	176	.497	1.000	254	.521	0.136
D3S1744	170.3	118	.478	1.000	190	.490	1.000	273	.507	0.363
D3S1763	190.7	95	.495	1.000	156	.501	0.490	222	.490	1.000
D3S1754	209.5	94	.435	1.000	158	.461	1.000	233	.476	1.000
D3S2398	229.3	88	.443	1.000	150	.467	1.000	204	.473	1.000
D3S2418	236.0	111	.495	1.000	186	.497	1.000	269	.503	0.418
Chromosome 5										
D5S1492	0.0	94	.486	1.000	155	.479	1.000	221	.487	1.000
D5S807	18.2	119	.475	1.000	195	.482	1.000	276	.497	1.000
D5S1473	34.3	76	.467	1.000	121	.461	1.000	182	.463	1.000
D5S819	45.9	94	.468	1.000	153	.460	1.000	219	.461	1.000
D5S1470	51.5	105	.493	1.000	174	.474	1.000	253	.474	1.000
D5S2507	65.8	108	.533	0.150	180	.497	1.000	262	.463	1.000
D5S2500	68.4	114	.492	1.000	190	.495	1.000	275	.467	1.000
D5S1711	71.6	107	.520	0.243	179	.533	0.070	260	.491	1.000
D5S1501	97.7	109	.517	0.292	170	.502	0.465	239	.517	0.204
D5S1726	106.0	111	.493	1.000	182	.490	1.000	257	.498	1.000
D5S1719	106.6	111	.459	1.000	181	.480	1.000	264	.491	1.000
D5S1462	113.8	118	.495	1.000	194	.485	1.000	278	.499	1.000
D5S1549	124.3	110	.500	1.000	177	.493	1.000	239	.503	0.444
D5S804	137.9	113	.487	1.000	180	.495	1.000	258	.495	1.000
D5S207	157.2	113	.528	0.111	187	.509	0.319	267	.514	0.191
D5S820	166.1	109	.553	**0.037**	185	.516	0.238	256	.525	0.098
GABRA1	174.0	109	.541	0.111	171	.515	0.279	240	.508	0.361
D5S1456	184.8	92	.545	**0.050**	155	.549	**0.015**	222	.548	**0.008**
Chromosome 15										
D15S817	0.0	103	.475	1.000	165	.502	0.475	240	.490	1.000
GABRB3	5.6	109	.482	1.000	177	.489	1.000	260	.489	1.000
D15S217	10.8	115	.519	0.249	190	.512	0.298	273	.505	0.393
GAAA1C11	27.4	88	.529	0.166	147	.537	0.059	208	.540	**0.022**
D15S659	44.2	104	.512	0.346	166	.501	0.491	235	.495	1.000
GATA153F11	50.1	118	.475	1.000	191	.471	1.000	271	.472	1.000
D15S643	55.4	99	.517	0.294	169	.521	0.191	248	.498	1.000
D15S644	63.6	96	.517	0.261	161	.518	0.201	230	.523	0.103
D15S818	79.7	96	.510	0.365	160	.513	0.289	231	.492	1.000
D15S652	106.1	88	.507	0.418	146	.507	0.394	210	.512	0.270
D15S230	126.0	111	.490	1.000	171	.489	1.000	252	.498	1.000
D15S642	141.4	107	.506	0.410	175	.483	1.000	254	.481	1.000
Chromosome 16										
D16S2618	0.0	101	.555	**0.028**	164	.530	0.099	240	.527	0.074
D16S2622	5.0	117	.512	0.306	191	.521	0.127	276	.513	0.212
D16S748	30.3	113	.542	0.060	185	.535	0.051	263	.521	0.128
D16S2619	35.8	108	.531	0.070	178	.541	**0.006**	252	.520	0.085
D16S749	49.9	109	.515	0.228	178	.529	**0.034**	257	.525	**0.028**
D16S757	67.0	108	.535	0.094	175	.528	0.079	257	.514	0.197
D16S3253	72.8	110	.496	1.000	177	.495	1.000	258	.493	1.000
D16S752	87.6	109	.518	0.264	180	.529	0.101	260	.532	**0.041**
D16S2624	88.7	102	.494	1.000	174	.499	1.000	254	.502	0.460
D16S750	102.2	119	.503	0.421	188	.497	1.000	273	.502	0.425
D16S539	130.4	105	.484	1.000	162	.494	1.000	224	.477	1.000
Chromosome 17										
D17S1308	0.0	96	.486	1.000	154	.507	0.378	217	.504	0.425
D17S1298	16.8	95	.472	1.000	160	.503	0.439	231	.499	1.000
D17S974	33.6	96	.474	1.000	164	.477	1.000	238	.471	1.000

(*Continued*)

TABLE I. *Continued*

Marker	Dist[a]	Model I BP1, SA/BP Pairs[b]	Model I BP1, SA/BP IBD[c]	Model I BP1, SA/BP *P*-value*	Model II BPI, SA/BP, BPII Pairs	Model II BPI, SA/BP, BPII IBD	Model II BPI, SA/BP, BPII *P*-value	Model III BPI, SA/BP, BPII, UPR Pairs	Model III BPI, SA/BP, BPII, UPR IBD	Model III BPI, SA/BP, BPII, UPR *P*-value
D17S969	39.9	120	.536	0.083	196	.539	**0.022**	280	.524	0.067
D17S1294	54.5	110	.501	0.488	171	.503	0.443	248	.503	0.444
D17S1293	65.4	74	.501	0.490	116	.510	0.358	169	.492	1.000
D17S1299	81.9	112	.527	0.146	177	.515	0.237	253	.504	0.415
D17S1290	101.6	105	.496	1.000	166	.508	0.371	238	.509	0.332
D17S968	116.5	112	.497	1.000	182	.501	0.469	265	.494	1.000
Chromosome 22										
F8VWFP	0.0	110	.491	1.000	175	.488	1.000	249	.486	1.000
D22S686	9.7	102	.471	1.000	166	.503	0.454	233	.514	0.232
D22S533	23.6	97	.487	1.000	156	.532	0.090	213	.531	0.061
D22S684	40.4	112	.520	0.220	183	.506	0.396	248	.506	0.366
D22S445	51.1	109	.508	0.391	183	.495	1.000	251	.499	1.000
D22S1267	55.5	111	.484	1.000	183	.491	1.000	264	.507	0.323

[a]Dist, distance in cM, calculated from our data.
[b]Pairs, all possible (n(n-1)/2) pairs.
[c]IBD, mean estimated identity by descent.
*P-value, test of significance of increased sharing from one-sided t-test (1.00 for all cases where sharing is <0.50). Models are explained in text. P-values <0.05 are in bold.

somewhat increased allele sharing, especially with the narrowest Model I, as did the next proximal marker, D5S820, which reached significance with $P < 0.04$ for Model I. Allele sharing was slightly elevated even at the next marker. Thus, a broad region of chromosome 5q showed increased allele sharing, although with only modest significance. ASPEX confirmed the increased allele sharing IBD in this region, with the highest sharing for Model I at GABRA1 and D5S1456, but greatest significance was for Model III, $P = <0.025$ for D5S1456. MOD score analysis (Fig. 1B) showed a peak of 2.46 for Model I at D5S820 ($P = 0.01$), with a secondary peak at D5S1456 ($P < 0.025$). The multipoint analysis (Fig. 2B), however, showed two regions with only very modest mLOD scores, <1.

Only one marker on chromosome 15, GAAA1C11, showed increased allele sharing which reached levels of $P = 0.022$ for Model III. This marker was difficult to genotype, and therefore there were fewer affected sib pairs in the analysis. MOD scores were elevated in this region (Fig. 1C), with a peak at D15S217 ($P < 0.025$ for Model I). Multipoint results did not show any regions of interest (Fig. 2C).

On chromosome 16, D16S2619 showed excess allele sharing significant at the $P = 0.006$ level for Model II; there was some excess allele sharing with other models that did not reach significance. Modest excess allele sharing extended to both sides of this marker, although it only reached lower levels of significance on the centromeric side. Two isolated markers on this chromosome reached low levels of significance ($P < 0.05$). ASPEX confirmed excess allele sharing IBD extending from the telomere beyond D16S749 for all three models, with the most telomeric marker, D16S2618, showing the highest level ($P < 0.05$ for Model I, mainly due to excess maternal sharing). MOD scores were modestly elevated in a broad region (Fig. 1D), with peaks at D16S748 and D16S749 (Models I and III, respectively, both with $P < 0.05$). The multipoint curve (Fig. 2D) shows only a small peak at D16S2622 in Models I and II, with mLOD <1.

Chromosome 17 had only one marker that showed significantly increased allele sharing, and that only with one of the models. D17S969 gave $P = 0.022$ with Model II in SIBPAL, but was not prominent in ASPEX. Adjacent markers did not show similar increases, although there was a gap of 14 cM proximal to this marker. Neither MOD scores nor multipoint results showed regions of interest (Figs. 1E, 2E).

D22S533 showed about 53% sharing with Models II and III in SIBPAL, which did not reach significance. However, this marker showed the most prominent level of allele sharing in ASPEX. The ASPEX IBD showed 62–63% sharing for all models, reaching significance for Model II ($P < 0.005$) and Model III ($P < 0.001$). Multipoint analyses (Fig. 2F) showed a strong peak at D22S533. The maximum LOD calculated for this marker was 2.46 for Model III; this was the highest mLOD calculated for any of the markers in this screen. MOD scores were very modestly elevated in a broad region that included D22S533 (Fig. 1F, Models II and III).

DISCUSSION

This initial scan of chromosomes 3, 5, 15, 16, 17, and 22 has located several regions that show modest evidence for linkage to bipolar disorder, although none rise to the level of significance suggested by Lander and Kruglyak [1995] for a genomic scan. We are encouraged by the finding of increased sharing in adjacent markers in some of these regions, as on chromosomes 3, 5, 16, and 22 (with latter with ASPEX). These areas are worth pursing with flanking markers and an increased set of affected sib pairs, since the size and composition of the samples genotyped to date might not provide sufficient power to detect some potentially significant loci at the marker spacing used to date.

There was a previous report of a possible locus for manic depressive illness at 16p13, with a recessive model of inheritance [Ewald et al., 1995]. Eiberg et al. [1993] had earlier reported evidence for linkage of

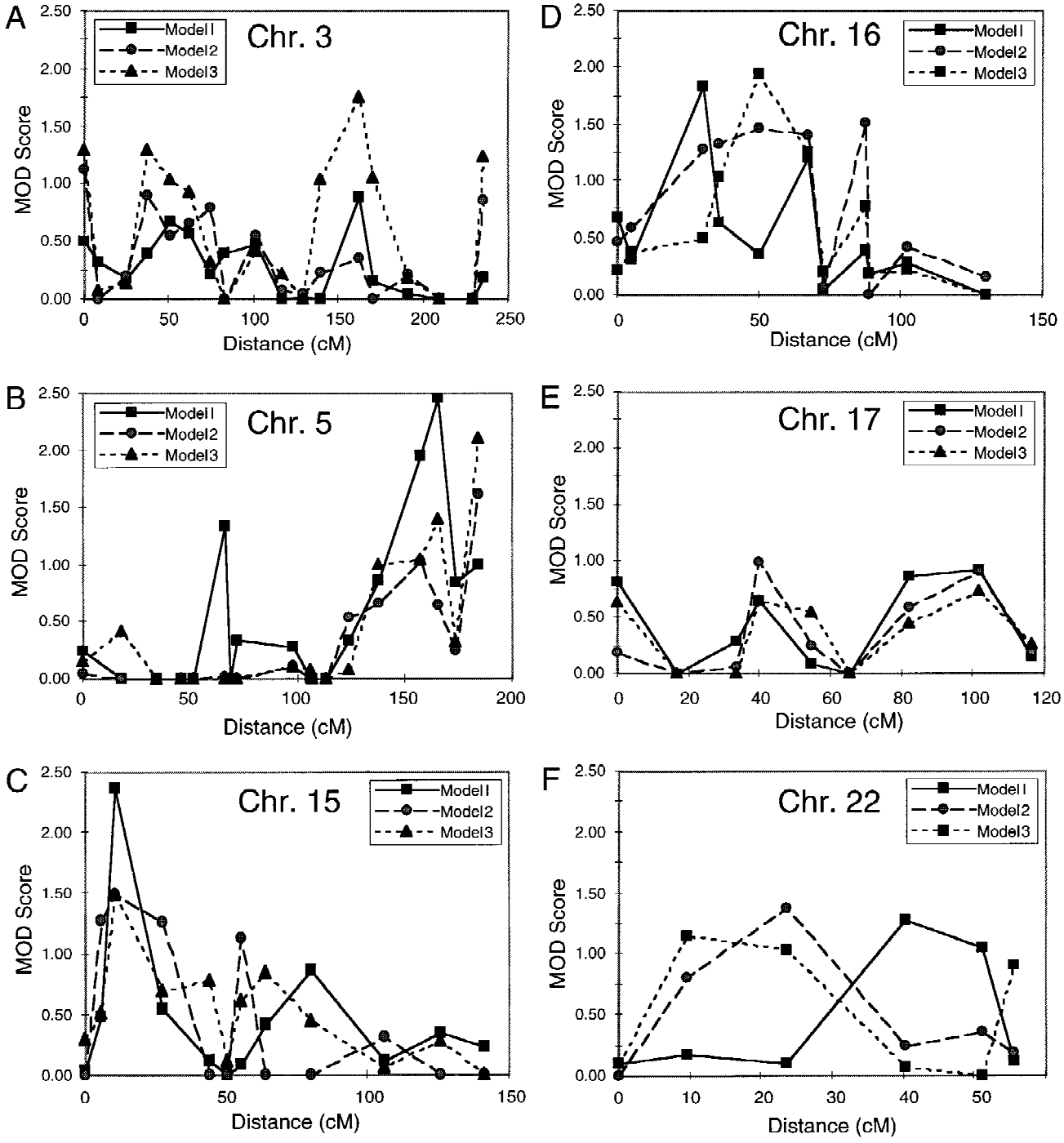

Fig. 1. **A–F:** Maps showing MOD score analysis under three models. Points are at the position of markers (order and distances in Table I); they are connected by straight lines for convenience. Ordinate = MOD score; values above 1.7 represent $P < 0.05$.

manic depressive illness to PGP, located in 16p13.3, testing an autosomal dominant model of inheritance, but that evidence was weakened in their later report. In the later report [Ewald et al., 1995], the highest two-point lod score (2.52 at $\theta = 0$) was observed for D16S510, using a recessive model. Three-point analyses showed the region of maximum LOD between D16S510 and D16S506 [Ewald et al., 1995]. A very recent report examined D16S510 in the Old Order Amish [LaBuda et al., 1996]. Using an autosomal dominant model with age-dependent penetrance, linkage could be excluded (LOD = −3 at $\theta = 0$); a recessive model did not allow exclusion, but also did not provide evidence for linkage [maximum LOD = 0.16 at $\theta = 0.05$; LaBuda et al., 1996]. Nonparametric analysis, however, showed an empirical *P*-value of 0.009 for D16S510 [LaBuda et al., 1996]. We have a broad area of interest in the same general region of chromosome

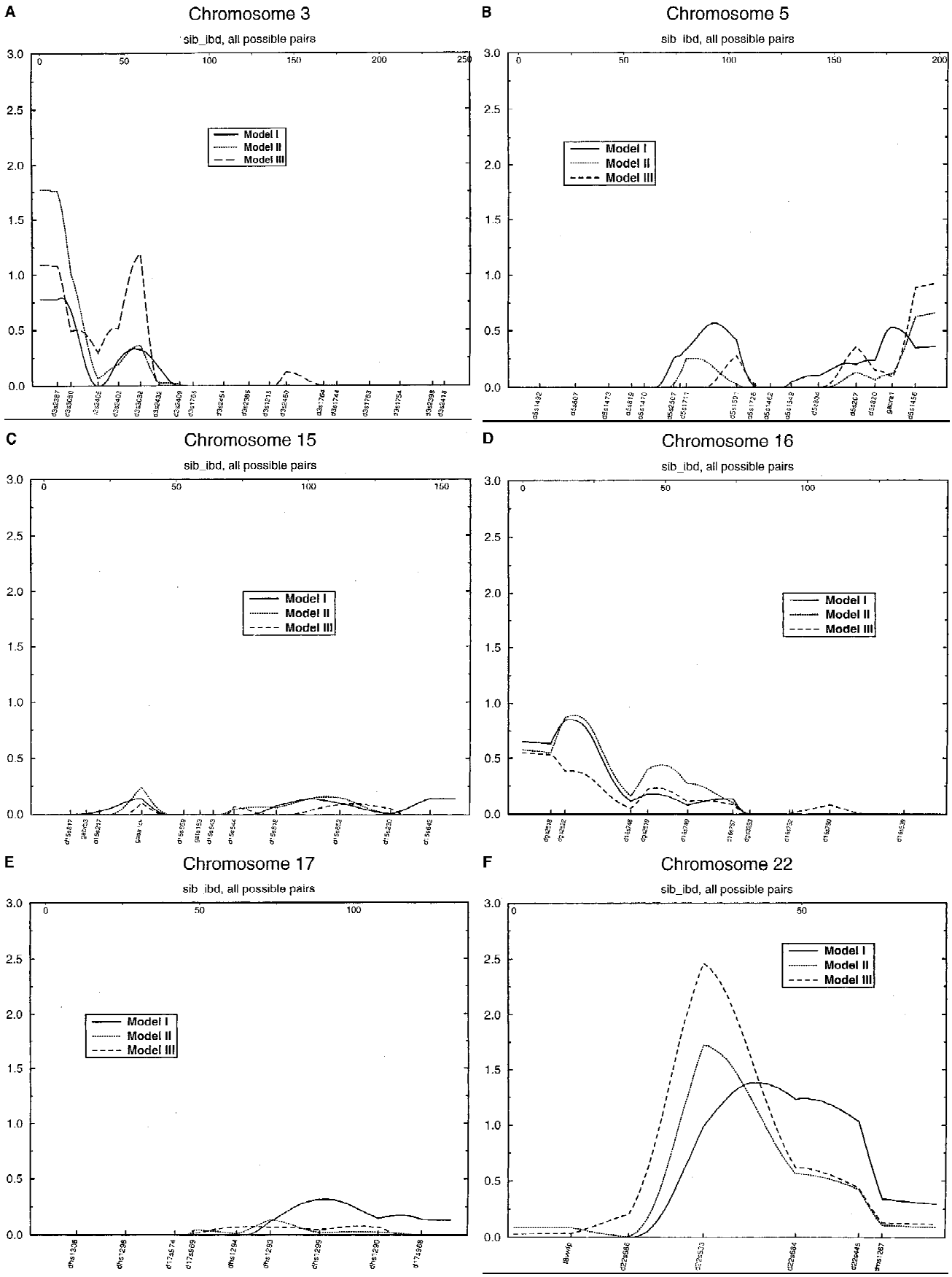

Fig. 2. **A-F:** Maps showing multipoint likelihood calculations done using ASPEX (D. Hines and N. Risch, unpublished) to analyze all possible (n(n-1)/2) sib pairs in each nuclear family. All three models are shown. Ordinate = calculated LOD. Markers are arrayed across the abscissa based upon their map positions (in cM at the top).

16. The nearest locus for which we have data is D16S2622, which is 2.7 cM telomeric to D16S510 according to the Marshfield Clinic map (and 5.4 cM telomeric to D16S506). Analysis of D16S2622 showed increased allele sharing, that did not quite reach significance at the $P = 0.05$ level in ASPEX. D16S748 is 13.5 cM below D16S2622; it flanks the previously reported pair of markers. D16S748 also shows some evidence of allele sharing ($P = 0.06$ and 0.051 for Models I and II, respectively). Our strongest region by two-point analysis is 5.5 cM below D16S748 at D16S2619. However, the sib_ibd option of ASPEX shows the highest levels of sharing at D16S2622 and above it at D16S2618 for all three models, and the multipoint curves (Fig. 2D) peak (at low levels) just below D16S2622, in the region of the previously reported markers. MOD score analyses show modest values throughout this broad region, with two peaks ($P < 0.05$) at D16S748 and D16S749. Thus, we have some weak support for linkage in the same general location reported by Ewald et al. [1995]. More markers must be tested in this region.

Coon et al. [1993] presented evidence for linkage to D5S62, in the region of *DRD1* and *GABRA1.* Although we did not test D5S62 itself, several markers in the broad region of 5q near this marker showed increased allele sharing. The increased sharing at D5S1456 was significant with all three models, with the multipoint analyses suggesting that the region extends telomeric to this marker. MOD score analysis gave the highest value at D5S820 ($P = 0.01$), in this region. This area, therefore, remains of great interest. We are testing additional markers in this region.

Kelsoe et al. [1995, 1996] found some suggestions of a susceptibility locus near the dopamine transporter gene on chromosome 5p15.3 [but see Coon et al., 1993]. We found no evidence of increased allele sharing at D5S1492, the closest marker to the dopamine transporter gene and D5S392 that we have tested to date, nor at any other marker in that region of chromosome 5. Neither MOD score nor multipoint analyses provided evidence for a locus near there.

Ginns et al. [1996] reported *P* values of 0.0003 (using SIBPAL) for D15S45 in a large pedigree from the Old Order Amish. This locus did not show evidence for linkage disequilibrium, however, and the maximum lod score was only 1.1 [Ginns et al., 1996]. We have not tested this RFLP marker itself, but have tested a marker that appears (from comparison of the flanking markers listed in Ginns et al. [1996] with current maps) to lie near it: D15S644. D15S644 does not show evidence for linkage ($P > 0.2$ in all models), nor does any nearby marker. There is a peak in the MOD score in another region of the chromosome, at D15S217, which is worth further analysis.

Although no marker on chromosome 22 showed significantly increased allele sharing in SIBPAL, the results from multipoint analysis (ASPEX, Fig. 2F) showed the highest peak at D22S533, with mLOD = 2.46. This was the highest mLOD obtained in our screening of these chromosomes. This region is worth further analysis.

The results of the initial scan of this subset of chromosomes should be viewed in the context of the whole genome scan of which it forms a part [see companion papers in this issue: Nurnberger et al., 1997; Detera-Wadleigh et al., 1997; Rice et al., 1997; Stine et al., 1997]. This genomic screen did not produce evidence that any one locus contributes a very large fraction of the increased risk for bipolar disorder. This suggests that several genes contribute, each accounting for a portion of the total variance; interaction among genes is also possible. Several regions of interest have been identified for further analysis, which will include examining potential interactions among loci. The results will be pursued using flanking markers, and by filling gaps in the coverage to reduce the average intermarker interval and increase our power to detect genes of smaller effect. Additional families will also be included.

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